Profiling of associated proteins reveals dual functions of the phosphatase ABI1 in abscisic acid signaling

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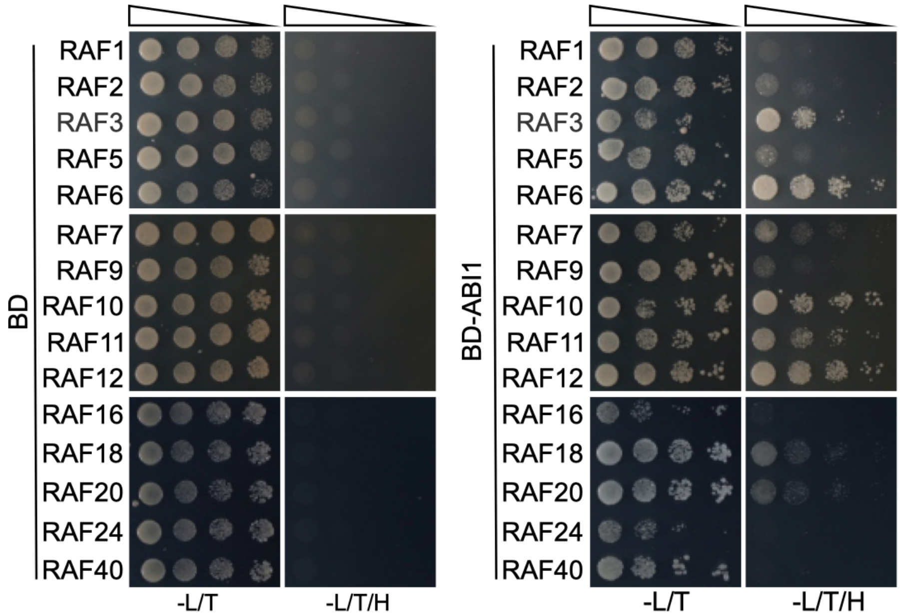
Supplemental Information

This file includes:

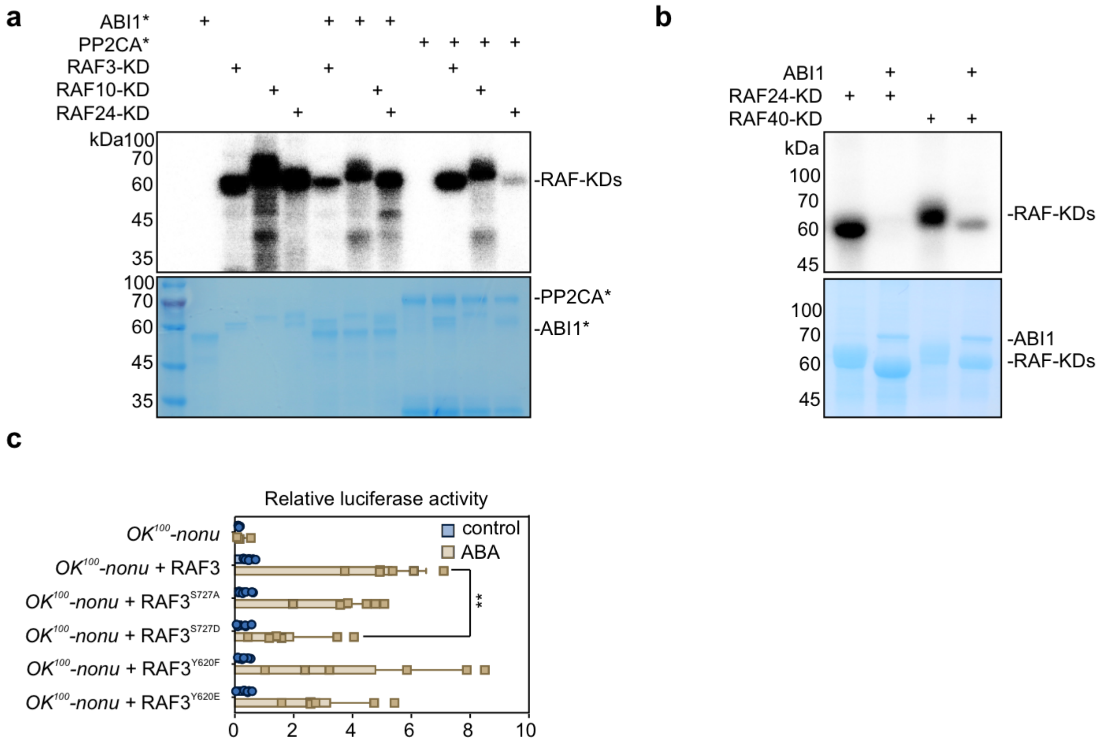
Supplementary Table 1
Supplementary Figure 1 to 3 with figure legends.

