Rodrigues_Fig S1

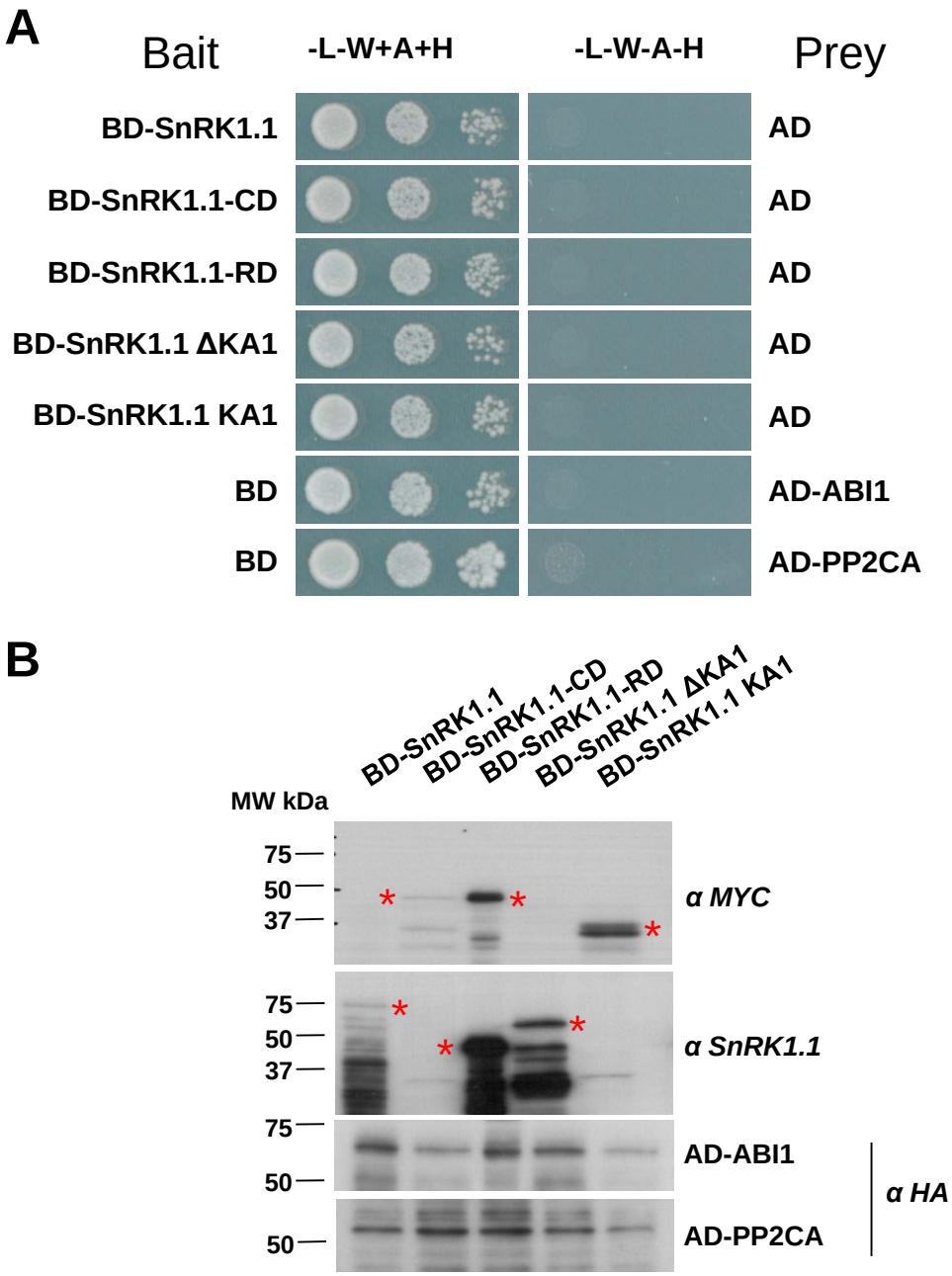
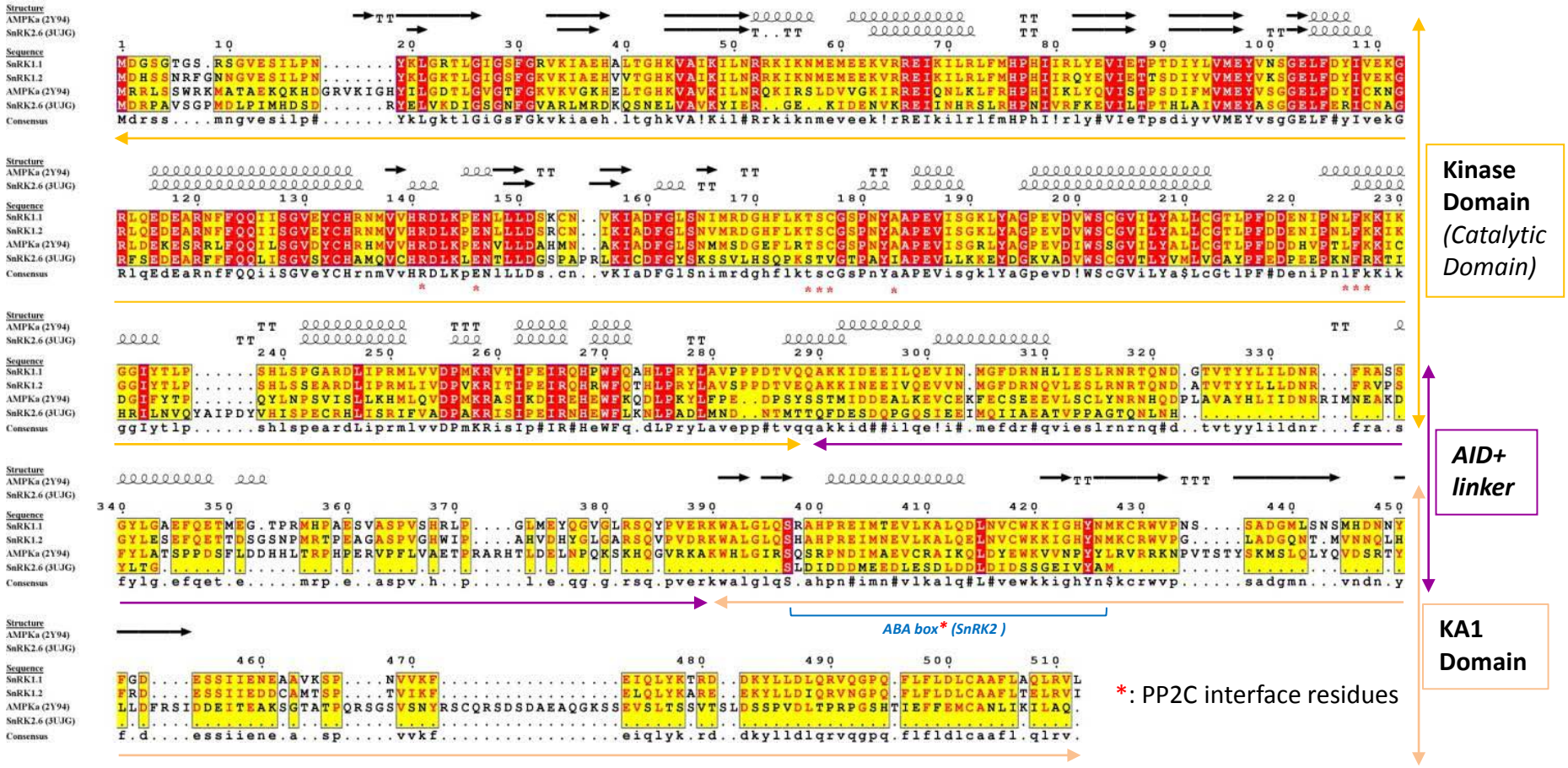


