

# Structural and Functional Analyses Explain Pea KAI2 Receptor Diversity and Reveal Stereoselective Catalysis During Signal Perception

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## Supplementary Materials

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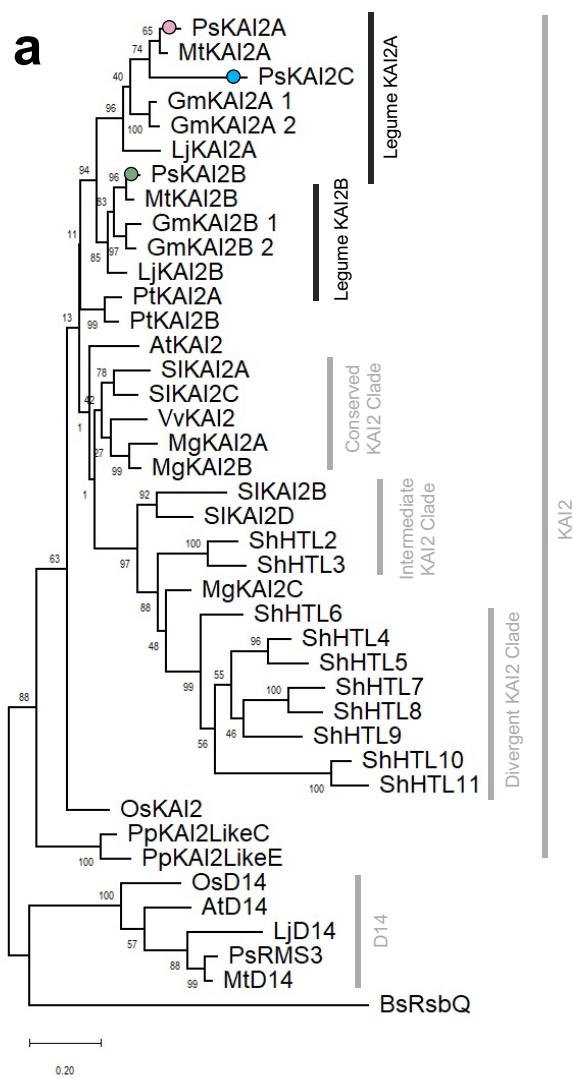
**Fig. S12.** Structural interrogation of the ligand bound PsKAI2B crystal structure.

**Fig. S13.** Mass spectrometry characterization of covalent PsKAI2-ligand complexes.

**Table S1.** PsKAI2 TILLING mutants.

**Table S2.** Data collection, phasing and refinement statistics.

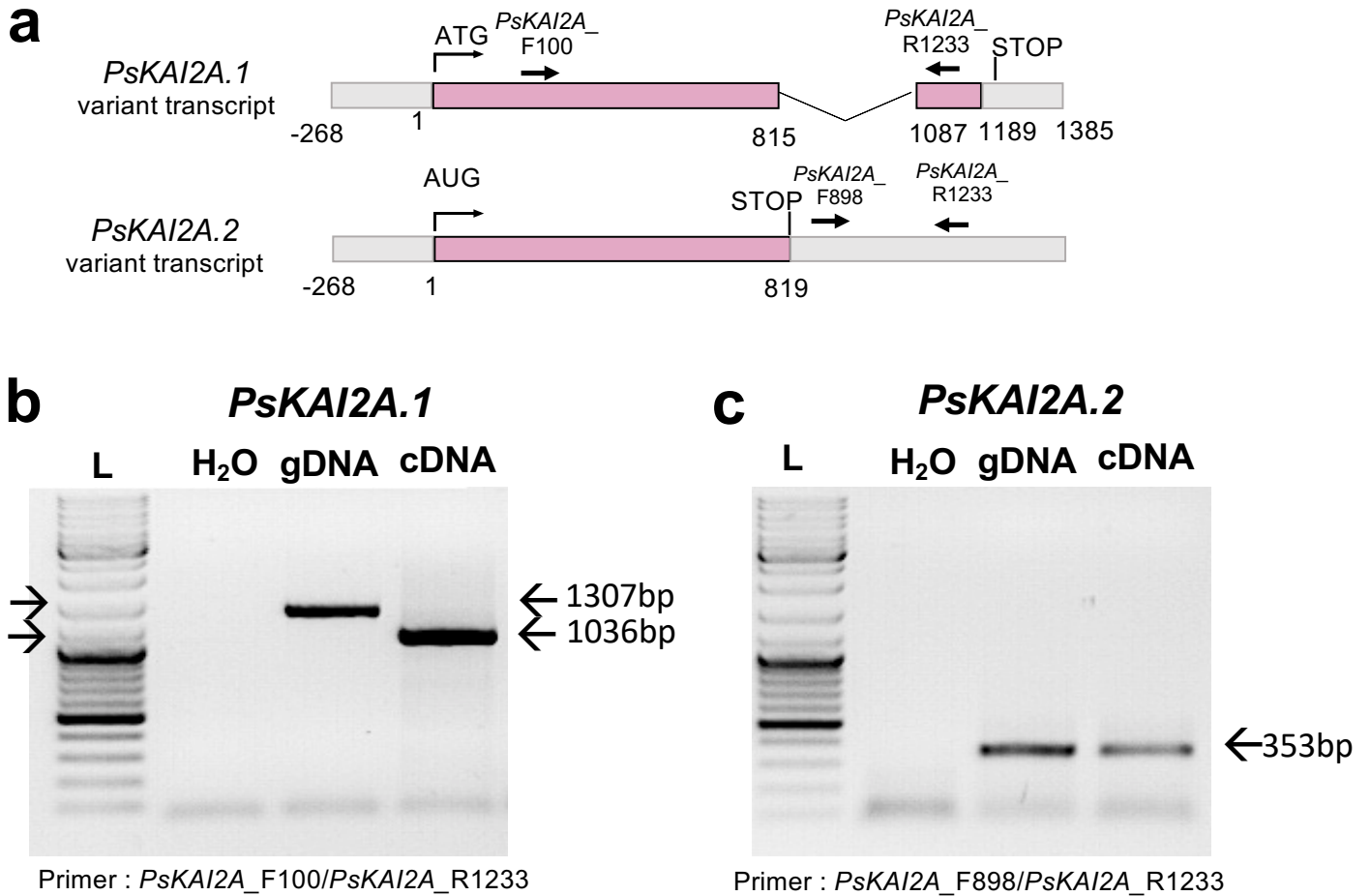
**Table S3.** Primer sequences used in study.



