Materials and methods

Plasmid constructs: A KING1 cDNA was cloned by PCR using a cDNA library (Fujii *et al.* 2007) as a template and primers are listed in Table 1 Suppl. The PCR fragment was cloned into the BamHI and EcoRI sites of pGEX4T1 (*Amersham*, Buckinghamshire, UK). The maltose-binding protein (MBP)-fused *Sucrose* non fermenting1 (SNF1)-related protein kinase (SnRK)2.6 was described in Fujii *et al*. (2009). Primer pairs (Table 1 Suppl.) were used for the first PCR in production of the chimeric protein. The second amplification was performed using the PCR products as templates. The final product was cloned into pMALc2x (BamHI-SalI sites).

Purification of recombinant proteins from *Escherichia coli* and *in vitro* binding assays: Recombinant proteins were purified as described previously (Fujii and Zhu 2009). Glutathione S-transferase (GST)-fused proteins were eluted from beads with 40 mM reduced glutathione (pH 8.0). The eluted proteins were dialyzed in an STE (150 mM NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM $EDTANa_2$) solution at 4 °C overnight. The MBP-fused proteins were incubated with the eluted proteins in 0.1 cm^3 of the STE solution at 23 °C for 1 h with slow rocking. After the beads were washed six times with 1 cm^3 of the STE solution, a Laemmli sample buffer was added on the beads. Proteins were separated on an SDS-PAGE gel and subjected to Western blot analysis with an anti-GST antibody (*Upstate/EMD*, *Millipore*, Billerica, MA, USA) according to the manufacturer's instructions. Purified proteins are shown in Fig. 3 Suppl.

***In vitro* kinase assay:** Recombinant proteins were produced in *E. coli* as described previously (Fujii and Zhu 2009). The MBP-fused SnRK2s were incubated with GST-fused ABA-responsive element binding factor (AREB)1a and either GST-fused KIN γ subunit (KING)1 or GST in 0.03 cm^3 of a reaction mixture (25 mM Tris, pH 7.4, 12 mM $MgCl_2$, 1 μM cold ATP, 0.185MBq [γ-^{32}P] ATP, 1 mM dithiotreitol, 1 mM Na_3VO_4, 5 mM NaF, and 0.03 mg cm^{-3} bovine serum albumin). Reaction to SnRK2.6 was tested with different amounts of GST-KING1. After incubation (30 °C for 45 min), reaction was stopped by adding a Laemmli sample buffer. After separation of the proteins by SDS-PAGE, radioactive bands were detected by autoradiography on the dried gel. The experiments were replicated three times. The *GIMP* (https://www.gimp.org/) was used for generating the figures for the *in vitro* assays.

Modeling of the SnRK2.6/KING1 complex: The heterotrimeric AMP-activated protein kinase (AMPK) complex (PDB ID: 4CFE) was used as a hetero-oligomeric template. Because of the moderate-to-low sequence similarity, we used a combined sequence/structural approach to ensure that all the "key" domain residues would be aligned at the correct domain positions. This approach involved a combination of multiple sequence alignments made with *ClustalW* (Larkin *et al*. 2007) and *Multalin* (Corpet 1988), combined with the data from the conserved domains database (*CDD*; Marchler-Bauer *et al*. 2017) and manual adjustment. *Modeller* (Sali and Blundell, 1993) and *Homodge* (Lehtonen *et al*. 2004) were used for homology modeling of domains based on known related structures. The *BIOVIA* discovery studio (http://accelrys.com) and the Schrödinger suite for molecular modeling (http://www.schrodinger.com/) were used for structure preparation and adjustments, additional structure modeling, superposition, viewing, and analysis. Models of KING1 and SnRK2.6 in its "activated" form were minimized and evaluated by *PROCHECK* (Laskowski *et al.* 1993) through the PDBsum database (De Beer *et al.* 2014). Analysis of the resulting Phi/Psi Ramachandran plots indicates that only three amino acids in each protein, Asp156, Asn176, and Phe247 in KING1, and Thr229, Val236, and Thr330 in SnRK2.6, had a disallowed geometry (see Ramachandran plots in Fig. 4 Suppl.). Fig. 4 Suppl. was manually modified with *CorelDRAWX7* to change fonts. The influence of this small number of amino acids on the conclusions derived in this manuscript can be considered as negligible.

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Table 1 Suppl. PCR-primer sequences.