Figure S1. Yeast-two-hybrid controls for the SnRK1.1 and PP2C interaction (Fig. 2*A*). **(A)** None of the AD and BD constructs activate the *ADE* and *HIS* reporters. Colony growth was assessed on medium lacking adenine and histidine (-A-H) using serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}) of saturated cultures. The different SnRK1.1 deletions are shown. CD=catalytic domain, residues 1-293; RD=regulatory domain, residues 294-512; KA1 domain=residues 390-512. AD=GAL4 activation domain, BD=GAL4 binding domain. **(B)** Expression of the indicated constructs in yeast as revealed by immunodetection with anti-HA (for AD-constructs) and anti-c-MYC (for BD-constructs) antibodies. Full-length SnRK1.1 and SnRK1.1 ΔKA1 have low expression levels and are more readily detected with the anti-SnRK1.1 antibody. Note that this antibody is against a peptide in the more proximal part of the RD-region and thus does not detect SnRK1.1-CD nor SnRK1.1 KA1. Red asterisks indicate the band with the expected molecular weight.

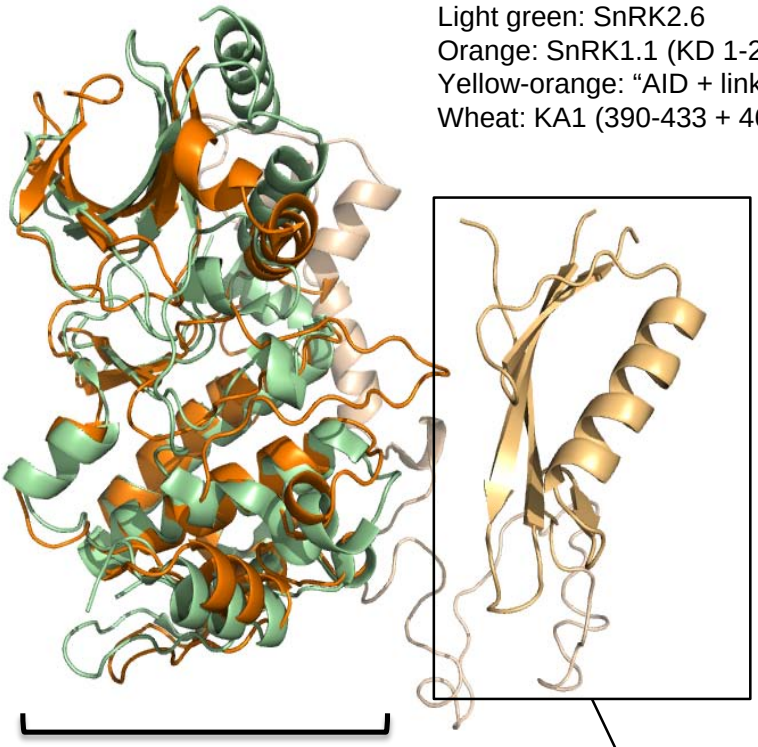
Rodrigues_Fig S2

A



Rodrigues_Fig S2 (cont.)

B

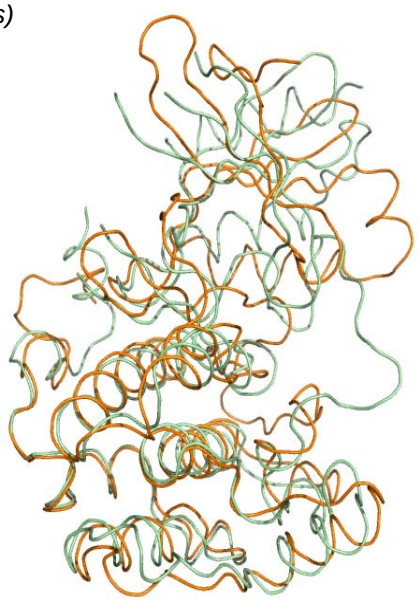


C

Kinase domain (KD)

Light green: SnRK2.6
Orange: SnRK1.1

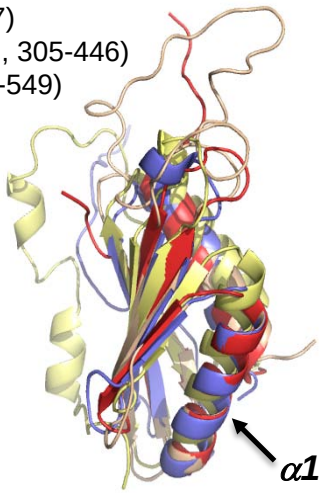
RMSD: 1.62Å (on 73% of atoms)