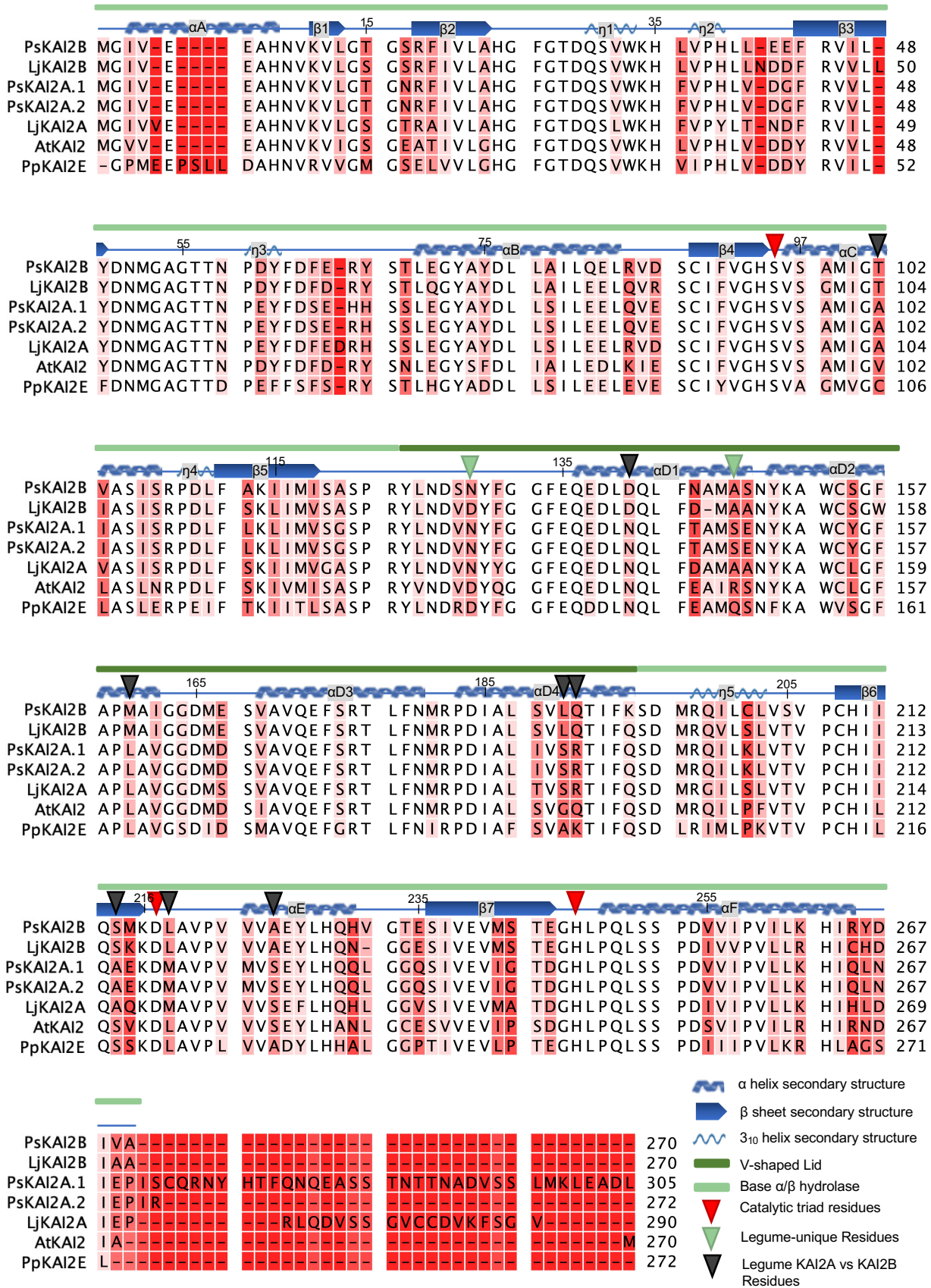
**b**

	Species	Gene name	Sequence ID	Source
KAI2	<i>Pisum sativum</i>	KAI2A	Psat2g169960	ANR GENOPEA
	<i>Pisum sativum</i>	KAI2B	Psat4g083040	ANR GENOPEA
	<i>Pisum sativum</i>	KAI2C	Psat3g014200	ANR GENOPEA
	<i>Oryza sativa</i>	KAI2	LOC_Os03g32270.1	Phytozome
	<i>Arabidopsis thaliana</i>	KAI2	AT4G37470.1	TAIR
	<i>Populus trichocarpa</i>	KAI2A	Potri.005G145000.1	Phytozome
	<i>Populus trichocarpa</i>	KAI2B	Potri.007G052000.1	Phytozome
	<i>Lotus japonicus</i>	KAI2A	Lj2g3v1931930.1	LORE1
	<i>Lotus japonicus</i>	KAI2B	Lj0g3v0117039.1	LORE1
	<i>Solanum Lycopersicon</i>	KAI2A	Solyc02g064770.2.1	Phytozome
	<i>Solanum Lycopersicon</i>	KAI2B	Solyc02g064760.1.1	Phytozome
	<i>Solanum Lycopersicon</i>	KAI2C	Solyc02g092770.2.1	Phytozome
	<i>Solanum Lycopersicon</i>	KAI2D	Solyc02g092760.2.1	Phytozome
	<i>Vitis vinifera</i>	KAI2	GSVIVT01000162001	Phytozome
	<i>Mimulus guttatus</i>	KAI2A	Migut.H00375.1	Phytozome
	<i>Mimulus guttatus</i>	KAI2B	Migut.H00378.1	Phytozome
	<i>Mimulus guttatus</i>	KAI2C	Migut.L00194.1	Phytozome
	<i>Physcomitrella patens</i>	KAI2-LikeC	6ATX	RCSB
	<i>Physcomitrella patens</i>	KAI2-LikeE	6AZB	RCSB
	KAI2	<i>Striga hermonthica</i>	HTL2	from Toh et al 2015
<i>Striga hermonthica</i>		HTL3	from Toh et al 2015	Toh et al 2015 <sup>56</sup>
<i>Striga hermonthica</i>		HTL4	from Toh et al 2015	Toh et al 2015 <sup>56</sup>
<i>Striga hermonthica</i>		HTL5	from Toh et al 2015	Toh et al 2015 <sup>56</sup>
<i>Striga hermonthica</i>		HTL6	from Toh et al 2015	Toh et al 2015 <sup>56</sup>
<i>Striga hermonthica</i>		HTL7	from Toh et al 2015	Toh et al 2015 <sup>56</sup>
