# The C-terminal domains SnRK2-box and ABA-box have a role in sugarcane SnRK2s auto-activation and activity

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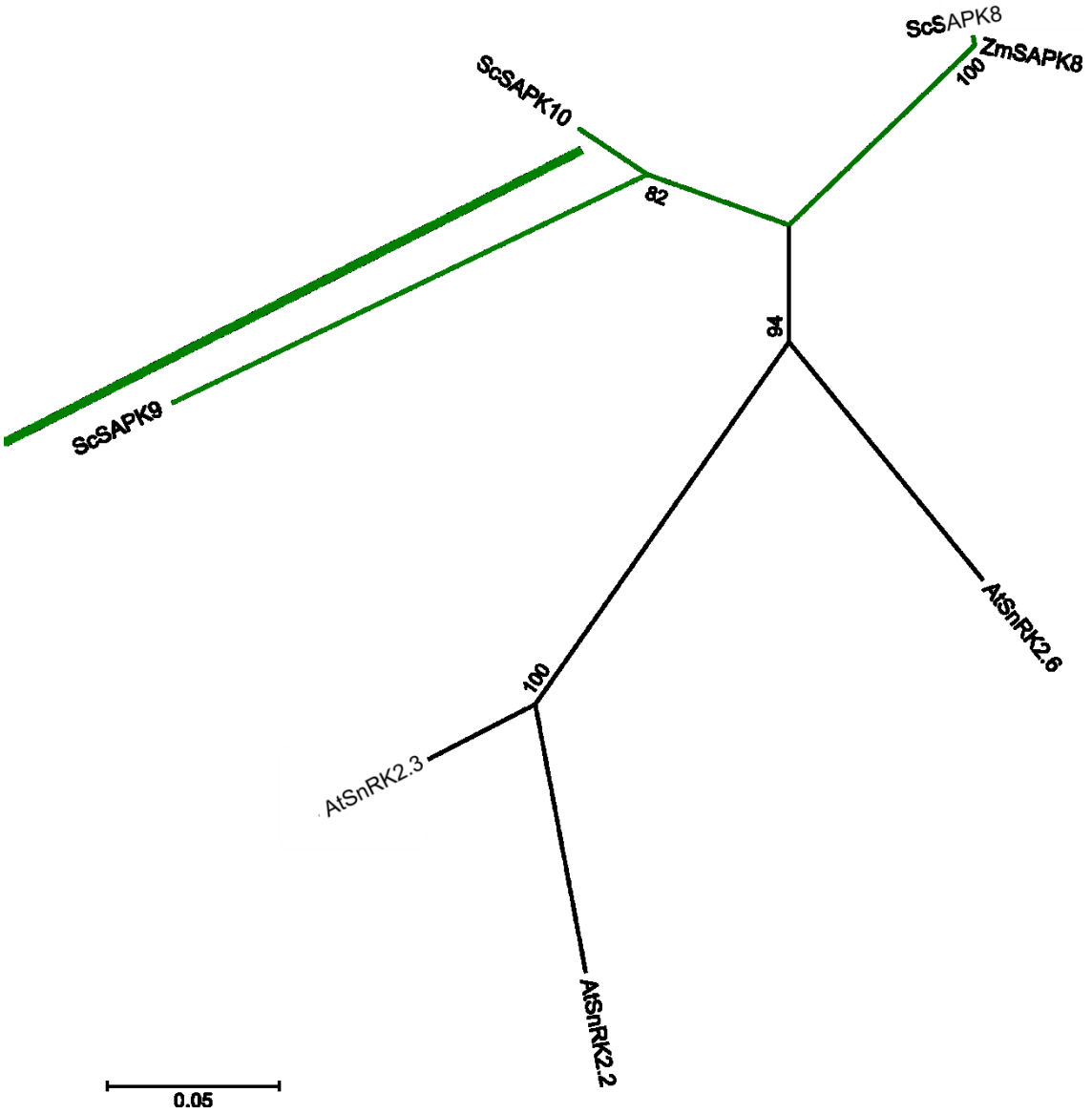
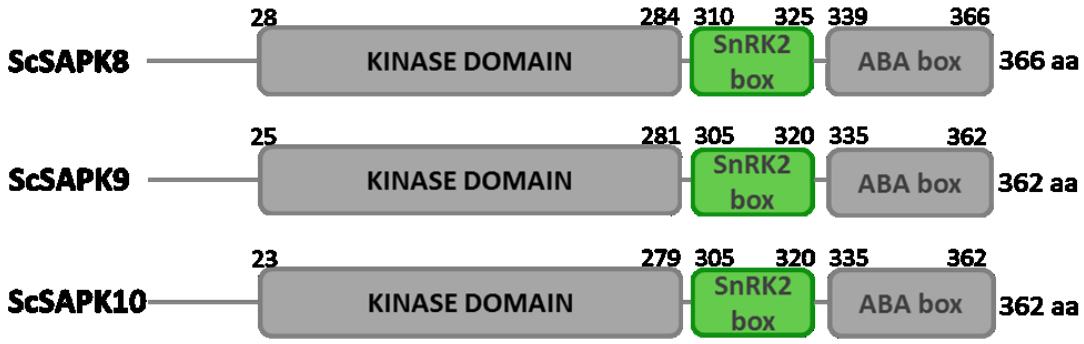
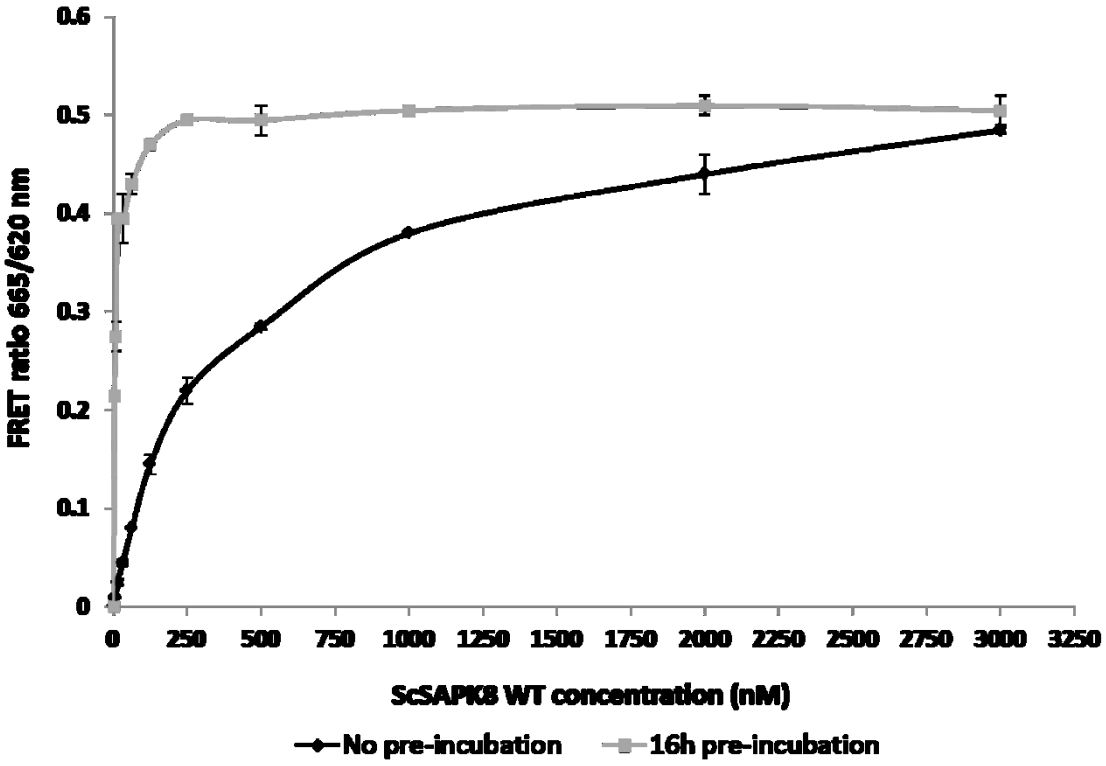
**SUPPLEMENTARY MATERIAL**