D

KA1 domains:

Wheat: SnRK1.1 (390-512)
Slate blue: MARK3 (1UL7)
Yellow: SnRK3.11 (2HEB, 305-446)
Red: AMPKα (2YA3, 397-549)



Rodrigues_Fig S2

Figure S2. Alignment and structural comparison of SnRK1 and SnRK2. **(A)** Alignment of SnRK1.1 (Q38997), SnRK1.2 (P92958), AMPKα (PDB: 2Y94-A) and SnRK2.6 (PDB: 3UJG-A) was performed with ClustalW and represented with ESPript (Gouet *et al.*, 1999), displaying the known secondary structures on the top. Residues fully conserved in all four sequences are in red and those conserved in three in yellow. Residues marked by a red asterisk are implicated in physical interaction with the HAB1 PP2C phosphatase (3UJG) (Soon *et al.*, 2012). Kinase Domain (KD, catalytic domain, CD; common to the four proteins) is marked by orange arrows and the KA1 domain (only for SnRK1 and AMPK) in marked by blue arrows. "AID + linker" (marked by purple arrows) stands for "Auto-Inhibitory Domain" followed by a linker region by analogy with the AMPKα (Hardie *et al.*, 2012). No function has been assigned to this sub-domain in plants. **(B)** Structural alignment of the SnRK1.1 model [performed from template 2Y94S (Xiao *et al.*, 2011) with Swiss-model (Arnold *et al.*, 2006)] with SnRK2.6 (3UJG-A). Colored as described, cartoon representation. **(C)** Structural alignment of the kinase domain of SnRK1.1 model with SnRK2.6. RMSD of kinase domain alignment is 1.62Å on 73% of aligned atoms, giving confidence on the conservation observed in alignment (see **A**). As almost all the important residues (* in **A**) are in loops, no more can be assessed for these. The other three are located in the αG helix of the kinase domain in its large lobe (subdomain XS) (Hanks & Hunter, 1995). The large lobe alignment of these kinases is good (RMSD=0.81Å on 74% of aligned atoms) giving confidence in these conservation. Colored as described, ribbon representation. **(D)** Validation of the Kinase Associated1 (KA1) domain model of SnRK1.1. KA1 domain from Uniprot database is annotated as shorter (486-512) than our considered model (390-512). Comparison of the actual structures of a SnRK3.11/SOS2 (2HEB) (Sánchez-Barrena *et al.*, 2007), MARK3 (1UL7) (Tochio *et al.*, 2006), the AMPKα "core complex" part (2YA3) (Xiao *et al.*, 2011) with a model of the last 122 residues of SnRK1.1 (part colored blue in **(A)** modeled by Phyre (Kelley & Stenberg, 2009). This part is clearly exhibiting a KA1 fold with a β-sheet (of four β-strands) and two α-helixes on the same side of the β-sheet. Colored as stated, cartoon representation. All images and structural alignment were generated with Pymol (from Delano Scientific). α1 refers to the α-helix part of the phosphatase interacting domain (PPI) (Sánchez-Barrena *et al.*, 2007).