<i>Striga hermonthica</i>		HTL8	from Toh et al 2015	Toh et al 2015 <sup>56</sup>
<i>Striga hermonthica</i>		HTL9	from Toh et al 2015	Toh et al 2015 <sup>56</sup>
<i>Striga hermonthica</i>		HTL10	from Toh et al 2015	Toh et al 2015 <sup>56</sup>
<i>Striga hermonthica</i>		HTL11	from Toh et al 2015	Toh et al 2015 <sup>56</sup>
KAI2		<i>Glycine max</i>	KAI2A 1	Glyma.01G191200.1.p
	<i>Glycine max</i>	KAI2A 2	Glyma.11G051000.1.p	Phytozome
	<i>Glycine max</i>	KAI2B 1	Glyma.17G164500.1.p	Phytozome
	<i>Glycine max</i>	KAI2B 2	Glyma.05G102800.1.p	Phytozome
	<i>Medicago truncatula</i>	KAI2A	Medtr5g016150.1	Phytozome
<i>Medicago truncatula</i>	KAI2B	Medtr4g095310.1	Phytozome	
D14	<i>Pisum sativum</i>	RMS3	Psat6g018320	ANR GENOPEA
	<i>Oryza sativa</i>	D14	3W04	RCSB
	<i>Arabidopsis thaliana</i>	D14	AT3G03990	TAIR
	<i>Lotus japonicus</i>	D14	Lj5g3v0310140.4	LORE1
	<i>Medicago truncatula</i>	D14	Medtr1g018320.1	Phytozome
RsbQ	<i>Bacillus subtilis</i>	RsbQ	WP_187001895	NCBI

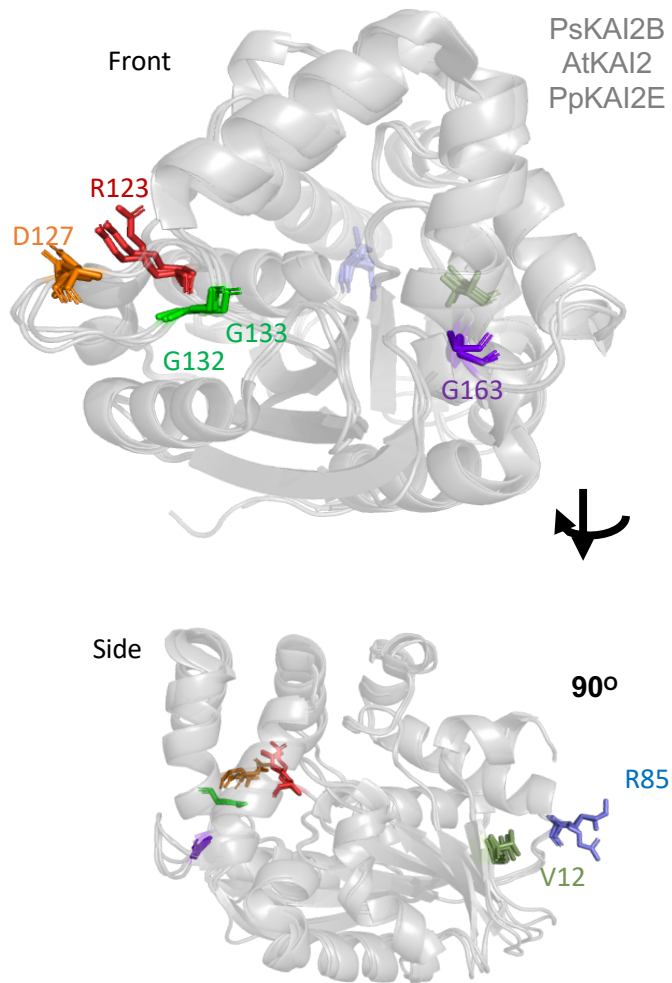
**Fig. S1. Proteins Sequence analysis of KAI2/D14 family.** (a) Maximum likelihood phylogeny of 41 KAI2/D14-family proteins with bacterial RsbQ as outgroup. Node values represent percentage of trees in which the associated taxa clustered together. Vertical rectangles highlight distinct KAI2/D14 family clades. Black circle indicates legume duplication event. Pink and green circles mark the position of PsKAI2As and PsKAI2Bs respectively. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. (b) Sequence identifiers and sources for each.