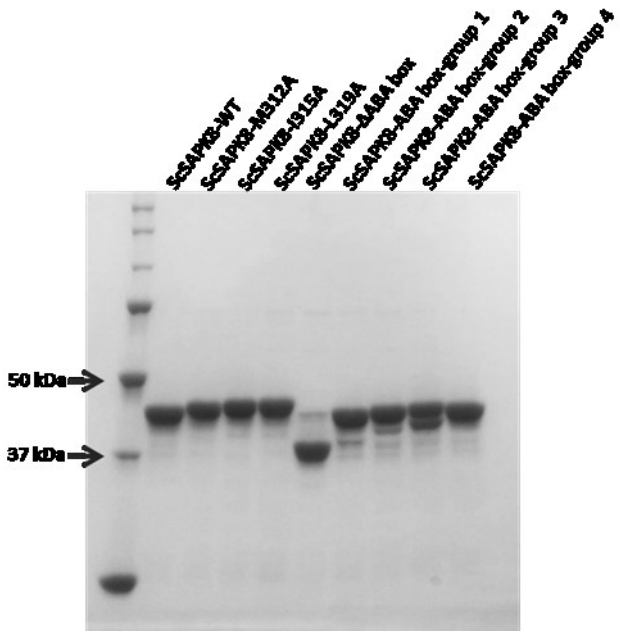

**Supplementary Figure S1: Unrooted phylogenetic tree of sugarcane, maize and Arabidopsis SnRK2s inferred by Maximum Likelihood.** The tree is drawn to scale, with branch lengths representing the number of substitutions per site. Bootstrapping analysis was performed 1000 times, and the values (in %) are shown at each node. Values above 80% indicate branches are well supported by our phylogenetic reconstruction. Branches corresponding to the conserved monocotyledons SnRK2 proteins are colored in green.



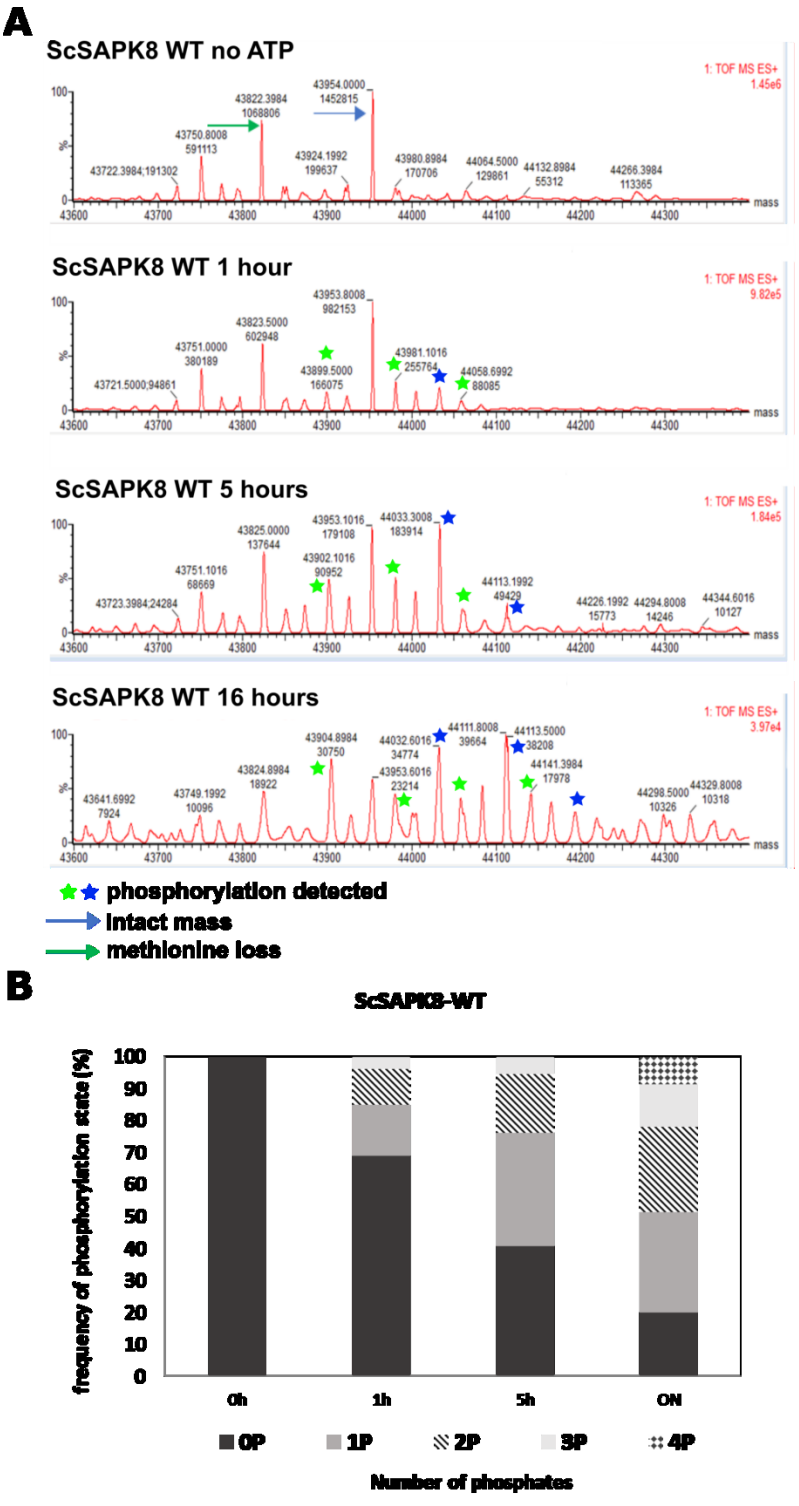

**Supplementary Figure S2: Schematic representation of the full-length ScSAPK8, ScSAPK9, and ScSAPK10.** Each protein has an N-terminal kinase domain and a C-terminal region containing the regulatory domains SnRK2-box and ABA-box. Numbers represent the amino acid positions of each protein domain.