Rodrigues_Fig S3

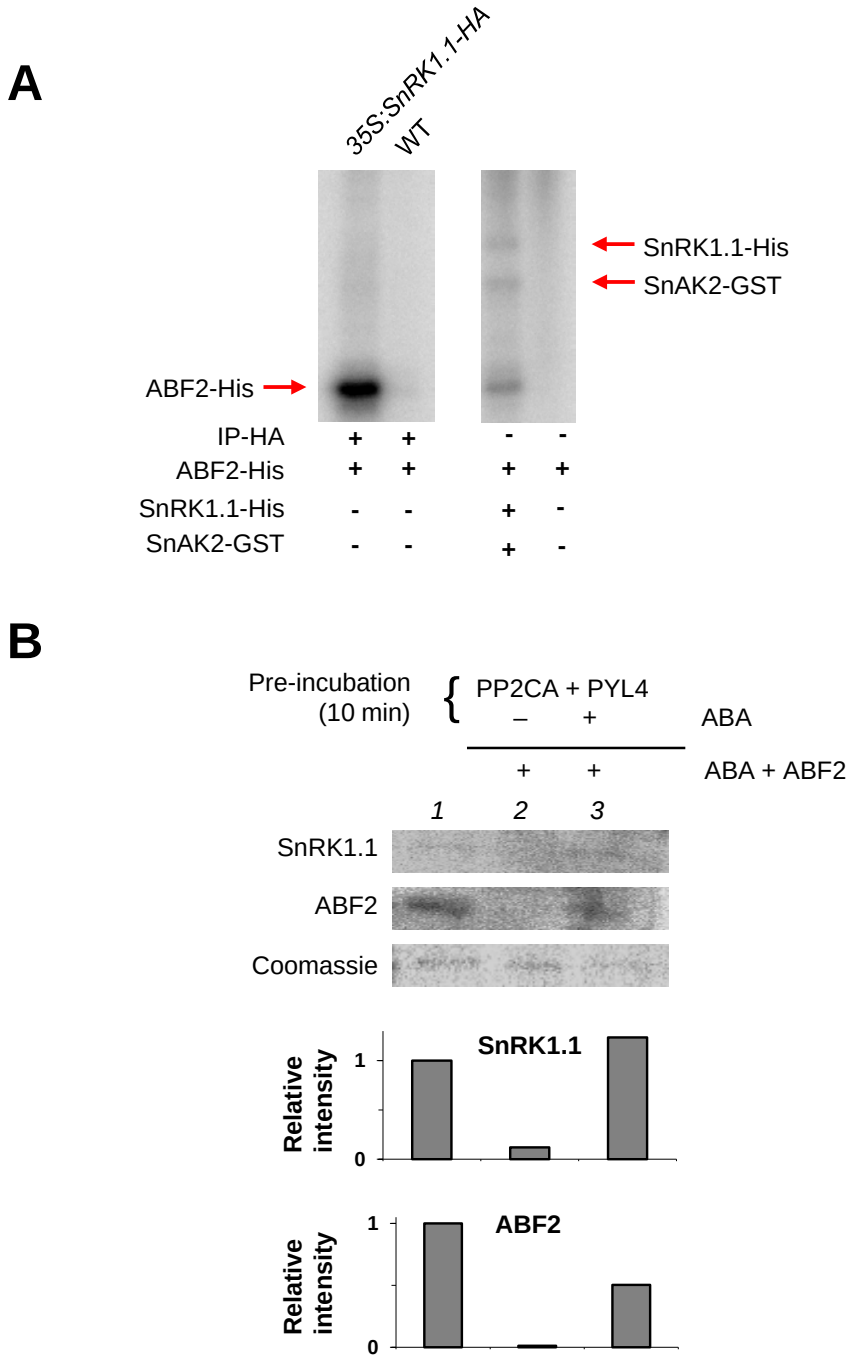


Figure S3. SnRK1.1 is inactivated by recombinant His-PP2CA *in vitro*. **(A)** Control HA-immunoprecipitation from WT plants retrieves no ABF2 phosphorylating activity, showing that the activity measured from *35:SnRK1.1-HA* plants is specific to SnRK1.1. Right panel, positive control showing that recombinant SnRK1-His preactivated with SnAK2-GST phosphorylates ABF2. **(B)** Where indicated SnRK1.1 was pre-incubated, for 10 min, with PP2CA and PYL4 in the absence (lane 2) or presence (lane 3) of ABA, to allow or prevent PP2CA activity, respectively. After this pre-incubation ABA was added to all samples to inactivate PP2CA, the ABF2 substrate was supplied, and the reaction was further incubated for 1h.

Rodrigues_Fig S4

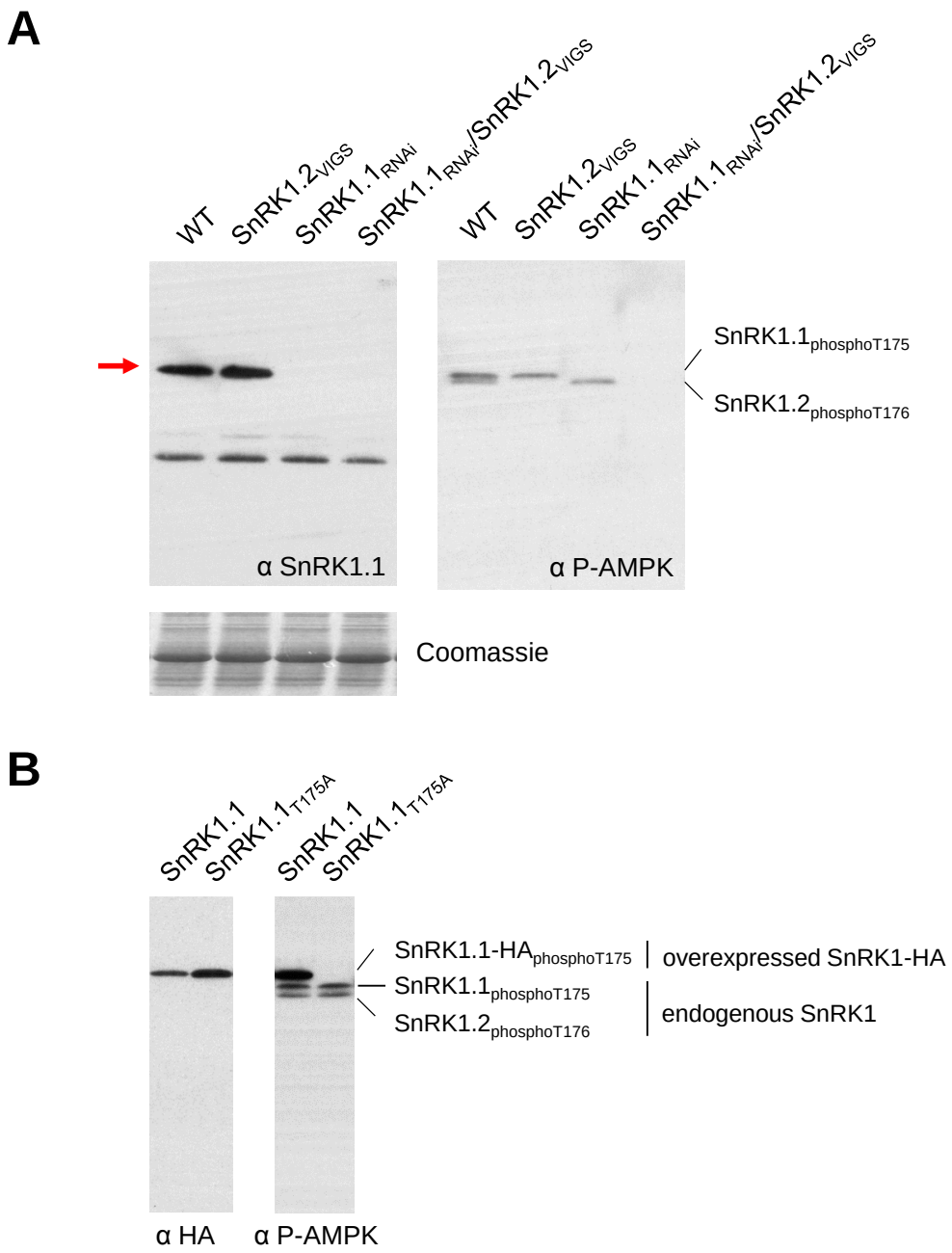


Figure S4. Specific detection of phosphorylated SnRK1. (A) The P-AMPK antibody recognizes specifically SnRK1.1 and SnRK1.2 in total protein extracts from *Arabidopsis* leaves. WT and SnRK1.1 RNAi plants were infiltrated with *Agrobacterium* containing viral vectors for a GFP control (WT) or for VIGS of SnRK1.2 and analyzed 3 weeks after, using anti-SnRK1.1 and anti-P-AMPK antibodies (Baena-González et al., 2007). The red arrow indicates the band corresponding to SnRK1.1. **(B)** Mutation of T175 to A abolishes SnRK1.1-HA recognition by the P-AMPK antibody. *Arabidopsis* mesophyll protoplasts were transfected with constructs expressing SnRK1.1-HA or SnRK1.1$_{T175A}$-HA and proteins were detected after SDS-PAGE by immunoblotting with anti-HA or anti-P-AMPK antibodies.