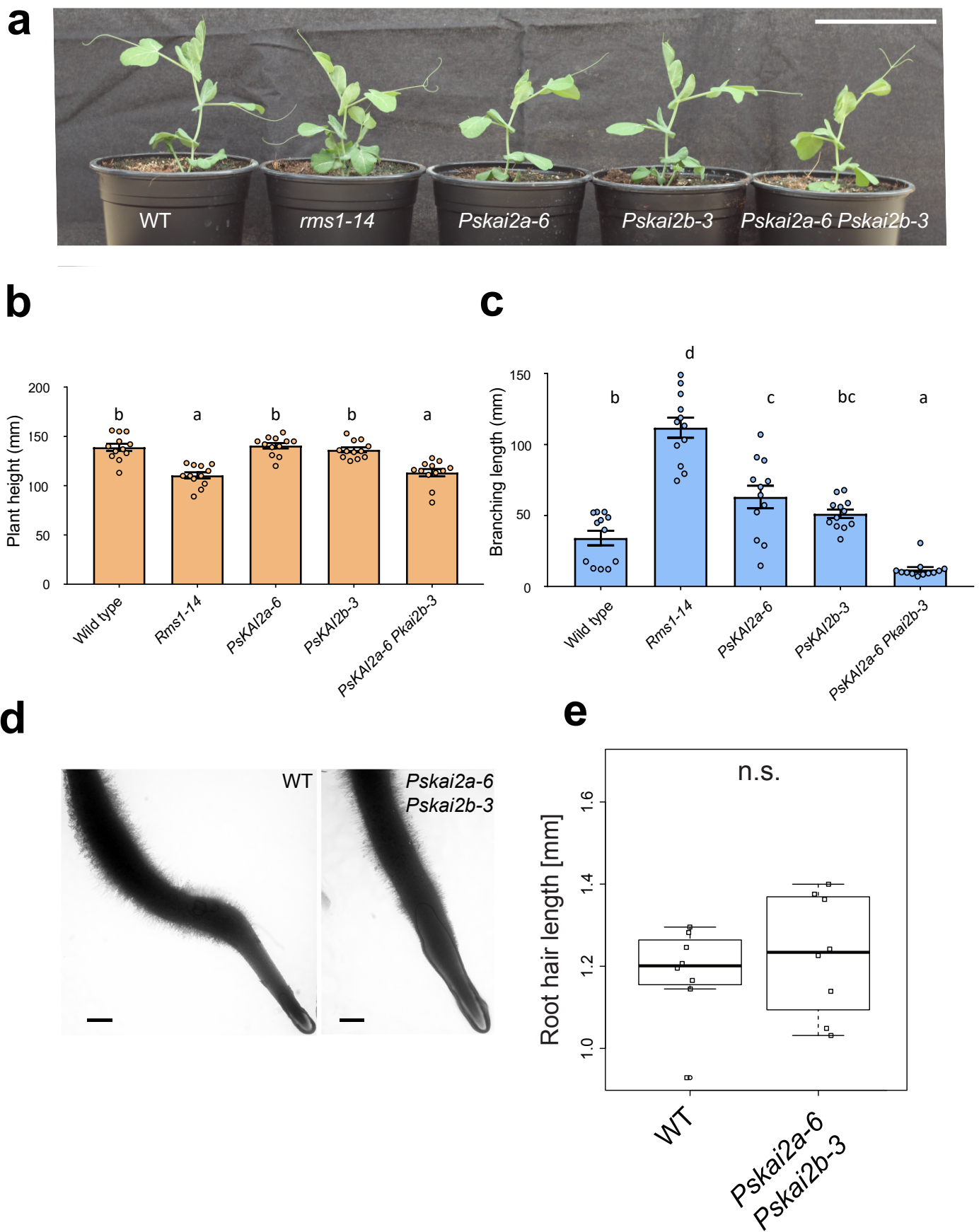
**Fig. S2. Alternative splicing of the *PsKAI2A* transcript.** (a) Schematic representation of the splicing events leading to *PsKAI2A.1* and *PsKAI2A.2* forms of mature transcripts. Exons are indicated as pink boxes. (b-c) Electrophoresis gel of PCR products obtained after amplification of the *PsKAI2A* coding sequence with primer specific to *PsKAI2A.1* form (b) and primer specific to *PsKAI2A.2* form (c) using genomic DNA (gDNA) or first-strand cDNA (cDNA) as template; H<sub>2</sub>O: negative control; Lane L: DNA ladder.