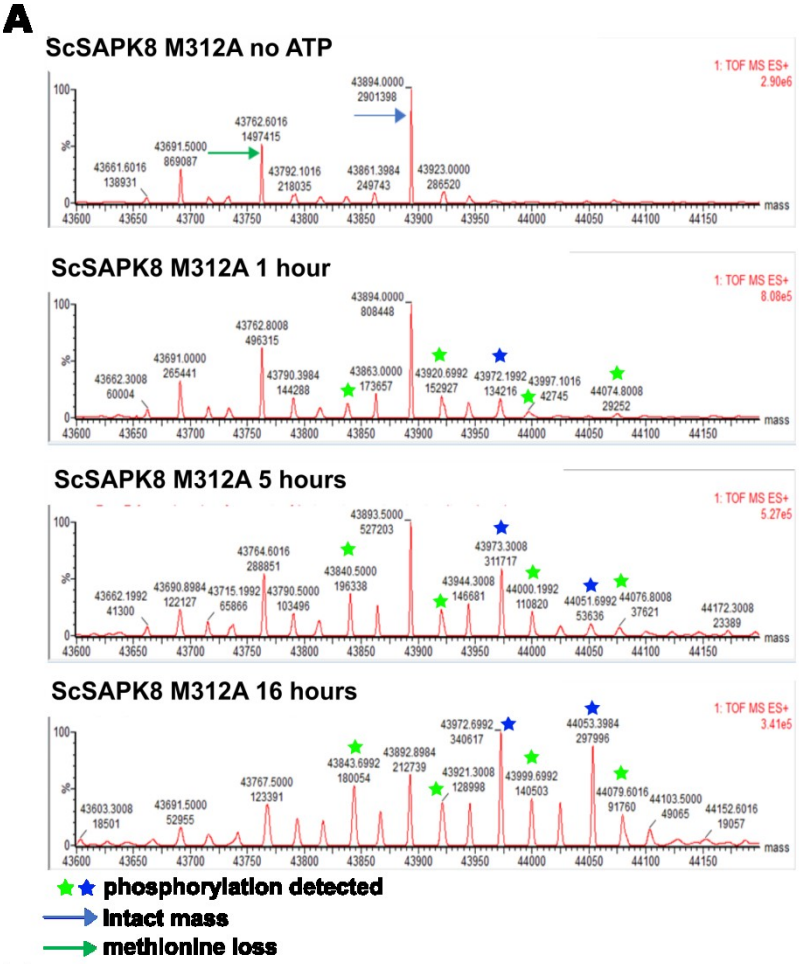

**Supplementary Figure S3: Enzymatic activity of ScSAPK8 WT under variable enzyme concentration and after ATP pre-incubation.** The data show the quantity of phosphorylated peptide produced after 1 hour, measured by the ratio of fluorescence intensity at 665 nm (streptavidin-XL665 emission excited by phospho-specific Eu-cryptate conjugated antibody) and 620 nm (Eu-cryptate emission). The ScSAPK8 activity was higher with ATP pre-incubation and also increased in an enzyme concentration-dependent manner.

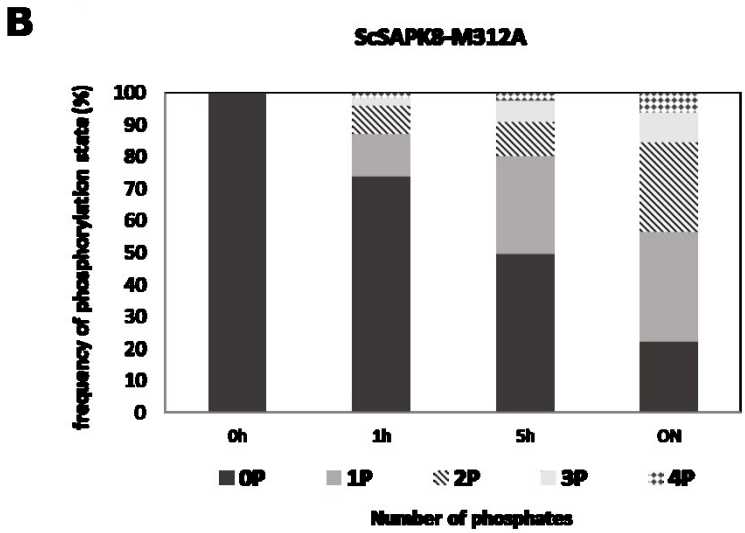



**Supplementary Figure S4: Coomassie-stained SDS-PAGE of ScSAPK8 WT and mutants.** The image represents all the proteins after affinity purification and dilution to 20 µM final concentration. The protein concentration was estimated by the Bradford method (Sigma-Aldrich) before gel loading.

