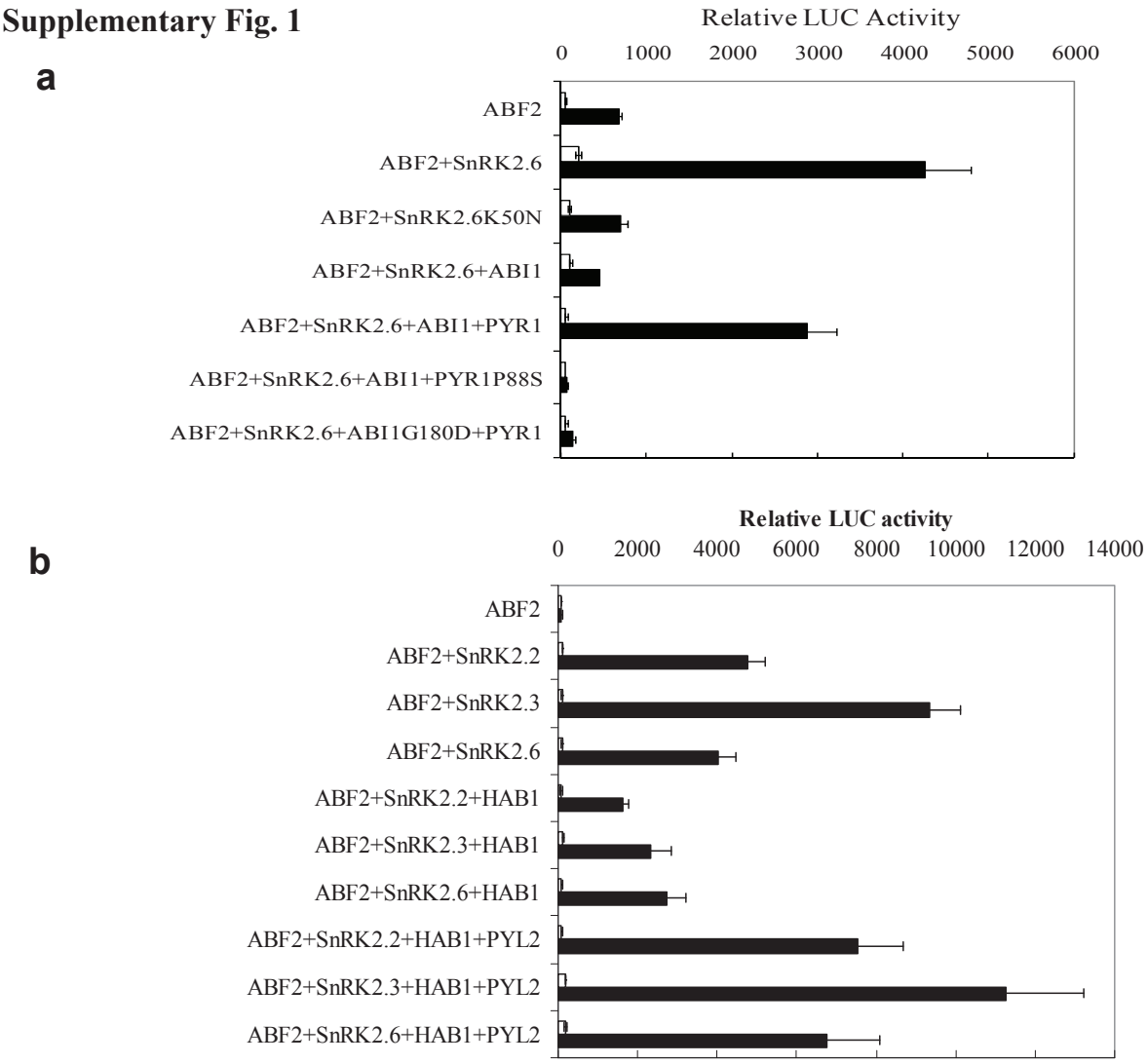
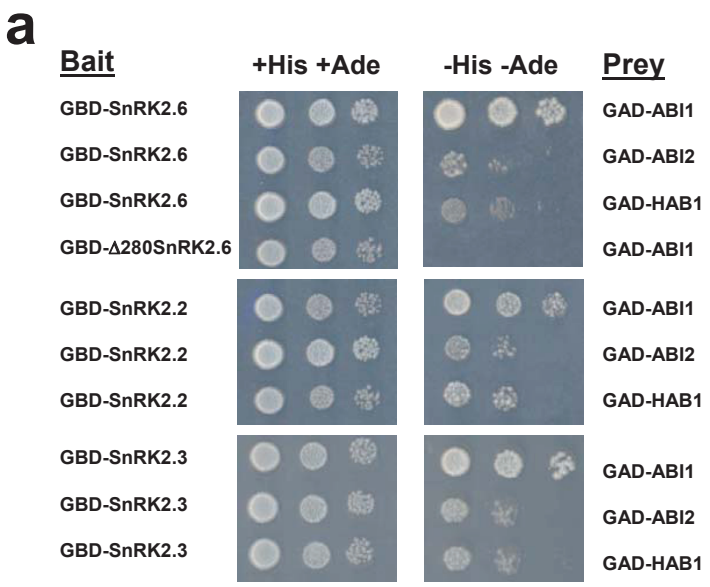
SUPPLEMENTARY INFORMATION