Rodrigues_Fig S5

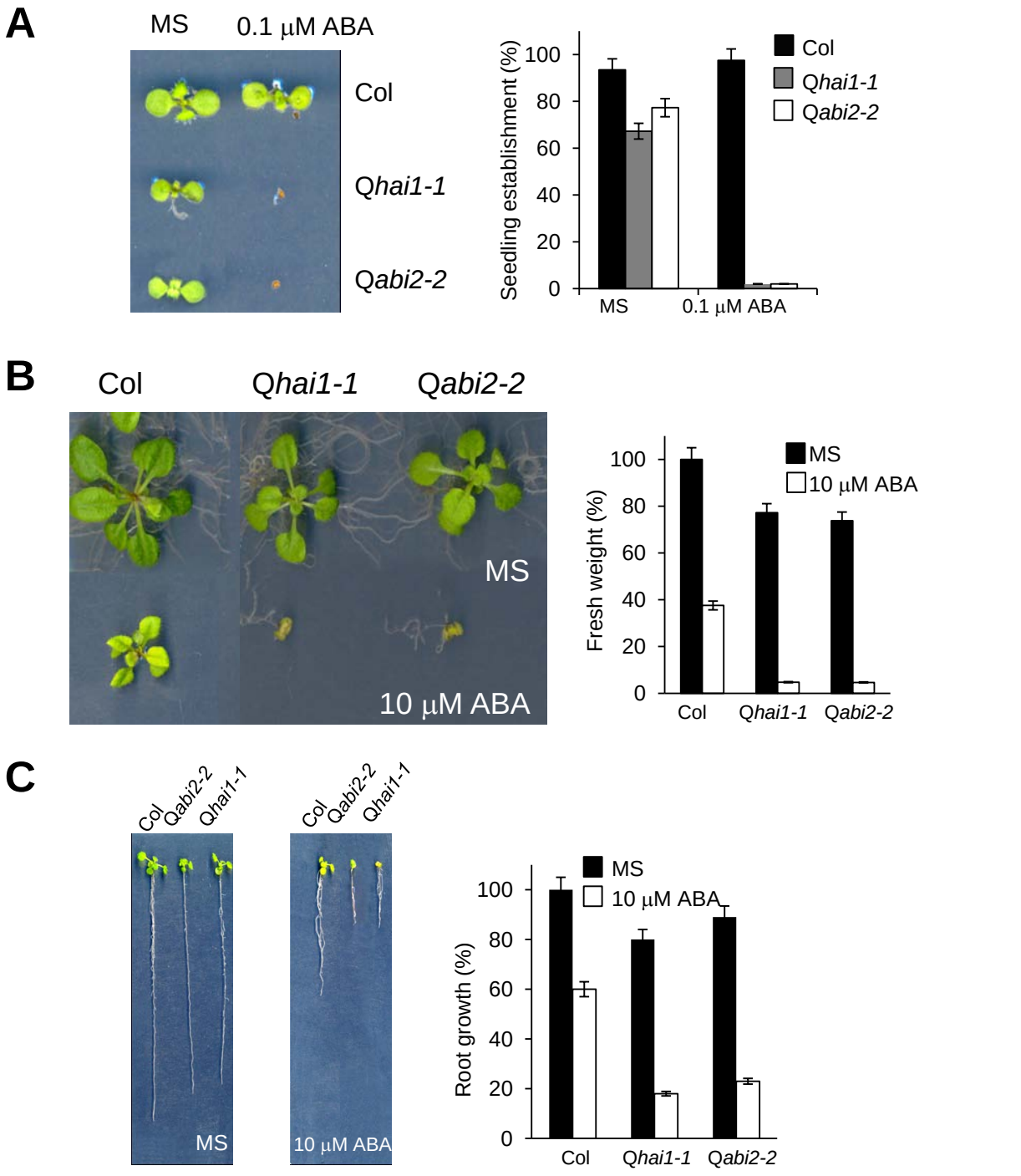


Figure S5. Clade A *pp2c* quadruple mutants are ABA–hypersensitive. **(A)** Enhanced sensitivity to inhibition of seedling establishment by ABA. Seeds were germinated and grown in medium lacking or supplemented with 0.1 µM ABA for 10 days (*n*=100). **(B)** The growth of the *pp2C* mutants is not strongly affected in control MS medium but is impaired in medium containing 10 µM ABA. Photographs were taken 20 days after transferring 5-day-old seedlings from MS medium to plates lacking or containing 10 µM ABA (*n*=15). **(C)** ABA-hypersensitive root growth inhibition of *pp2c* mutants. Photographs were taken 10 days after transferring 4-day-old seedlings to MS plates lacking or supplemented with 10 µM ABA (*n*=15). *Col*, Columbia wild-type; Q*hai1-1, hab1-1 abi1-2 pp2ca-1 hai1-1*; Q*abi2-2, hab1-1 abi1-2 pp2ca-1 abi2-2* (Antoni *et al.*, 2013). Values represent means±SEM.