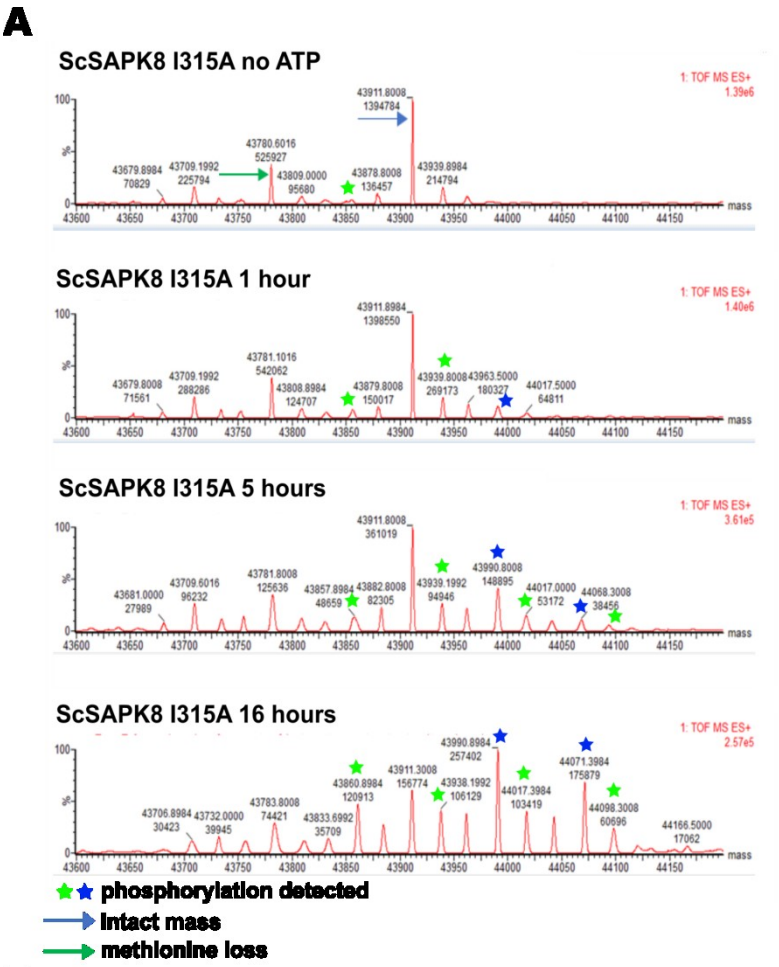



**Supplementary Figure S5: ScSAPK8 WT autophosphorylation. A:** Deconvoluted mass spectrum at time 0, 1 hour, 5 hours and overnight, generated by MAX ENT1 software (Waters). The blue arrow represents the intact kinase mass, and the blue stars represent the detected masses with one or more phosphorylations. The green arrows indicate the exact kinase mass with the loss of methionine that could occur during protein expression. The green stars represent masses of this kinase form with one or more phosphorylations. **B:** Graphical representation of protein autophosphorylation over time. The percentage values were calculated using the ion counts extracted from the deconvoluted spectrum.

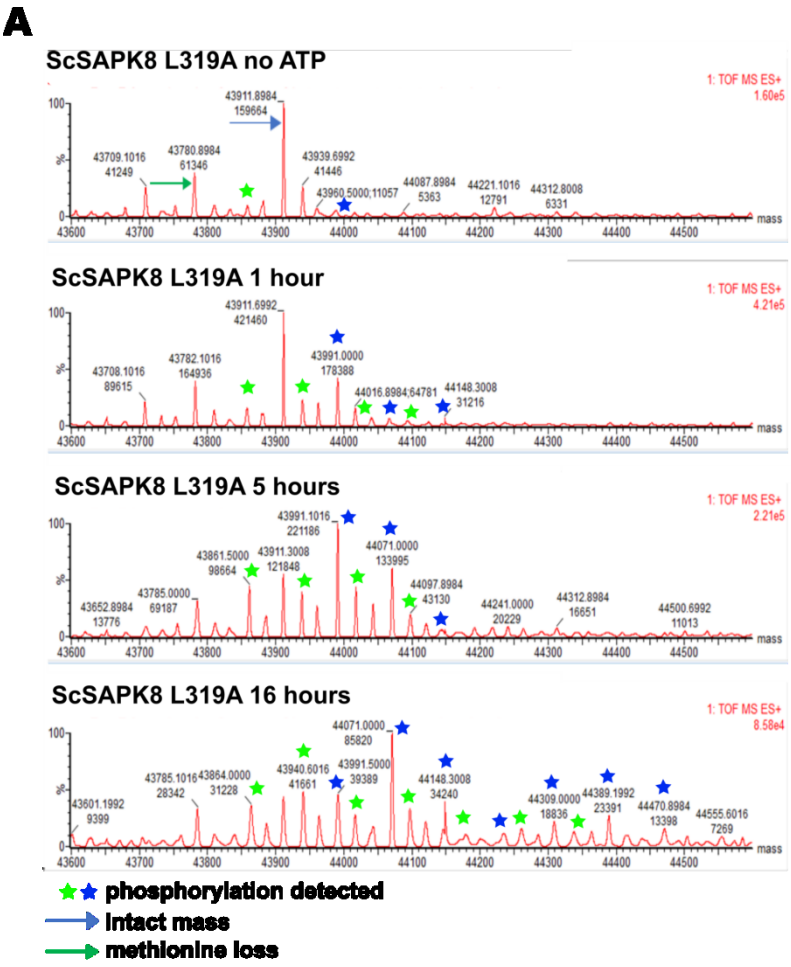


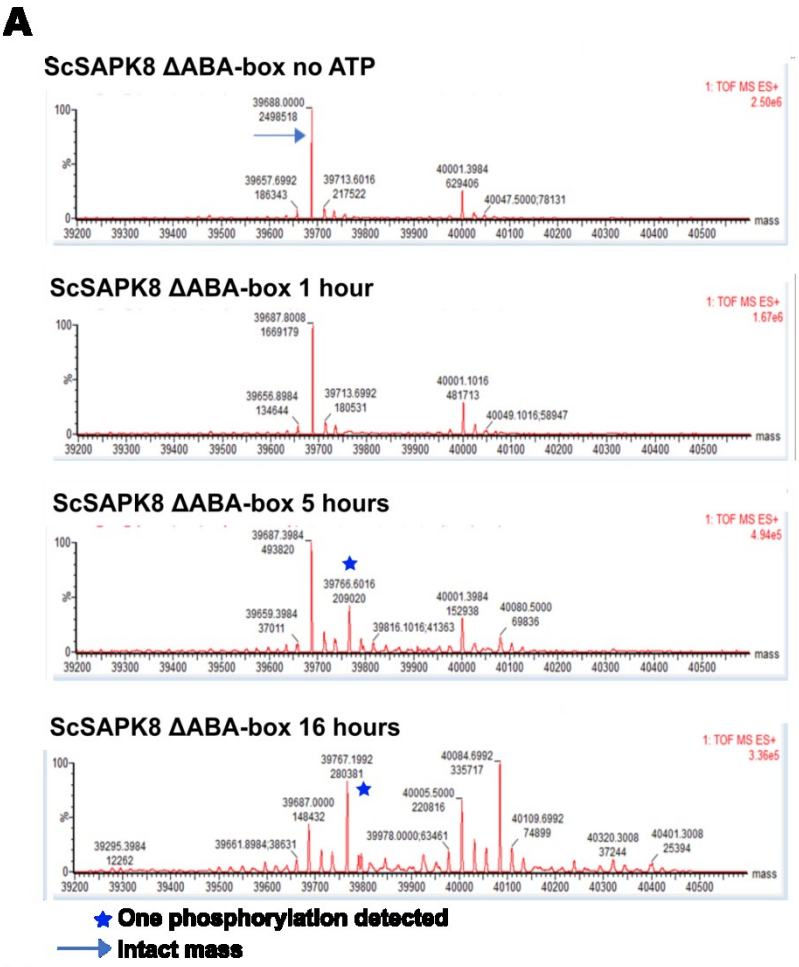

**Supplementary Figure S6: ScSAPK8-M312A autophosphorylation. A:** Deconvoluted mass spectrum at time 0, 1 hour, 5 hours and overnight, generated by MAX ENT1 software (Waters). The blue arrow represents the intact kinase mass, and the blue stars represent the detected masses with one or more phosphorylations. The green arrows indicate the exact kinase mass with the loss of methionine that could occur during protein expression. The green stars represent masses of this kinase form with one or more phosphorylations. **B:** Graphical representation of protein autophosphorylation over time. The percentage values were calculated using the ion counts extracted from the deconvoluted spectrum.