Primers	Forward	Reverse
KING1	AAGGATCCATGGCGACTGTTCCGGAG	AGAATTCAGACTCGGTAGTTTTCGG
pMAL	CGCAGACTAATTCGAGCTCGAAC	GCGATTAAGTTGGGTAACGCC
2622Nterm	AGAAGAAATTATGCAGATAATATCGGAGGCTAC	CCGATATTATCTGCATAATTTCTTCTATGCTTTGG

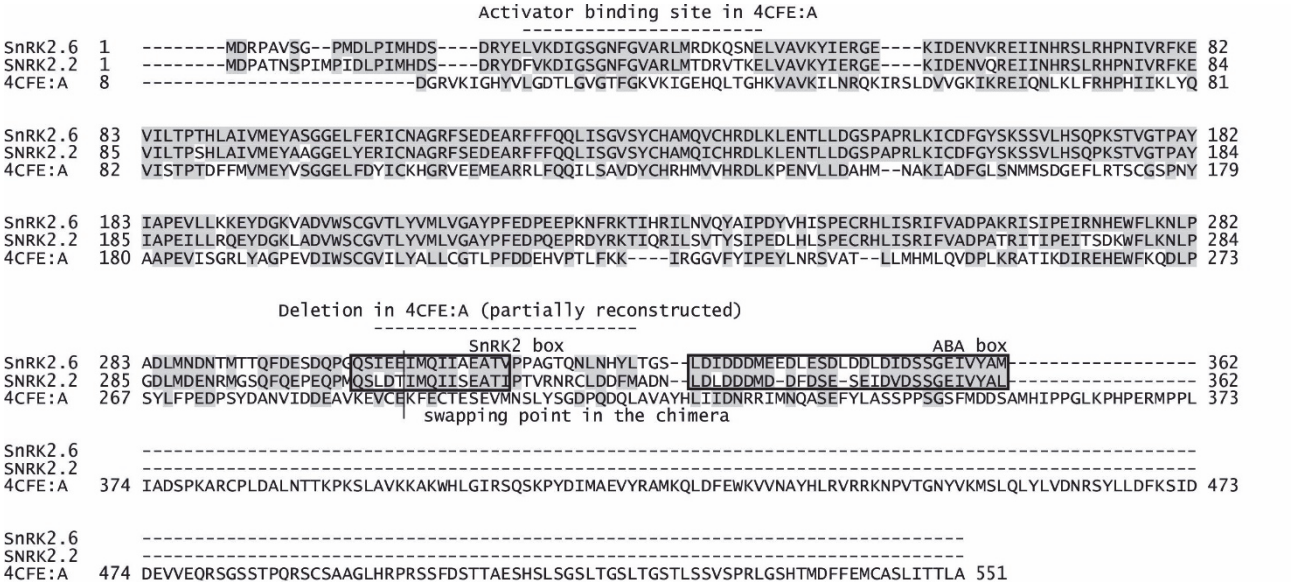

Fig 1 Suppl. The hybrid sequence and structural alignment of SnRK2.6.

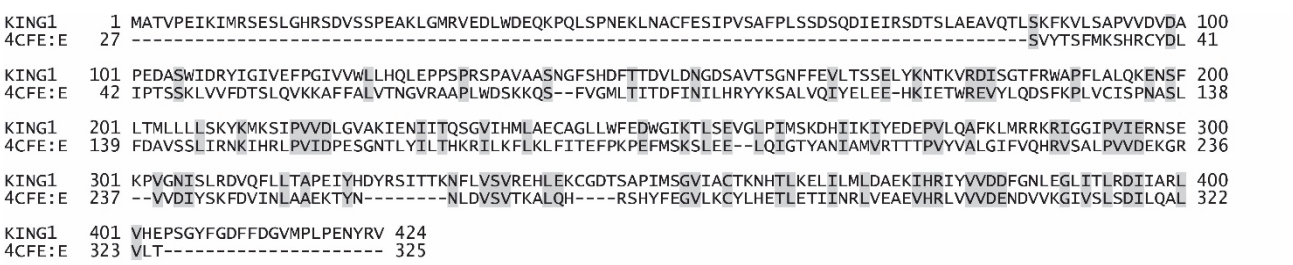

Fig. 2 Suppl. The hybrid sequence and structural alignment of KING1.