Rodrigues_Fig S6

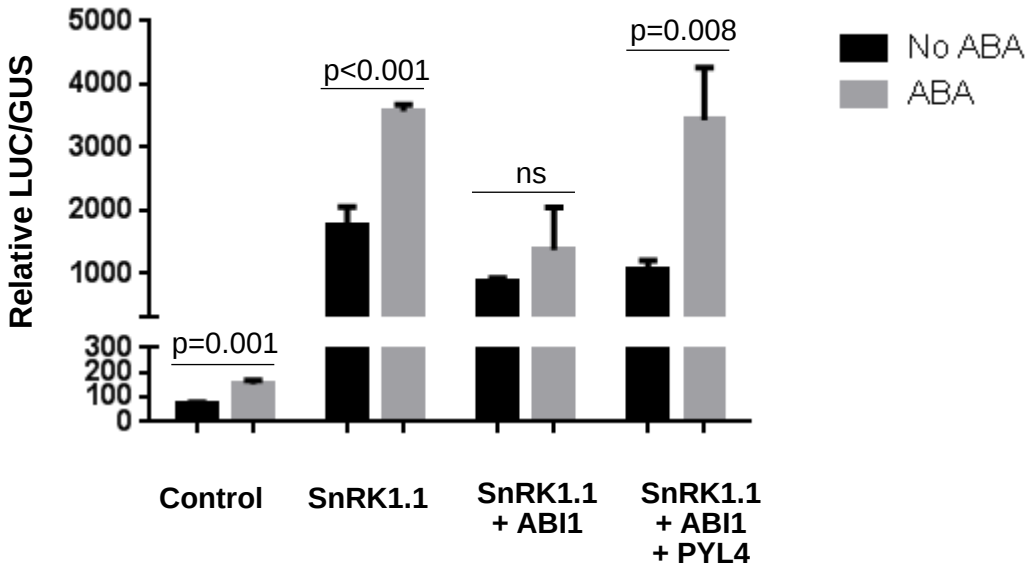


Figure S6. ABA promotes SnRK1 signaling in protoplasts. Cells were transfected with control DNA, or with plasmids expressing SnRK1.1 alone or in combination with ABI1 and the PYL4 receptor. In the absence of overexpressed PYL4, ABA and the endogenous receptors are not sufficient to inhibit overexpressed ABI1. Samples are the same as in Fig. 2A, but instead of normalizing the mock and ABA sets to their corresponding controls, all samples were normalized to the mock control (n=3). Values represent means±SEM. p-values, multiple t-test with Holm-Sidak correction.

Rodrigues_Fig S7

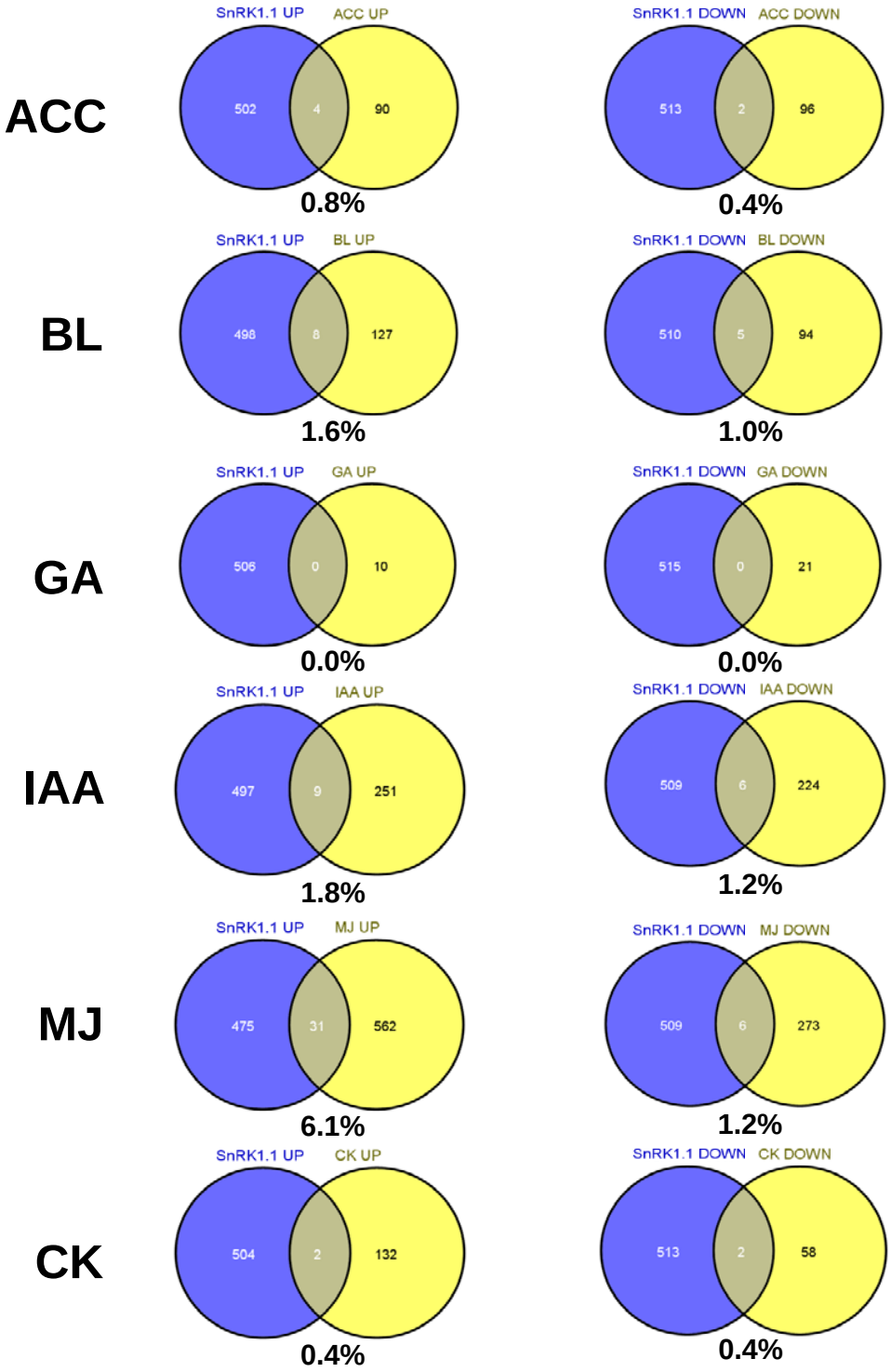


Figure S7 (cont.) Overlap between transcriptional changes induced by SnRK1.1 (Baena-González, Rolland, *et al.*, 2007) and indicated hormone treatments (Nemhauser et al., 2006; AtGenExpress). UP and DOWN denote the set of up- or down-regulated genes, respectively, in the indicated datasets. Percentage values refer to the number of overlapping genes per total number of upregulated or downregulated SnRK1.1 targets. ACC, 1-aminocyclopropane-1-carboxylic acid (ethylene precursor); BL, brassinolide; GA, gibberellic acid; IAA, indole-3-acetic acid (auxin); MJ, methyl jasmonate; CK, cytokinin

Rodrigues_Fig S7 (cont.)