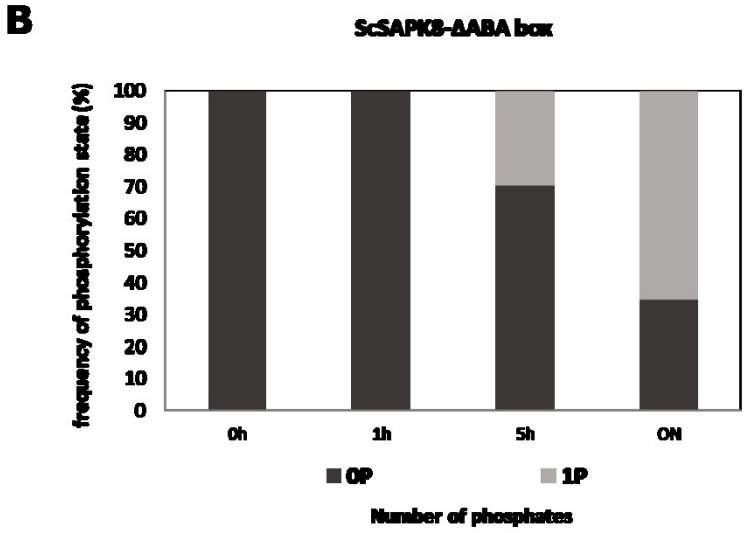



**Supplementary Figure S7: ScSAPK8-I315A autophosphorylation. A:** Deconvoluted mass spectrum at time 0, 1 hour, 5 hours and overnight, generated by MAX ENT1 software (Waters). The blue arrow represents the intact kinase mass, and the blue stars represent the detected masses with one or more phosphorylations. The green arrows indicate the exact kinase mass with the loss of methionine that could occur during protein expression. The green stars represent masses of this kinase form with one or more phosphorylations. **B:** Graphical representation of protein autophosphorylation over time. The percentage values were calculated using the ion counts extracted from the deconvoluted spectrum.

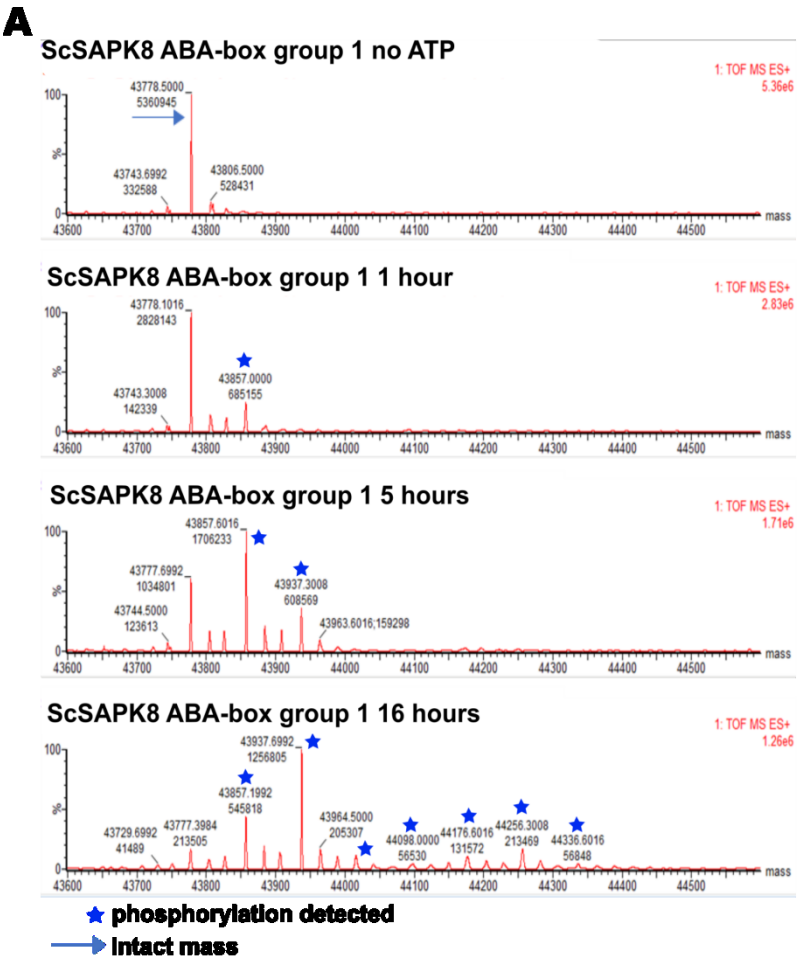



**Supplementary Figure S8: ScSAPK8-L319A autophosphorylation. A:** Deconvoluted mass spectrum at time 0, 1 hour, 5 hours and overnight, generated by MAX ENT1 software (Waters). The blue arrow represents the intact kinase mass, and the blue stars represent the detected masses with one or more phosphorylations. The green arrows indicate the exact kinase mass with the loss of methionine that could occur during protein expression. The green stars represent masses of this kinase form with one or more phosphorylations. **B:** Graphical representation of protein autophosphorylation over time. The percentage values were calculated using the ion counts extracted from the deconvoluted spectrum.