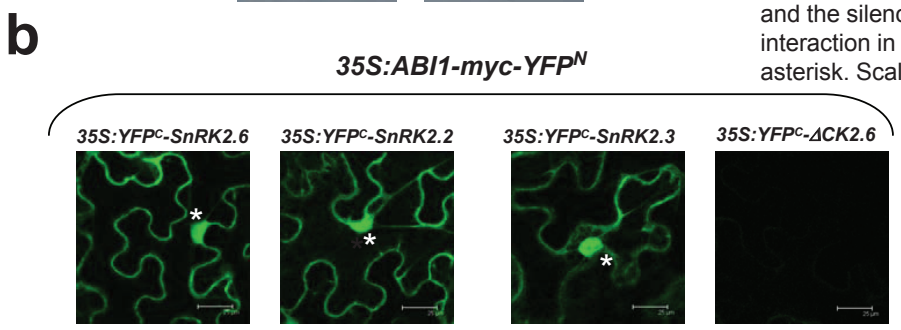
Supplementary Fig. 1

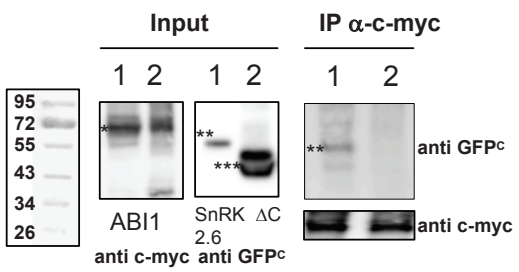


Reconstitution of ABA signaling pathway for stress responsive gene expression in *Arabidopsis* protoplasts. (a) Reconstitution of ABA signaling pathway using PYR1, ABI1, SnRK2.6 and ABF2 in wild type protoplasts. (b) Reconstitution using PYL2, HAB1, different SnRK2s and ABF2 in protoplasts from the *snrk2.2/3/6* triple mutant. The *RD29B* promoter fused with *LUC* coding sequence was used as an ABA-responsive reporter (7 µg per transfection). *ZmUBQ*::*GUS* was included in each sample as an internal control (3 µg per transfection). ABF2-HA, SnRK2s-Flag, His-PYR1/PYL2 and HAB1-myc plasmid constructs were used at 3 µg per transfection, while ABI1 was used at 2 µg per transfection. After transfection, protoplasts were incubated for 5 h under light and in the presence of 0 (open bars) or 5 µM (solid bar) ABA. Data shown are mean ± s.e.m. (n=3).

Supplementary Fig. 2



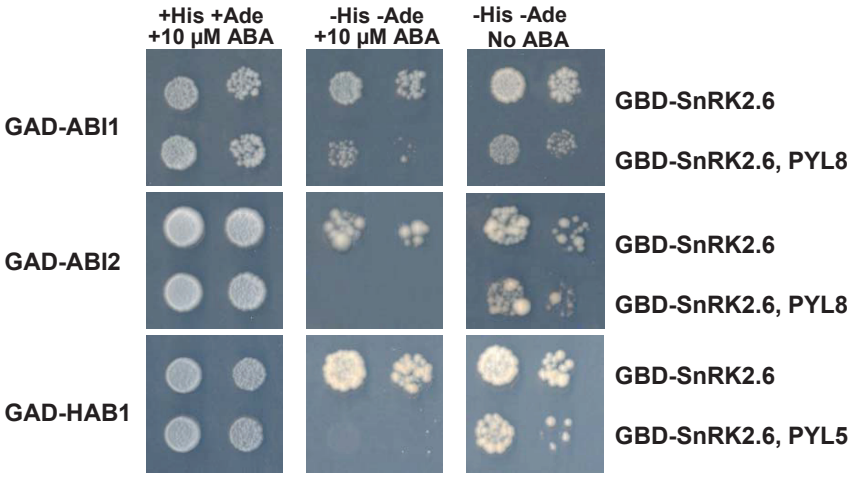




Interaction of clade A PP2Cs with SnRK2s in yeast and plants.
(a) Clade A PP2Cs interact with SnRK2s in a yeast two hybrid assay. SnRK2.2, 2.3 and 2.6 fused to the GAL4-DNA binding domain (GBD) were used as baits and PP2Cs fused to the GAL4-activating domain (GAD) were used as preys. A C-terminal deletion of SnRK2.6 lacking amino acids 280-362 (GBD-Δ280SnRK2.6) did not interact with ABI1. Interaction was determined by growth assay on media lacking His and Ade. Dilutions (10-1, 10-2, 10-3) of saturated cultures were spotted onto the plates and photographs were taken after 5 days. (b) BiFC analysis shows that ABI1 interacts with SnRK2.6, SnRK2.2 and SnRK2.3 in both the nucleus and cytosol of tobacco epidermal cells. Interaction of ABI1 and the three SnRK2s in *Agrobacterium*-infiltrated tobacco (*N. benthamiana*) leaves is shown in epifluorescence images of epidermal leaf cells infiltrated with a mixture of *Agrobacterium* suspensions harboring the indicated constructs and the silencing suppressor p19. The interaction in the nucleus is marked with an asterisk. Scale bar corresponds to 25 μm.