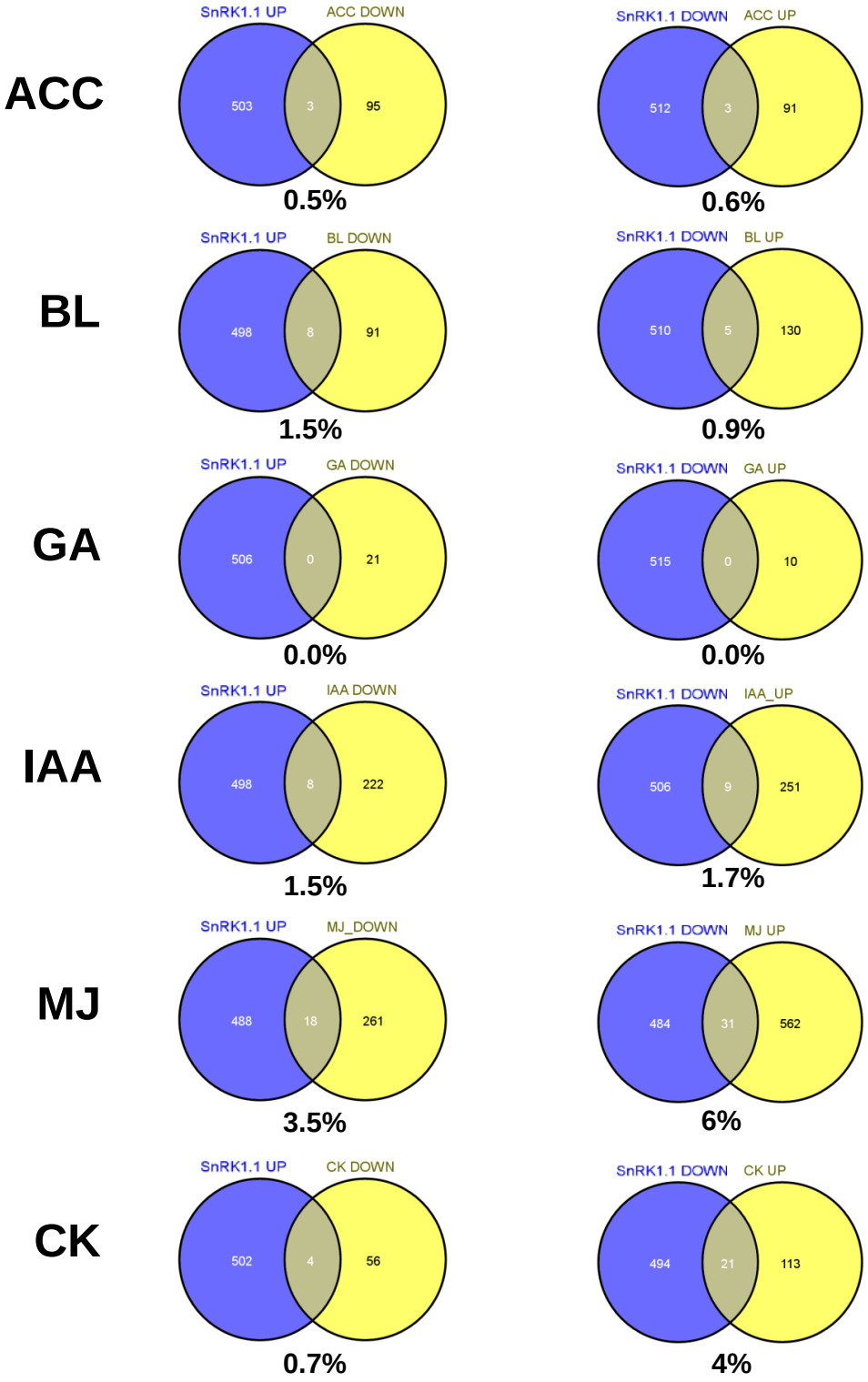


Figure S7 (cont.) Overlap between transcriptional changes induced by SnRK1.1 (Baena-González, Rolland, *et al.*, 2007) and indicated hormone treatments (Nemhauser et al., 2006; AtGenExpress). UP and DOWN denote the set of up- or down-regulated genes, respectively, in the indicated datasets. Percentage values refer to the number of overlapping genes per total number of upregulated or downregulated SnRK1.1 targets. ACC, 1-aminocyclopropane-1-carboxylic acid (ethylene precursor); BL, brassinolide; GA, gibberellic acid; IAA, indole-3-acetic acid (auxin); MJ, methyl jasmonate; CK, cytokinin

Rodrigues_Fig S7 (cont.)

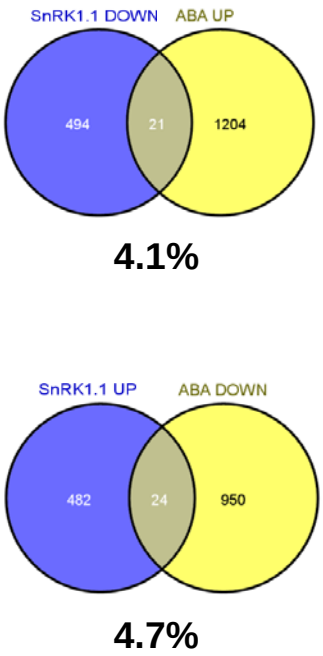


Figure S7. Overlap between transcriptional changes induced by SnRK1.1 (Baena-González, Rolland, *et al.*, 2007) and ABA (Nemhauser et al., 2006; AtGenExpress). Overlap between the genes induced by SnRK1.1 and repressed by ABA, and between the genes repressed by SnRK1.1 and induced by ABA. UP and DOWN denote the set of up- or down-regulated genes, respectively, in the indicated datasets. Percentage values refer to the number of overlapping genes per total number of upregulated or downregulated SnRK1.1 targets.