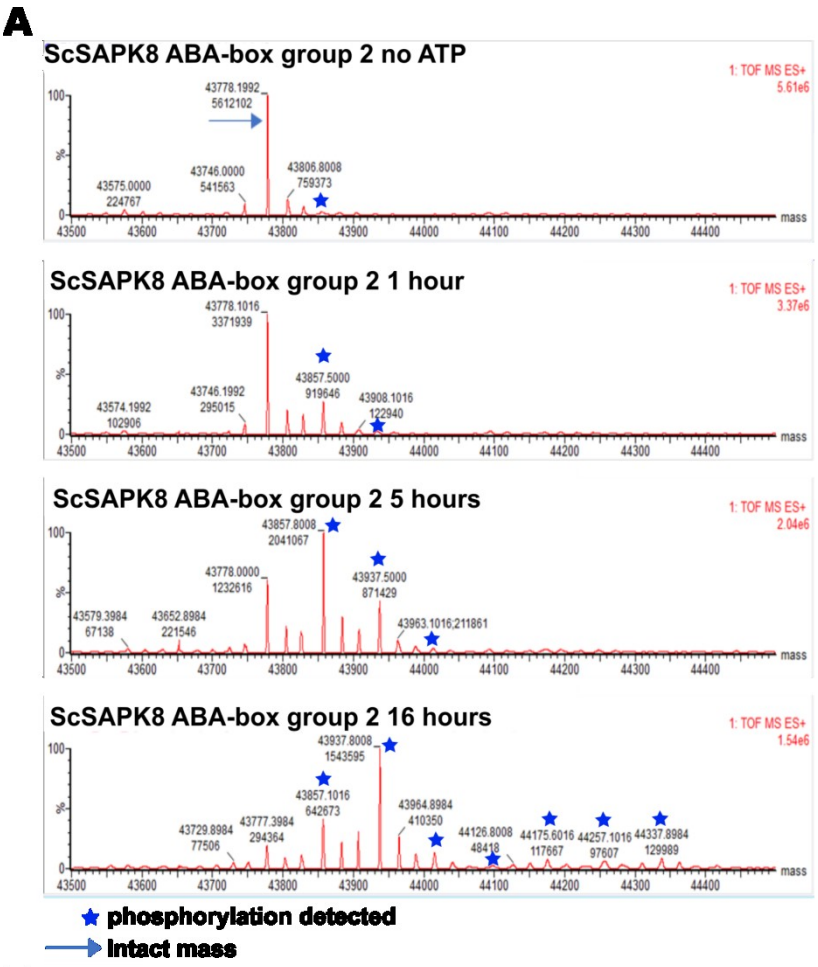


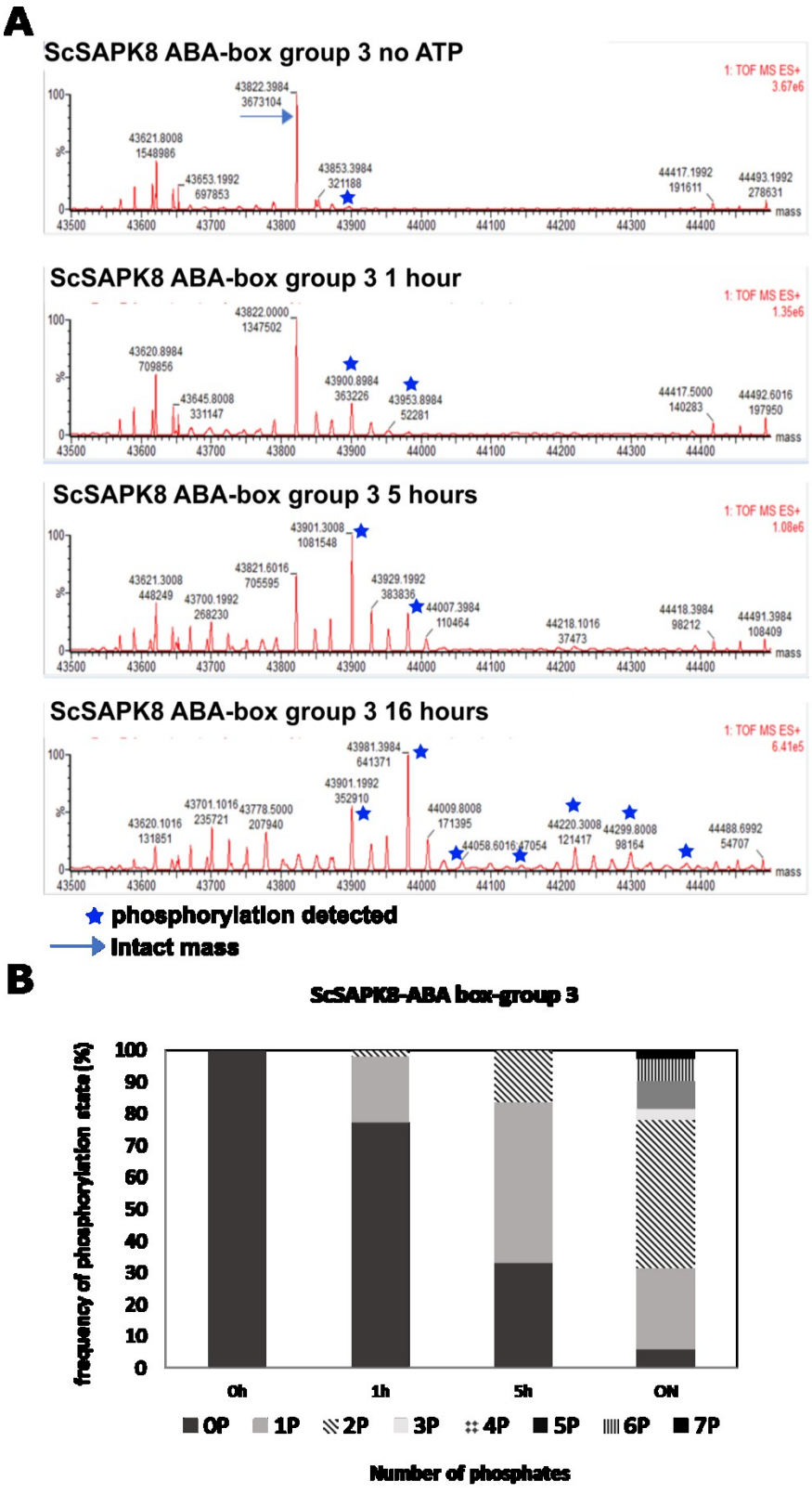

**Supplementary Figure S9: ScSAPK8-ΔABA-box autophosphorylation. A:** Deconvoluted mass spectrum at time 0, 1 hour, 5 hours and overnight, generated by MAX ENT1 software (Waters). The blue arrow represents the intact kinase mass, and the blue stars represent the detected masses with one or more phosphorylations. **B:** Graphical representation of protein autophosphorylation over time. The percentage values were calculated the ion counts extracted from the deconvoluted spectrum.