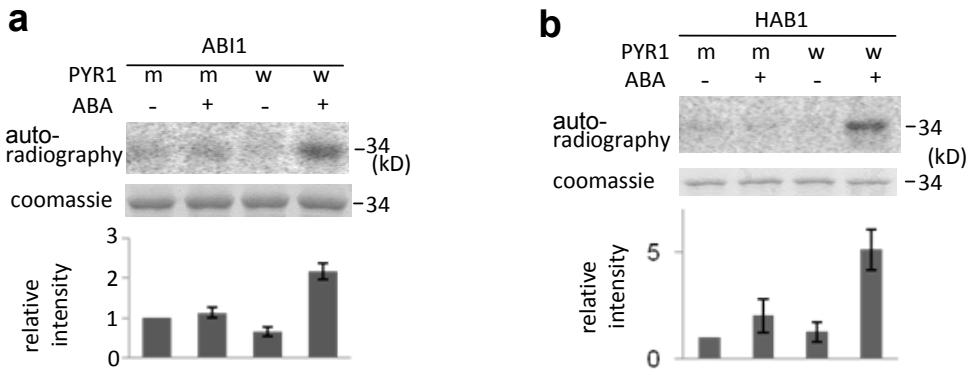
(c) Expression of ABI1 and SnRK2.6 in *Agrobacterium*-infiltrated tobacco (*N. benthamiana*) leaves and co-immunoprecipitation analysis. Protein gel blot analysis demonstrates the expression of the different fusion proteins containing ABI1, the SnRK2.6 and the C-terminal deletion (ΔC) of SnRK2.6 (Input). Protein extracts obtained from tobacco leaves infiltrated with *Agrobacterium* harboring the construct 35S:ABI1-myc-YFPN/35S:YFPC-SnRK2.6 (lane 1) or 35S:ABI1-myc-YFPN/ 35S:YFPC-Δ280SnRK2.6 (lane 2), were analyzed by immunoblots using anti-c-myc or anti-GFPC antibodies. Anti-myc immunoprecipitates (IP α-c-myc) were probed with anti-GFPC antibodies. Fusion proteins are marked with asterisks.

Supplementary Fig. 3



PYLs disrupt the interaction between the PP2Cs and SnRK2.6 in an ABA-dependent manner. Co-expression of ABA receptors abrogates or reduces the interaction of ABI1, ABI2 and HAB1 with SnRK2.6. Interaction was determined by yeast growth assay on media lacking His and Ade. Dilutions (10-2, 10-3) of saturated cultures were spotted onto the plates and photographs were taken after 4 days (ABI1) or six days (ABI2 and HAB1). Full length PP2Cs were fused to the GAD, whereas SnRK2.6 was fused to the GBD in the pBridge three-hybrid vector. When indicated, the ABA-receptors PYL8 and PYL5 were cloned in the SnRK2.6-pBridge construct and co-expressed with SnRK2.6.

Supplementary Fig. 4



Reconstitution of ABA signalling. (a) Reconstitution of ABA signalling. with ABA-activated SnRK2.6 from plant extracts. FLAG-SnRK2.6, which is pulled down from extracts of transgenic plants after ABA treatment and treated with GST-ABI1 and His-tagged wild type PYR1 (w) or mutated PYR1P88S (m) in the absence (-) or presence (+) of 2 μM (+)-ABA, was incubated with GST-ABF2 fragment in the presence of [γ32P]-ATP. Coomassie staining, autoradiography and relative radioactivities of GST-ABF2 fragment are shown. Radioactivities of GST-ABF2 fragment were normalized, taking the radioactivity of the band with PYR1P88S in the absence of ABA as 1 (mean ± s.e.m., n = 7). (b) Reconstitution of ABA signalling with HAB1 instead of ABI1. MBP-SnRK2.6 treated with GST-HAB1 and His-tagged wild type PYR1 (w) or mutated PYR1P88S (m) in the absence (-) or presence (+) of 2 μM (+)-ABA was incubated with the GST-ABF2 fragment in the presence of [γ32P]-ATP. Coomassie staining, autoradiography and relative radioactivities ofGST-ABF2 fragment are shown. Radioactivities of GST-ABF2 fragment were normalized, taking the radioactivity of the band with PYR1P88S in the absence of ABA as 1 (mean ± s.e.m., n = 3).