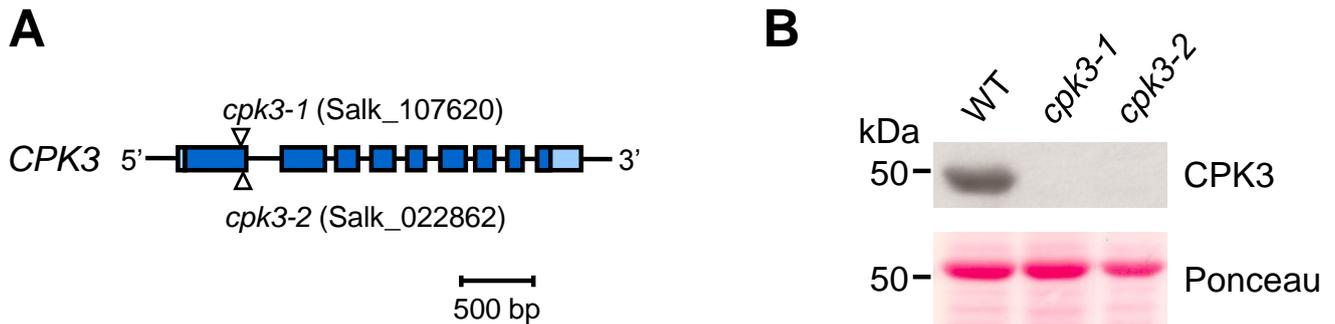


14-3-3 zeta ( <i>Homo sapiens</i> )	53	GARRSSWRVVS	63
BMH1 ( <i>Saccharomyces cerevisiae</i> )	55	GARRASWRIVS	65
BMH2 ( <i>Saccharomyces cerevisiae</i> )	55	GARRASWRIVS	65
14-3-3 chi ( <i>Arabidopsis thaliana</i> )	62	GARRASWRIIS	72
14-3-3 omega ( <i>Arabidopsis thaliana</i> )	57	GARRASWRIIS	67
14-3-3 psi ( <i>Arabidopsis thaliana</i> )	56	GARRASWRIIS	66
14-3-3 phi ( <i>Arabidopsis thaliana</i> )	63	GARRASWRIIS	73
14-3-3 upsilon ( <i>Arabidopsis thaliana</i> )	59	GARRASWRIIS	69
14-3-3 lambda ( <i>Arabidopsis thaliana</i> )	60	GSLRAAWRIVS	70
14-3-3 nu ( <i>Arabidopsis thaliana</i> )	57	GARRASWRIIS	67
14-3-3 kappa ( <i>Arabidopsis thaliana</i> )	60	GSLRAAWRIVS	70
14-3-3 mu ( <i>Arabidopsis thaliana</i> )	57	GSRRASWRIFS	67
14-3-3 epsilon ( <i>Arabidopsis thaliana</i> )	55	GARRASWRILS	65
14-3-3 omicron ( <i>Arabidopsis thaliana</i> )	55	GARRASWRILS	65
14-3-3 iota ( <i>Arabidopsis thaliana</i> )	60	GARRASWRIMS	70
14-3-3 pi ( <i>Arabidopsis thaliana</i> )	55	EAKRVSLRVIS	65

**Figure S1. A serine phosphorylation site is conserved among various members of the 14-3-3 family**

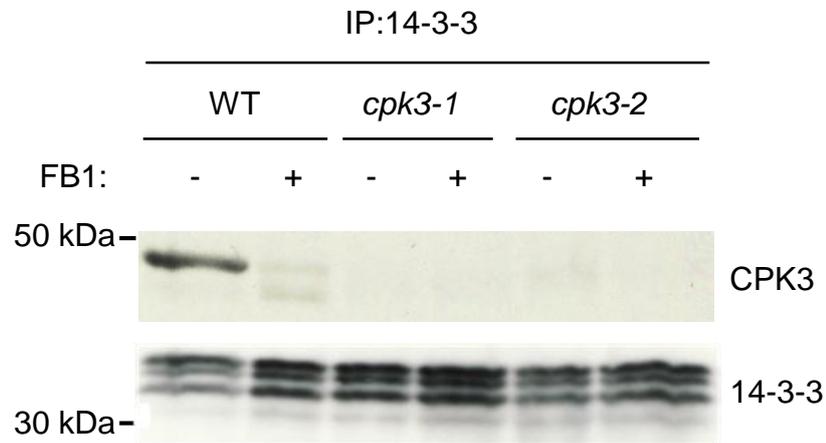
The region flanking the residue Ser58 in human 14-3-3 zeta ( $\zeta$ ) was aligned with that of all 14-3-3 isoforms from *Saccharomyces cerevisiae* and *A. thaliana*. The conserved serine residue is highlighted in black.