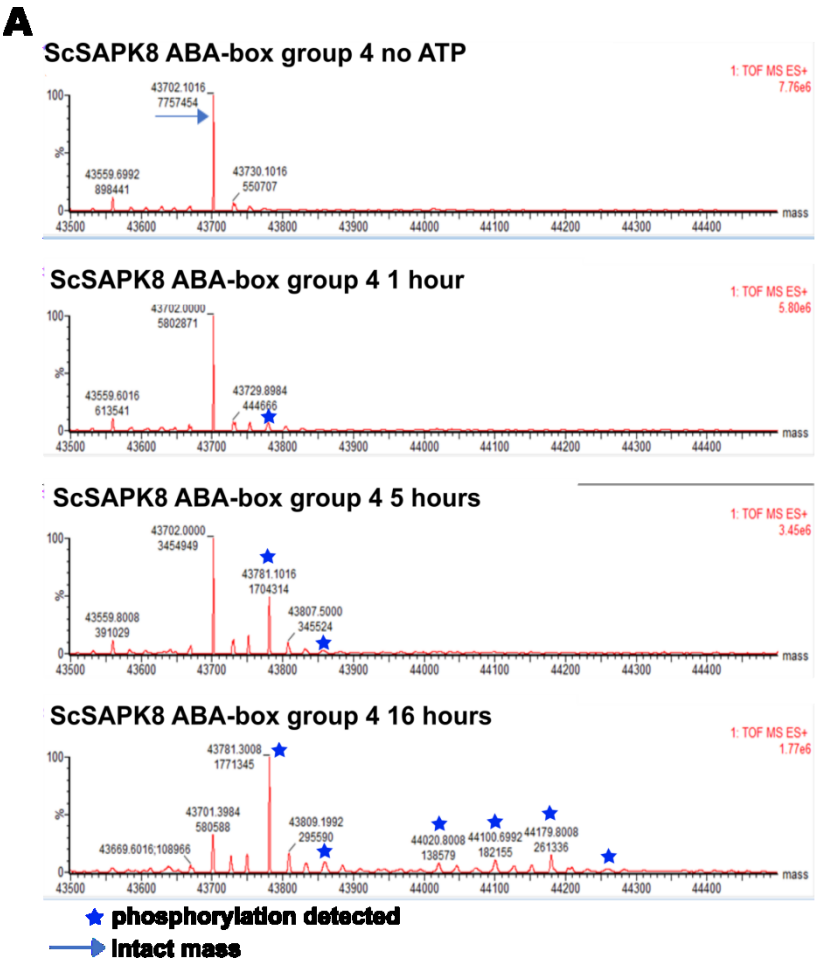

**Supplementary Figure S10: ScSAPK8-ABAbox-group1 autophosphorylation. A:** Deconvoluted mass spectrum at time 0, 1 hour, 5 hours and overnight, generated by MAX ENT1 software (Waters). The blue arrow represents the intact kinase mass, and the blue stars represent the detected masses with one or more phosphorylations. **B:** Graphical representation of protein autophosphorylation over time. The percentage values were calculated the ion counts extracted from the deconvoluted spectrum.




**Supplementary Figure S11: ScSAPK8-ABAbox-group2 autophosphorylation. A:** Deconvoluted mass spectrum at time 0, 1 hour, 5 hours and overnight, generated by MAX ENT1 software (Waters). The blue arrow represents the intact kinase mass, and the blue stars represent the detected masses with one or more phosphorylations. **B:** Graphical representation of protein autophosphorylation over time. The percentage values were calculated the ion counts extracted from the deconvoluted spectrum.

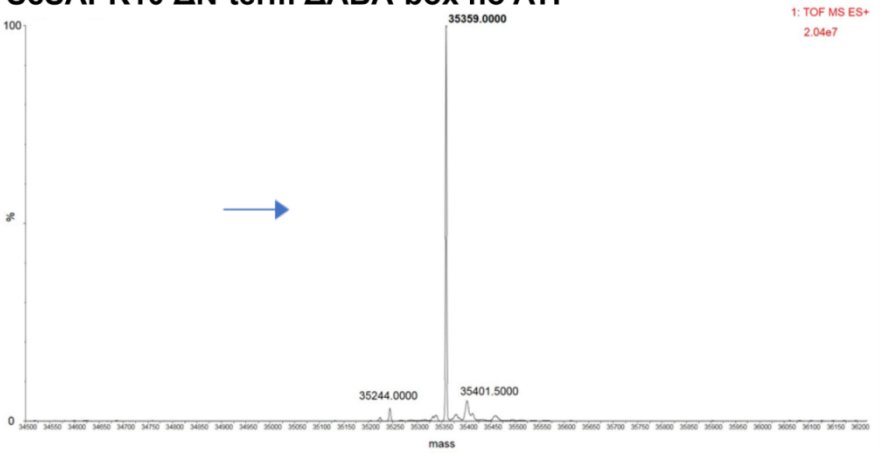



**Supplementary Figure S12: ScSAPK8-ABAbox-group3 autophosphorylation. A:** Deconvoluted mass spectrum at time 0, 1 hour, 5 hours and overnight, generated by MAX ENT1 software (Waters). The blue arrow represents the intact kinase mass, and the blue stars represent the detected masses with one or more phosphorylations. **B:** Graphical representation of protein autophosphorylation over time. The percentage values were calculated the ion counts extracted from the deconvoluted spectrum.

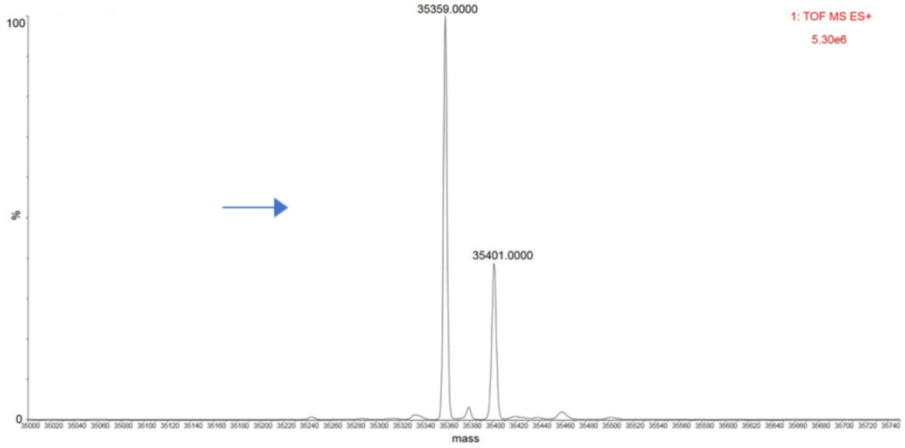



**Supplementary Figure S13: ScSAPK8-ABAbox-group4 autophosphorylation. A:** Deconvoluted mass spectrum at time 0, 1 hour, 5 hours and overnight, generated by MAX ENT1 software (Waters). The blue arrow represents the intact kinase mass, and the blue stars represent the detected masses with one or more phosphorylations. **B:** Graphical representation of protein autophosphorylation over time. The percentage values were calculated the ion counts extracted from the deconvoluted spectrum.