Rodrigues_Supplementary Table S1
Cloning primers
Restriction sites introduced by PCR are marked in blue

Name	Primer sequence	Vector	Description	Sites used for the cloning
SnRK1.1 Fw	CGGGATCCATGGATGGATCAGGCACAGG	pHBT95	SnRK1.1 overexpression in protoplasts	BamHI/StuI
SnRK1.1 Rev	AAGGCCTGAGGACTCGGAGCTGAGC	pHBT95		
ABI1 BamHI Fw	TTTGGATCCATGGAGGAAGTATCTCCGGCG	pHBT95	ABI1 overexpression in protoplasts	BamHI/SmaI (insert)
ABI1 SmaI Rev	TTTCCCGGGGTTCAAGGGTTTGCTCTTGAG	pHBT95		BamHI/StuI (vector)
PP2CA Fw	GCGGATCCATGGCTGGGATTTGTTGC	pHBT95	PP2CA overexpression in protoplasts	BamHI/StuI
PP2CA Rev	AAGGCCTAGACGACGCTTGATTATTCCT	pHBT95		
At1g03590 BamHI Fw	CGCGGATCCATGGGAGGTTGTATCTCTAAG	pHBT95	At1g03590 (URP) overexpression in protoplasts	BamHI/StuI
At1g03590 StuI Rev	GAAGGCCTAGTCTTTGGTTCCTCTCCAGG	pHBT95		
PYL4_StuI_Fw	AAGGCCTCTTGCCGTTCACCGTCCTT	pHBT95	PYL4 overexpression in protoplasts	StuI/PstI
PYL4_PstI_Rev	AACTGCAGTCACAGAGACATCTTCTTC	pHBT95		
2CASal2bFW	AAAGTCGACTCATGGCTGGGATTTGTTGCGGT	pGEX-4T1	Production of recombinant PP2CA-GST	SalI/NotI
2CANotI Rev	AAAGCGGCCGCTTAAGACGACGCTTGATTATTC	pGEX-4T1		
SnRK1.1BamHI-F	CGGGATCCGATGGATCAGGCACAGGCAG	pET28a	Production of recombinant His-T7-SnRK1.1CD	BamHI/EcoRI
SnRK1.1I293_EcoRI_RP_STOP	CCGGAATTCTCAAATCTTTTTTGCCTGTTGC	pET28a		
SnRK1.1D294EcoRIFw	CCGGAATTCGACGAGGAGATTCTCCAAGAAG	pET28a	Production of recombinant His-T7-SnRK1.1RD	EcoRI
SnRK1.1EcoRI-R	CGGAATTCTCAGAGGACTCGGAGCTGAG	pET28a		
PP2CA_NdeI_Fw	TTTGTCGACTACATATGGCTGGGATTTGTTGCGGT	pGADT7	Expression GAL4 AD-PP2CA for Y2H	NdeI/SmaI
PP2CA_SmaI_Rev	TTTGTCGACTTACCCGGGAGACGACGCTTGATTATTCC1	pGADT7		
SnRK1.1EcoRIFw	CCGGAATTCATGGATGGATCAGGCACAGGC	pGBKT7	Expression of GAL4 BD-SnRK1.1 full-length for Y2H	EcoRI/BamHI
SnRK1.1NOSTOPBamRev	CGCGGATCCGAGGACTCGGAGCTGAGCAAG	pGBKT7		
SnRK1.1D294EcoRIFw	CCGGAATTCGACGAGGAGATTCTCCAAG	pGBKT7	Expression of GAL4 BD-SnRK1.1 RD for Y2H	EcoRI/BamHI
SnRK1.1NOSTOPBamRev	CGCGGATCCGAGGACTCGGAGCTGAGCAAG	pGBKT7		
BD adapt1	TATGGGATCCATGGAAGCTTTAGGCCTCTGCA	Adaptors to create BamHI and StuI sites in the pGBKT7 MCS		
BD adapt2	GAGGCCTAAAGCTTCCATGGATCCCA	to generate the following BD-KIN delections		
SnRK1.1BamNdeFw	CGGGATCCCATATGGATGGATCAGGCACAGGC	pGBKT7	Expression of GAL4 BD-SnRK1.1 CD for Y2H	BamHI/StuI
SnRK1.1CatDStuRev	TAGGCCTGTCAATCTTTTTTGCCTGTTG	pGBKT7		
SnRK1.1BamNdeFw	CGGGATCCCATATGGATGGATCAGGCACAGGC	pGBKT7	Expression of GAL4 BD-SnRK1.1 ΔKA1 for Y2H	BamHI/StuI
SnRK1.1D390StuRev	TAGGCCTTCTCTCAACAGGGTATTGAG	pGBKT7		
SnRK11 KA1BamH1-Fw	CGGGATCCAAATGGGCTCTTGGACTTCAG	pGBKT7	Expression of GAL4 BD-SnRK1.1 KA1 for Y2H	BamHI/StuI
SnRK1.1D390StuRev	TAGGCCTTCTCTCAACAGGGTATTGAG	pGBKT7		

Mutagenesis primers

ABI1_D177A_Fw	CATTTCTTCGGTGTTTACGCTGGCCATGGCGGTTCTCAGG	To generate a catalytically inactive ABI1
ABI1_D177A_Rev	CCTGAGAACCGCCATGGCCAGCGTAAACACCGAAGAAATG	
PP2CA_D142A_Fw	CATTTCTACGGTGTCTTTGCTGGCCATGGCTGCTCTCATG	To generate a catalytically inactive PP2CA
PP2CA_D142A_Rev	CATGAGAGCAGCCATGGCCAGCAAAGACACCGTAGAAATG	

qPCR primers

EIF4 A	TCATAGATCTGGTCCTTAAACC	amplifying eIF4, house-keeping gene
EIF4 B	GGCAGTCTCTTCGTGCTGAC	
DIN6 A	AACTTGTCGCCAGATCAAGG	amplifying DIN6, SnRK1.1 activated marker gene
DIN6 B	GGAACACGTGCCTCTAGTCC	
SEN5 A	GCGAAACTCTCTCCGACTTC	amplifying SEN5, SnRK1.1 activated marker gene
SEN5 B	CCACAGAACAACCTTTGACG	
AXP A	CTTCGACAAGCCTTCTCACC	amplifying AXP, SnRK1.1 activated marker gene
AXP B	TCGTCGCTGTATAGCCAATC	
RAB18 A	TGGCTTGGGAGGAATGCTTCA	amplifying RAB18, ABA activated marker gene
RAB18 A	CCATCGCTTGAGCTTGACCAGA	
RD29B A	CTTGGCACCACCGTTGGGACTA	amplifying RD29B, ABA activated marker gene
RD29B B	TCAGTTCCCA GAATCTTGAACT	