**Figure S2. Two different T-DNA insertion lines for CPK3, *cpk3-1* and *cpk3-2*, are null mutants at the protein level**

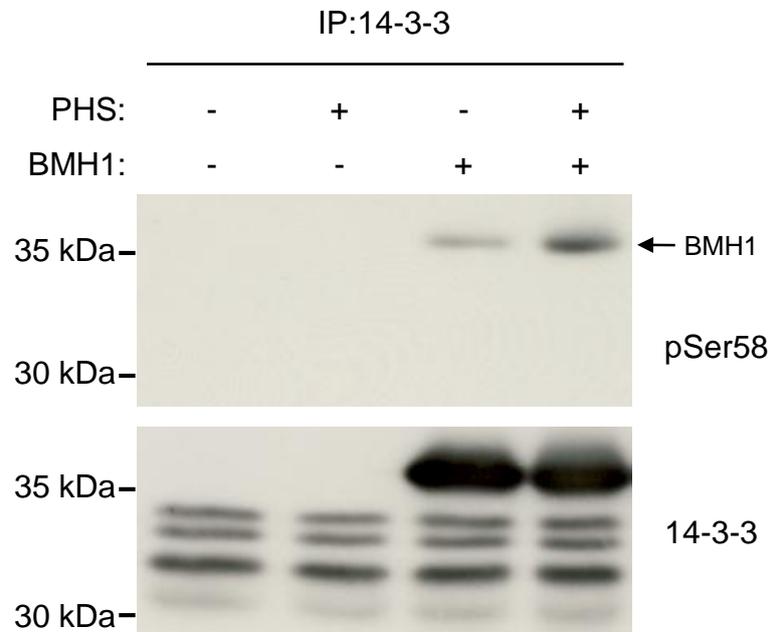
**(A)** Schematic representation of the *Arabidopsis* CPK3 gene and its alleles *cpk3-1* and *cpk3-2*. Boxes indicate exons and dark blue represents the coding region. The positions of T-DNA insertions are marked by triangles.

**(B)** Protein extracts from leaves of *Arabidopsis* wild type (WT) or mutant plants (*cpk3-1*, *cpk3-2*) were analysed by immunoblotting with an anti-CPK3 antibody and Ponceau staining. The results presented are representative of 3 separate experiments.



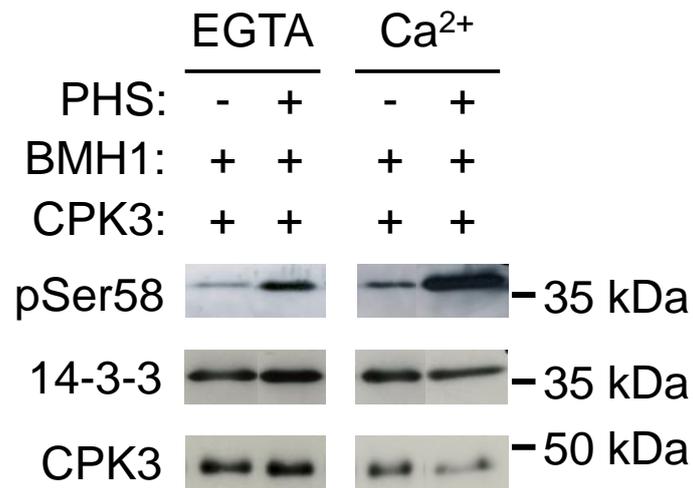
**Figure S3. CPK3 is not associated with 14-3-3s in *cpk3* mutant plants**

*Arabidopsis* leaves of wild type (WT) or mutant plants (*cpk3-1*, *cpk3-2*) were treated +/- 10  $\mu$ M FB1 for 48 hr. 14-3-3 immunoprecipitates were immunoblotted with the indicated antibodies. The results presented are representative of 3 separate experiments.



**Figure S4. A 14-3-3-associated kinase phosphorylates 14-3-3 Ser58 site of the yeast 14-3-3 BMH1 in a PHS-dependent manner**

Proteins from untreated *Arabidopsis* cells were immunoprecipitated with an anti-14-3-3 protein antibody. Immunoprecipitates were used as kinase sources in nonradioactive *in vitro* phosphorylation assays performed in the presence or not (+/-) of recombinant BMH1 and +/- 25  $\mu$ M PHS. Proteins from these assays were blotted with the indicated antibodies. The results presented are representative of 3 separate experiments. The upper panel showing detection of pSer58 corresponds to a shorter exposure than the blot in Figure 4A, left panel.



**Figure S5. CPK3 phosphorylates 14-3-3 Ser58 site of the yeast 14-3-3 BMH1 in a PHS- and calcium-dependent manner**

Kinase assays were performed with recombinant *Arabidopsis* CPK3 and BMH1 (used as the substrate) in the presence or absence of 25  $\mu$ M PHS, and in the presence (Ca<sup>2+</sup>) or absence (EGTA) of Ca<sup>2+</sup>. Proteins were analysed by immunoblotting with the indicated antibodies. The results presented are representative of 3 separate experiments. The right upper panel showing detection of pSer58 corresponds to a longer exposure than the blot in Figure 4C.