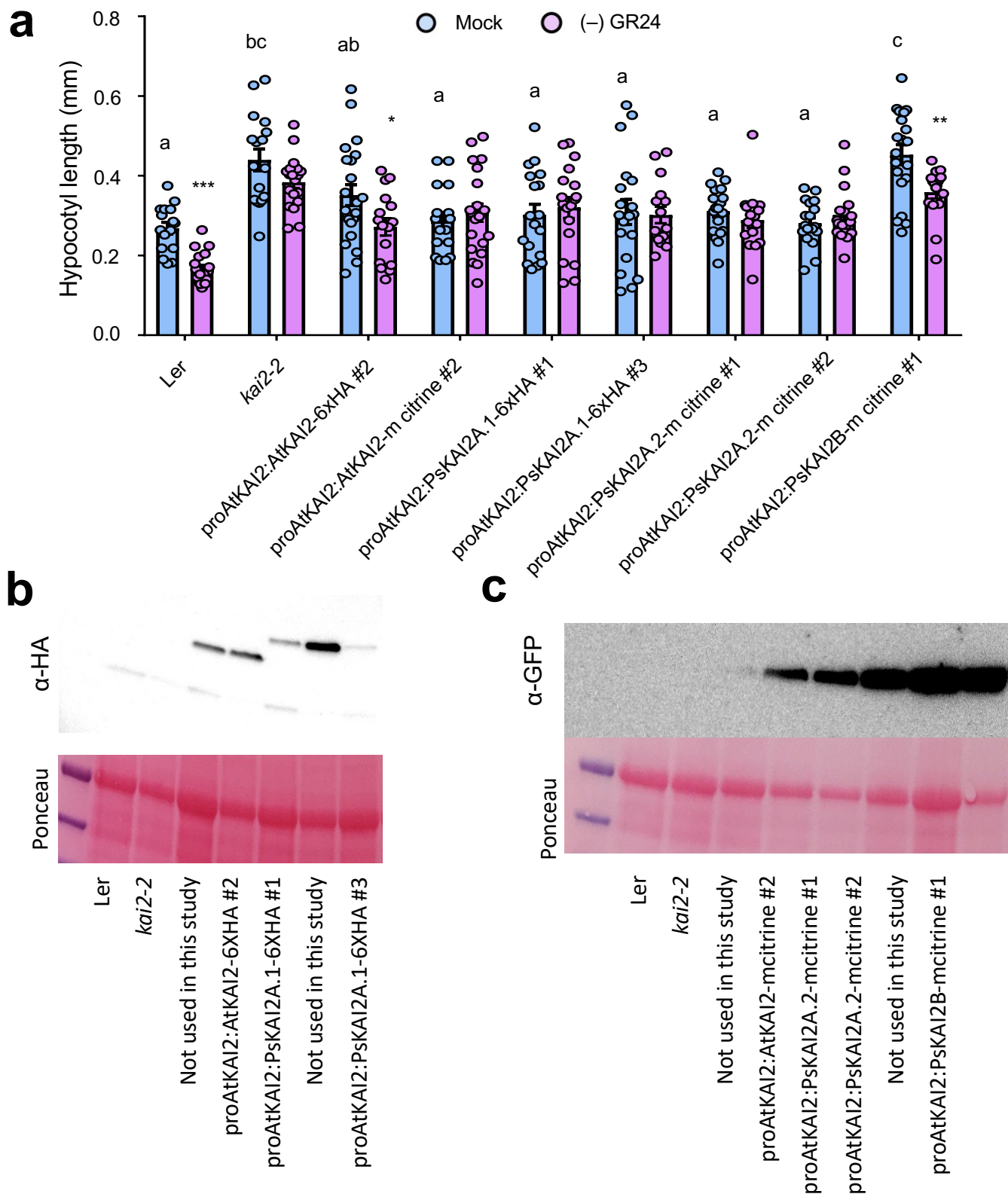
**Fig. S3. Multiple sequence alignment and conservation analysis of representative legume and non-legume KAI2s.** Multiple sequence alignment and conservation analysis of selected KAI2s. Amino acid alignment of 7 plant KAI2 proteins. Intensity of red behind residues shows degree of divergence. Numbers on residues refer to position in PsKAI2B sequence. PsKAI2B lid (forest green) and base (light green) domains are indicated above alignment. Secondary structure of PsKAI2B sequence is shown above sequence in blue as alpha helices ( $\alpha$ A-  $\alpha$ F, base; and  $\alpha$ D1-  $\alpha$ D4, lid), beta sheets ( $\beta$ 1-  $\beta$ 7),  $3_{10}$  helices ( $\eta$ 1-  $\eta$ 5) and non-secondary structure-containing loops. Red arrows indicate catalytic triad residues, green arrows indicate legume KAI2-unique residues as shown in Fig. 6, dark grey arrows indicate residues diverged between legume KAI2A and KAI2B as described in Fig. 7 and Fig. S10.



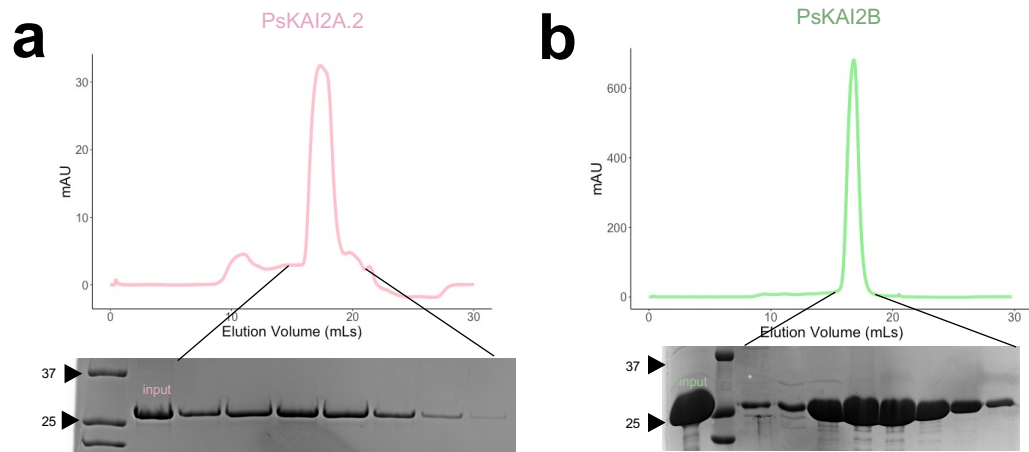
**Fig. S4. TILLING mutant residues position on KAI2 structures.** Superposition of PsKAI2B, AtKAI2, and PpKAI2E structures are shown in grey. The identified TILLING mutant residues are highlighted and labelled in rainbow colors using PsKAI2B as reference for amino acid sequence position.