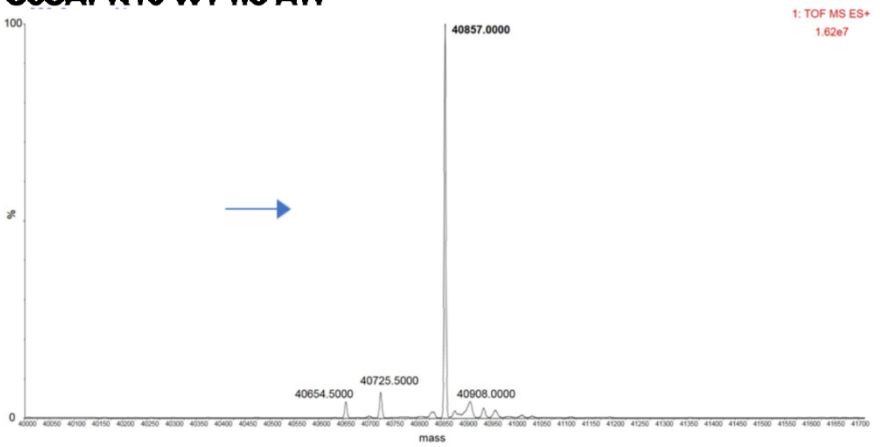
**ScSAPK10 ΔN-term ΔABA-box no ATP**



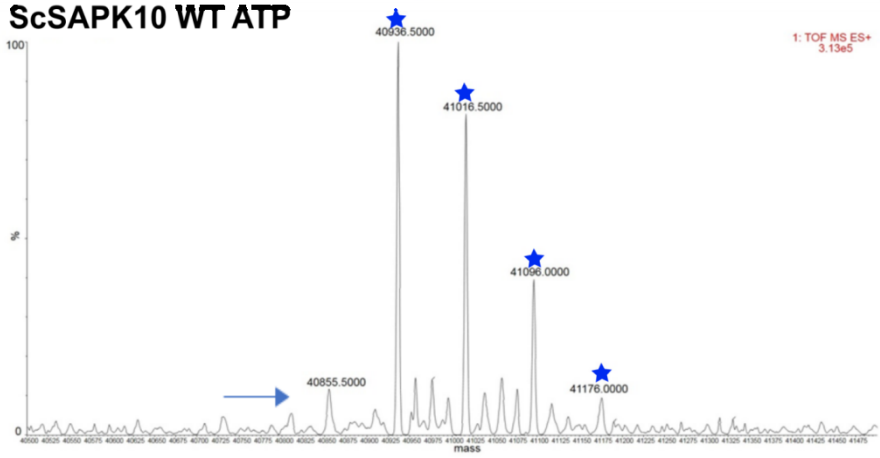

**ScSAPK10 ΔN-term ΔABA-box ATP**




**ScSAPK10 WT no ATP**




**ScSAPK10 WT ATP**




→ **Intact mass** ★ **phosphorylation detected**

**Supplementary Figure S14: ScSAPK10 WT and mutant ScSAPK10 ΔN-term ΔABA-box autophosphorylation.** Deconvoluted mass spectrum at time 1 hour generated by MAX ENT1 software (Waters). The blue arrow represents the intact kinase mass, and the blue stars represent the detected masses with one or more phosphorylations.

**Table S1: List of primer sequences**

| Primer Name | Sequence 5' - 3' | Used for |
|---|---|---|
| Sc_SAPK8_MluI_NdeI_F | ACGCGTCATATGATGGCAGGGCCGGCGCCG | isolation from cDNA and cloning |
| Sc_SAPK8_NotI_R | GCGGCCGCCATTGCGTACACAATCTCACC | |
| Sc_SAPK9_MluI_NdeI_F | ACGCGTCATATGATGGCGAGGACGCCGGCA | |
| Sc_SAPK9_NotI_R | GCGGCCGCCATGGCATACACTATCTCTCC | |
| Sc_SAPK10_Mlu_NdeI_F | ACGCGTCATATG ATGGACCGGGCGGCGCTC | |
| Sc_SAPK10_NotI_R | GCGGCCGCCATAGCATACACGATCTCCC | |
| SAPK8_full_S | TACTTCCAATCCATGGCAGGGCCGGCG | cloning in pNIC28-Bsa4 vector |
| SAPK8_full_AS | TATCCACCTTTACTGTCACATTGCGTACACAATCTCACC | |
| SAPK9_full_S | TACTTCCAATCCATGGCGAGGACGCCG | |
| SAPK9_full_AS | TATCCACCTTTACTGTCACATGGCATACACTATCTCTCC | |
| SAPK10_full_S | TACTTCCAATCCATGGACCGGGCGGCG | |
| SAPK10_full_AS | TATCCACCTTTACTGTCACATAGCATACACGATCTCCCC | |
| SAPK10_dm_S | TACTTCCAATCCATGGACATGCCCATAATGCAC | ScSAPK10 truncation |
| SAPK10_dm_AS | TATCCACCTTTACTGTCATGGAATGGTCGCCTCGG | |
| ScSAPK8_M312A_S | AATGCAGACCGCGGATCAGATCA | SnRK2-box site-directed mutagenesis |
| ScSAPK8_M312A_AS | TGATCTGATCCGCGGTCTGCATT | |
| ScSAPK8_I315A_S | ATGGATCAGGCCATGCAGATTTTG | |
| ScSAPK8_I315A_AS | CAAAATCTGCATGGCCTGATCCATGG | |
| ScSAPK8_L319A_S | CATGCAGATTGCGACAGAGGCCA | |
| ScSAPK8_L319A_AS | TGGCCTCTGTCGCAATCTGCATG | |
| ScSAPK8_group1_S | GATGGATTGGCCATGGCCGCCGCCATGGATGAT | ABA-box site-directed mutagenesis |
| ScSAPK8_group1_AS | ATCATCCATGGCGGCGGCCATGGCCAATCCATC | |
| ScSAPK8_group2_S | CGACGACATGGCTGCTCTTGCCTCCGCCTCAGATCTTG | |
| ScSAPK8_group2_AS | CAAGATCTGAGGCGGAGGCAAGAGCAGCCATGTCGTCG | |
| ScSAPK8_group3_S | TCCGACTCAGCTCTTGCTGTTGCCAGCAGCGGT | |
| ScSAPK8_group3_AS | ACCGCTGCTGGCAACAGCAAGAGCTGAGTCGGA | |
| ScSAPK8_group4_S | AGCAGTGGAGCGGCTGTGGCCGCAGCGTGACAGTAAAGGTGGATA | |
| ScSAPK8_group4_AS | TATCCACCTTTACTGTCACGCTGCGGCCACAGCCGCTCCACTGCT | |
| SAPK8_full_S | TACTTCCAATCCATGGCAGGGCCGGCG | ABA-box deletion |
| SAPK8_dm_AS | TATCCACCTTTACTGTCAAGGTGGTATGGTGGCCTC | |