Supplementary Table I. Known components in ABA signaling present in our list of ABI1-associated proteins

AGI ID	Name	Annotated Function	Reference
Components in ABA receptor-coupled core signaling			
AT2G38310	PYL4/RCAR10	ABA receptor	
AT2G40330	PYL6/RCAR9	ABA receptor	(Ma et al., 2009; Park et al., 2009)
AT4G01026	PYL7/RCAR2	ABA receptor	
AT5G53160	PYL8/RCAR3	ABA receptor	
AT3G50500	SnRK2.2	Positive regulator in ABA signaling, phosphorylates substrates to transduce ABA signaling	(Fujii et al., 2007; Fujita et al., 2009)
AT5G57050	ABI2	Clade A PP2C, dephosphorylates and inhibits SnRK2 in absence of ABA, inhibited by PYLs	(Merlot et al., 2001)
Other components			
AT5G11850	RAF3	Phosphorylates and activates ABA-dependent SnRK2s	(Lin et al., 2020; Soma et al., 2020)
AT1G73660	RAF5/SIS8	Phosphorylates and activates ABA-dependent SnRK2s; the knock-out mutant *sis8* is insensitive to sugar	(Huang et al., 2014; Lin et al., 2020; Soma et al., 2020)
AT4G04720	CPK21	Inhibited by ABI1, phosphorylates SLAC1 to activate the guard cell anion channel	(Geiger et al., 2011)
AT3G18040	MPK9	Expressed preferentially in guard cells and involved in ROS-mediated ABA signaling	(Jammes et al., 2009)
AT2G23070	CKA4	Plastid casein kinase 2 for protein phosphorylation in the chloroplast stroma, *cka4* mutant showed reduced sensitivity to ABA	(Wang et al., 2014)
AT5G53000	TAP46	A PP2A-associated protein, *tap46* knockdown mutants are less sensitive to ABA	(Hu et al., 2014)
AT5G52200	AT-I2	Inhibitor of PP1 phosphatase, negatively regulates ABA signaling	(Hou et al., 2016)
AT2G35330	PIR1	RING finger E3 ligase, mediates the degradation of PP2CA	(Baek et al., 2019)
AT1G61210	DWA3	Negatively regulates ABA responses	(Lee et al., 2011)
AT3G51780	BAG4	Regulates the KAT1 potassium channel and controls stomatal movement	(Locascio et al., 2019)
AT1G77180	SKIP	Transcription factor involved in ABA signaling and salt/osmotic stress tolerance	(Lim et al., 2010)
AT2G41430	EDR15	A marker ABA responsive gene, negatively regulates ABA signaling	(Kariola et al., 2006)
AT2G21660	GRP7, CCR2	A glycine-rich RNA-binding protein involved in ABA and stress response	(Cao et al., 2006)
AT1G18080	RAFK1A	Involvement of ABA response by negatively regulating protein translation	(Guo et al., 2011)
AT4G16830	AtRGGA	A Hyaluronan mRNA binding protein, overexpressing plants show ABA tolerance	(Ambrosone et al., 2015)
AT3G22380	TIC	A circadian regulator, *tic* is hypersensitive to ABA and resistant to drought stress	(Sanchez-Villarreal et al., 2013)
AT2G13370	CHR5	Chromatin-remodeling factor, associated with the promoters of ABI3	(Shen et al., 2015)
AT1G13180	ARPC2/DIS1	Involved in actin organization resulting in a distorted trichome phenotype and ABA-insensitivity in guard cell movement, also involved in epidermal development	(Jiang et al., 2012)
AT2G34150	WAVE1	Encodes a member of the SCAR family	
AT1G29170	WAVE2	Encodes a member of the SCAR family	
AT5G01730	WAVE3	Encodes a member of the SCAR family	
AT5G18410	OPAL5	Encoding the PIR/SRA1/KLK subunit of the Arabidopsis SCAR/WAVE complex, the opal5 mutant does not close stomata in response ABA and darkness	(Isner et al., 2017)