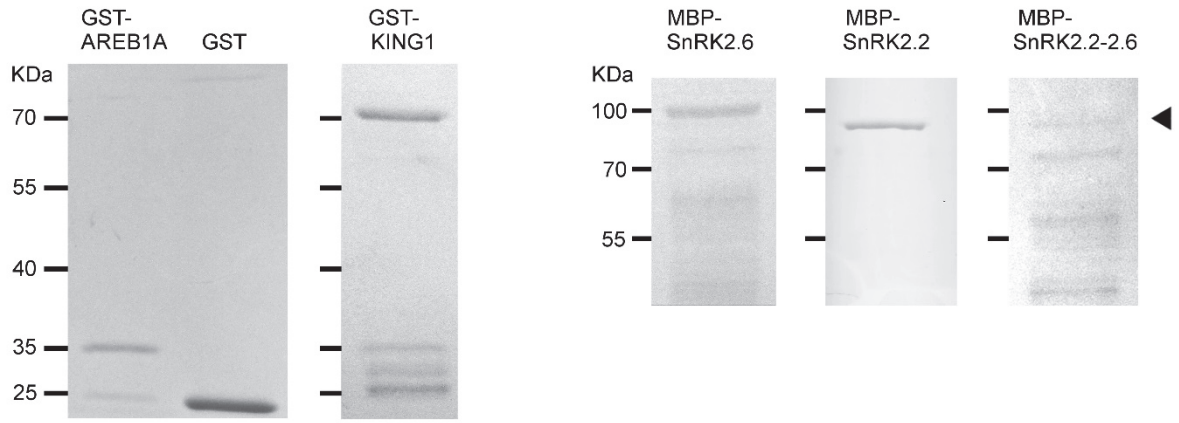

Fig. 3 Suppl. Purified proteins used for *in vitro* kinase assays. Proteins were run in SDS-PAGE gels and stained with Coomassie blue. The predicted size is indicated by a *black arrowhead*.

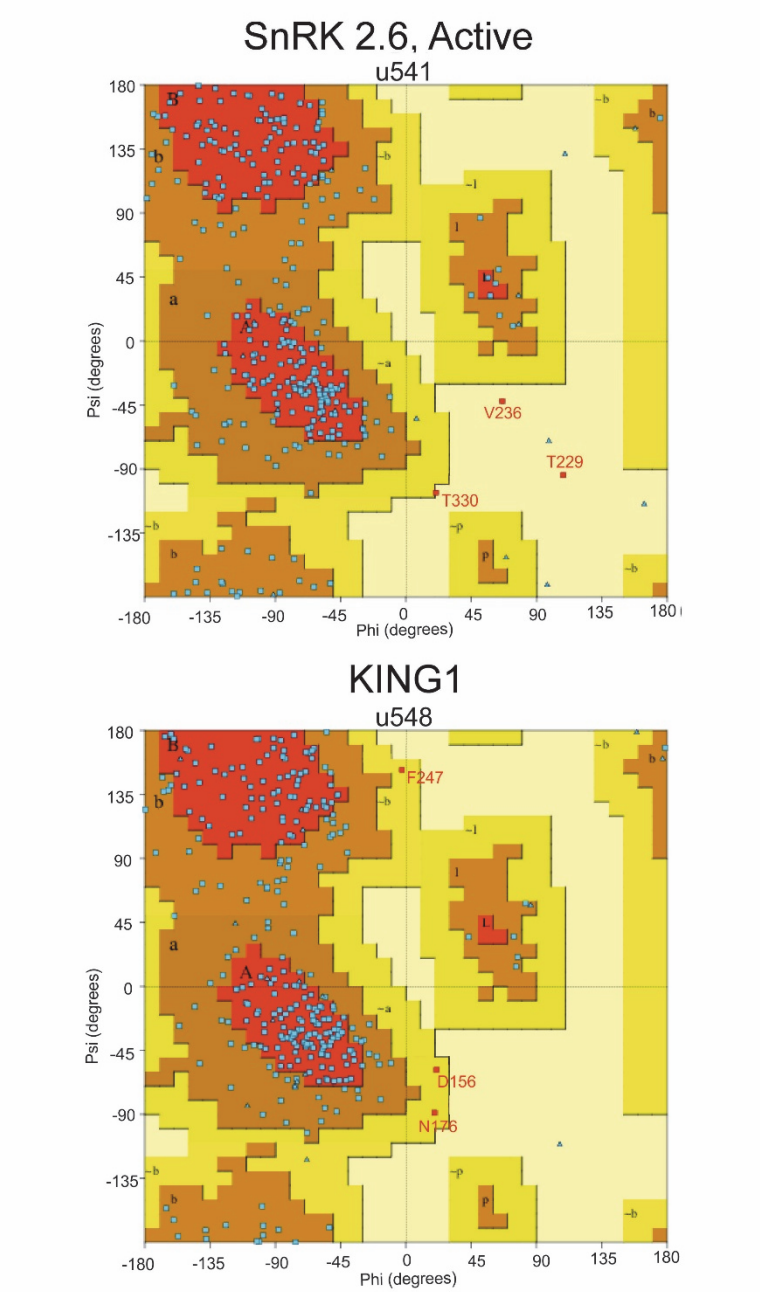

Fig. 4 Suppl. Ramachandran plots demonstrating results of the structural validation of models with only three amino acids with bad geometries in each structure.