**Fig. S5. Branching and root hair phenotype *Pskai2* mutants.** (a) Phenotype and quantification of (b) plant height and (c) plant lateral length (mm) of 10-day-old *Pisum sativum* plant. Bar, 8.5 cm. Data are means  $\pm$  SE (n=11-12). Statistical differences were determined using a one-way ANOVA with a Tukey multiple comparison of means post-hoc test, statistical differences of  $P < 0.05$  are represented by different letters. (d) Root hair length phenotype and quantification (e) of 10 day old WT and *Pskai2a-6 Pskai2b-3* seedlings. Bars, 2 mm. Data are means of measurements (n=8) of individual root (n=9), Statistical analysis Welch t-test, p-value  $< 0.05$

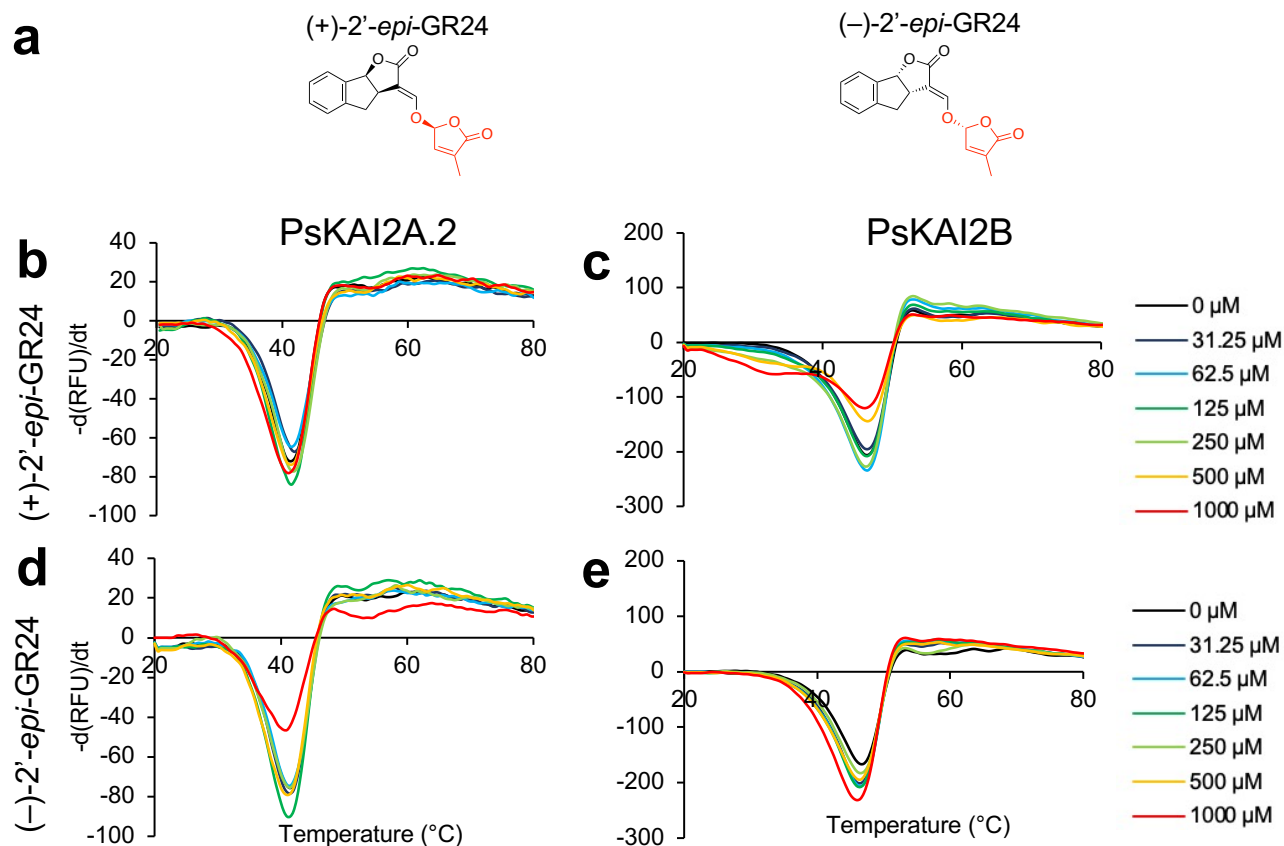


**Fig. S6. Hypocotyl elongation in *kai2-2* mutant and KAI2s protein expression in complementation assay.** (a) Hypocotyl length of 7-day-old seedlings grown under low light at 21 °C. Data are means  $\pm$  SE (n = 20-24; 2 plates of 10-12 seedlings per plate). Light blue bars: Mock (DMSO), lavender bars: (-)GR24 (1 $\mu$ M). Complementation assays using the *AtKAI2* promoter to express *AtKAI2* (control) or *PsKAI2* genes in the null *kai2-2* mutant background (Ler ecotype) as noted above the graph. Proteins were tagged with 6xHA epitope or mCitrine protein. For DMSO controls, statistical differences were determined using a one-way ANOVA with a Tukey multiple comparison of means post-hoc test, statistical differences of  $P < 0.05$  are represented by different letters. Means with asterisks indicate significant inhibition compared to mock-treated seedlings with \*\*\* corresponding to  $p \leq 0.001$  and \* to  $p \leq 0.01$ , as measured by t-test. (b-c) *AtKAI2* and *PsKAI2* level analyzed by immunoblot using  $\alpha$ -HA antibody (b) or  $\alpha$ -GFP antibody (c) in Col-0 (wild type), *kai2-2* and transformed *kai2-2* plants, expressed under the control of *AtKAI2* promoter. Protein extracts from 10-d-old seedlings were separated by 10% SDS-PAGE. Ponceau staining is included for loading reference.