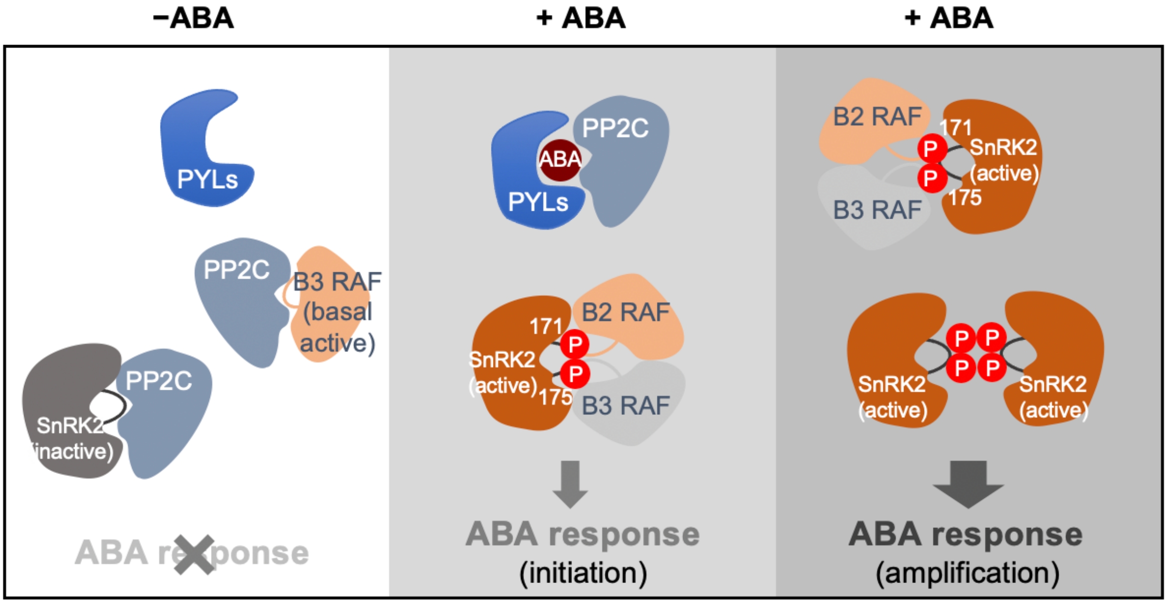


Supplementary Fig. 1 Yeast-two-hybrid assay shows the interaction between ABI1 and some B2, B3, and B4 RAFs.



Supplementary Fig. 2 ABI1 regulates RAFs activity by dephosphorylation.

a. The recombinant RAF3-KD, RAF10-KD, or RAF24-KD incubated with boiled inactive recombinant ABI1* or PP2CA* purified from *E. coli* in the presence of [γ-^{32}p]ATP. Autoradiograph (upper) and Coomassie staining (lower) show phosphorylation and loading of purified RAF-KDs and PP2Cs, respectively. Images shown are representative of at least two independent experiments. **b.** ABI1 regulates kinase activity of recombinant B4 RAFs. The recombinant RAF24-KD and RAF40-KD were incubated with ABI1 purified from *E. coli* in the presence of [γ-^{32}p]ATP. Autoradiograph (upper) and Coomassie staining (lower) show phosphorylation and loading of purified RAF-KDs and PP2Cs, respectively. Images shown are representative of at least two independent experiments. **c.** Activation of the *RD29B-LUC* reporter gene by wild type and the mutated RAF3 in the protoplasts of *OK^{100}-nonu*. Error bars, SD (n = 6 individual transfections). Two-tailed paired *t*-tests, **$p < 0.01$.



Supplementary Fig. 3 A model illustrating the dual functions of ABI1 in ABA signaling. Under unstressed conditions, ABI1 binds to and inhibits SnRK2 protein to prevent the transphosphorylation by activated RAFs, while dephosphorylates B2 and B3 RAFs to maintain their basal level activation that essential for initiation and amplification of SnRK2 activation in the presence of ABA. Figure adapted from Fig. 7 in Lin *et al.,* 2021.

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