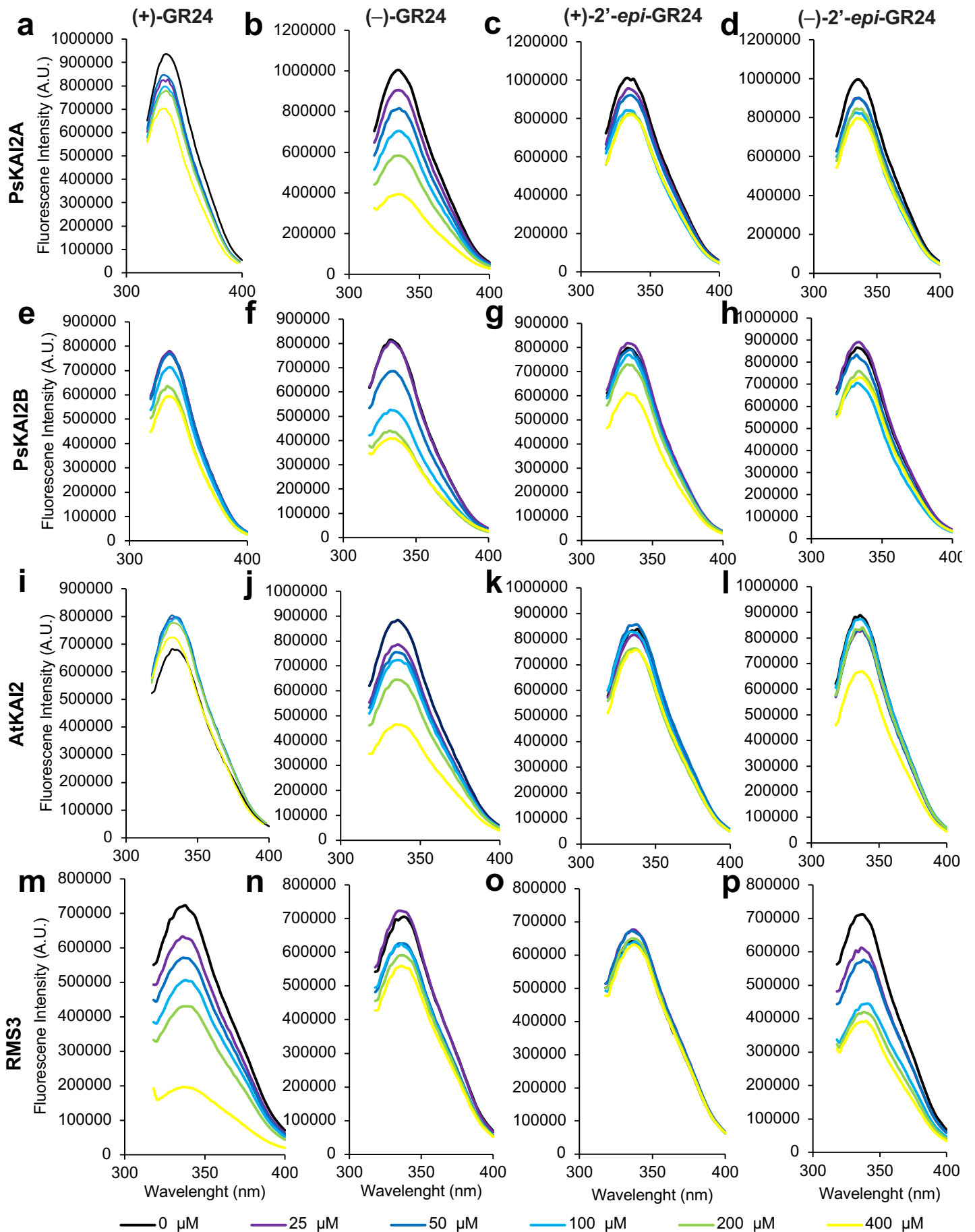


**Fig. S7. Purification of PsKAI2s.** Size exclusion peaks eluates for PsKAI2A.2 (**a**) and PsKAI2B (**b**). Proteins were resolved on SDS-PAGE gels and visualized via Coomassie stain. Molecular weight (MW) markers are labeled to show the relative size of proteins.

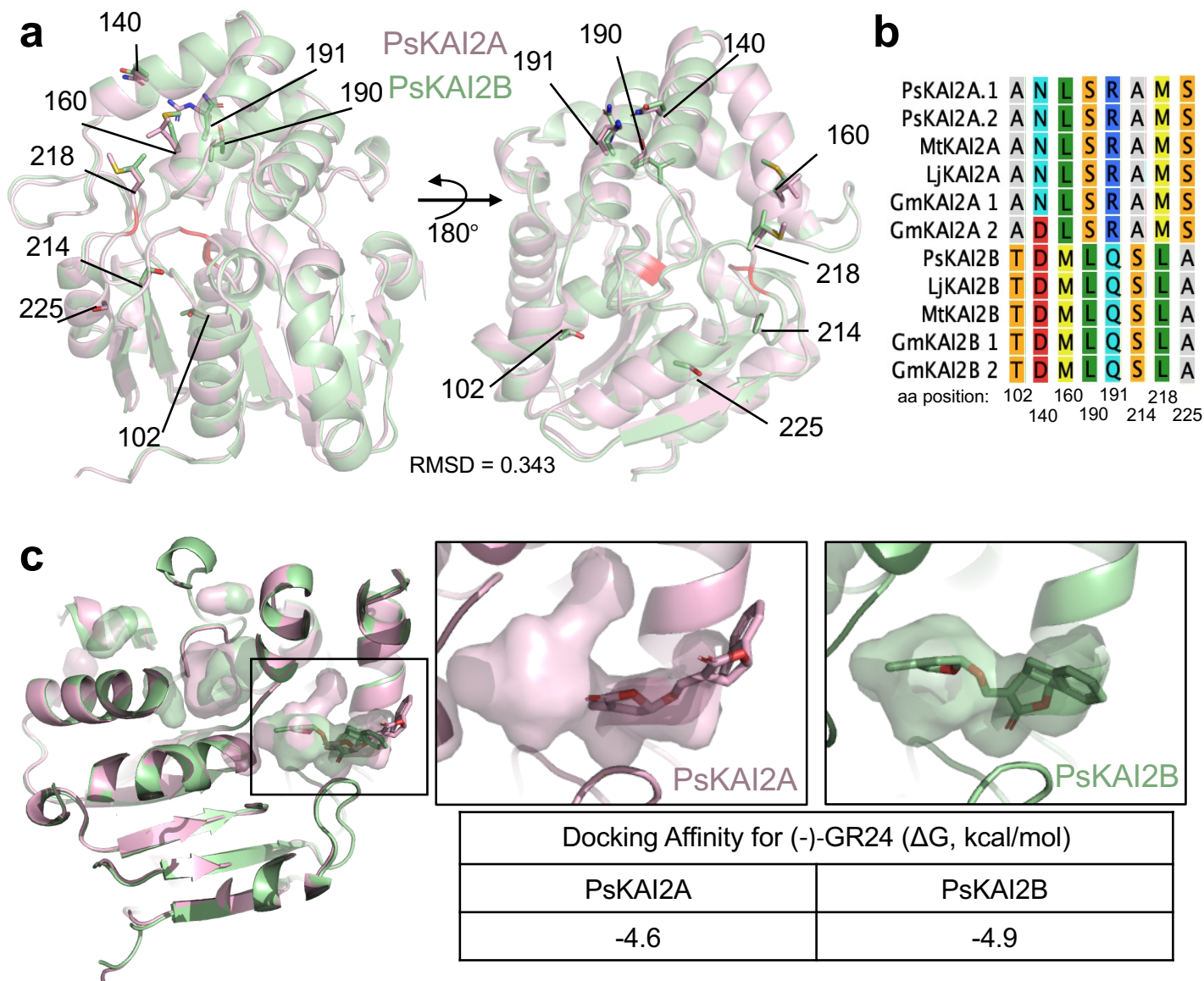




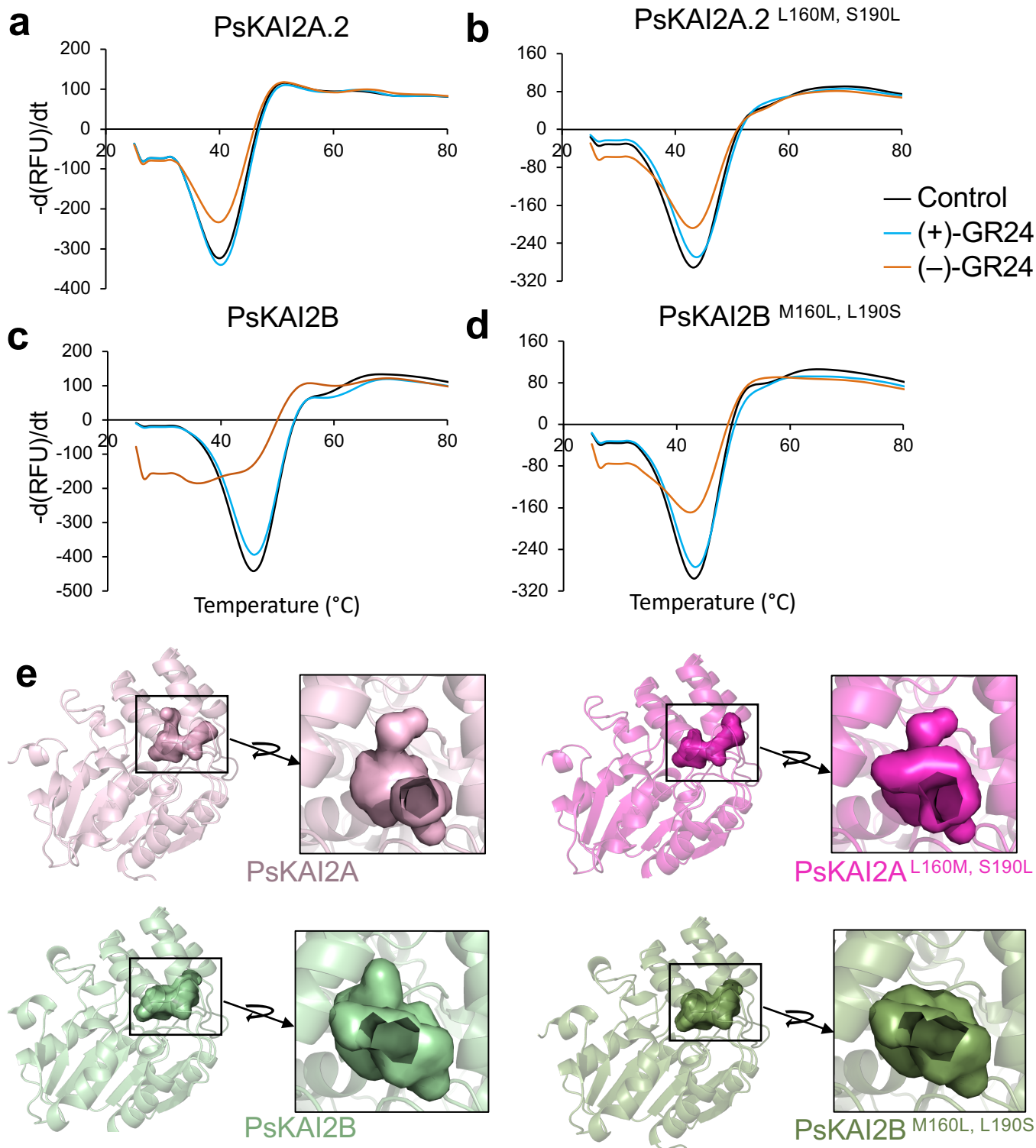
**Fig. S8. Biochemical analysis of the interaction between PsKAI2 proteins and the (+)-2'-*epi*-GR24 and the (-)-2'-*epi*-GR24 isomers ligands by DSF.** (a) Chemical structure of ligands used in DSF assay. The melting temperature curves of 10  $\mu\text{M}$  PsKAI2A.2 (b, d) or PsKAI2B (c, e) with (+)-2'-*epi*-GR24 (b-c), or (-)-2'-*epi*-GR24 (d-e) at varying concentrations are shown as assessed by DSF. Each line represents the average protein melt curve for three technical replicates and the experiment was carried out twice.



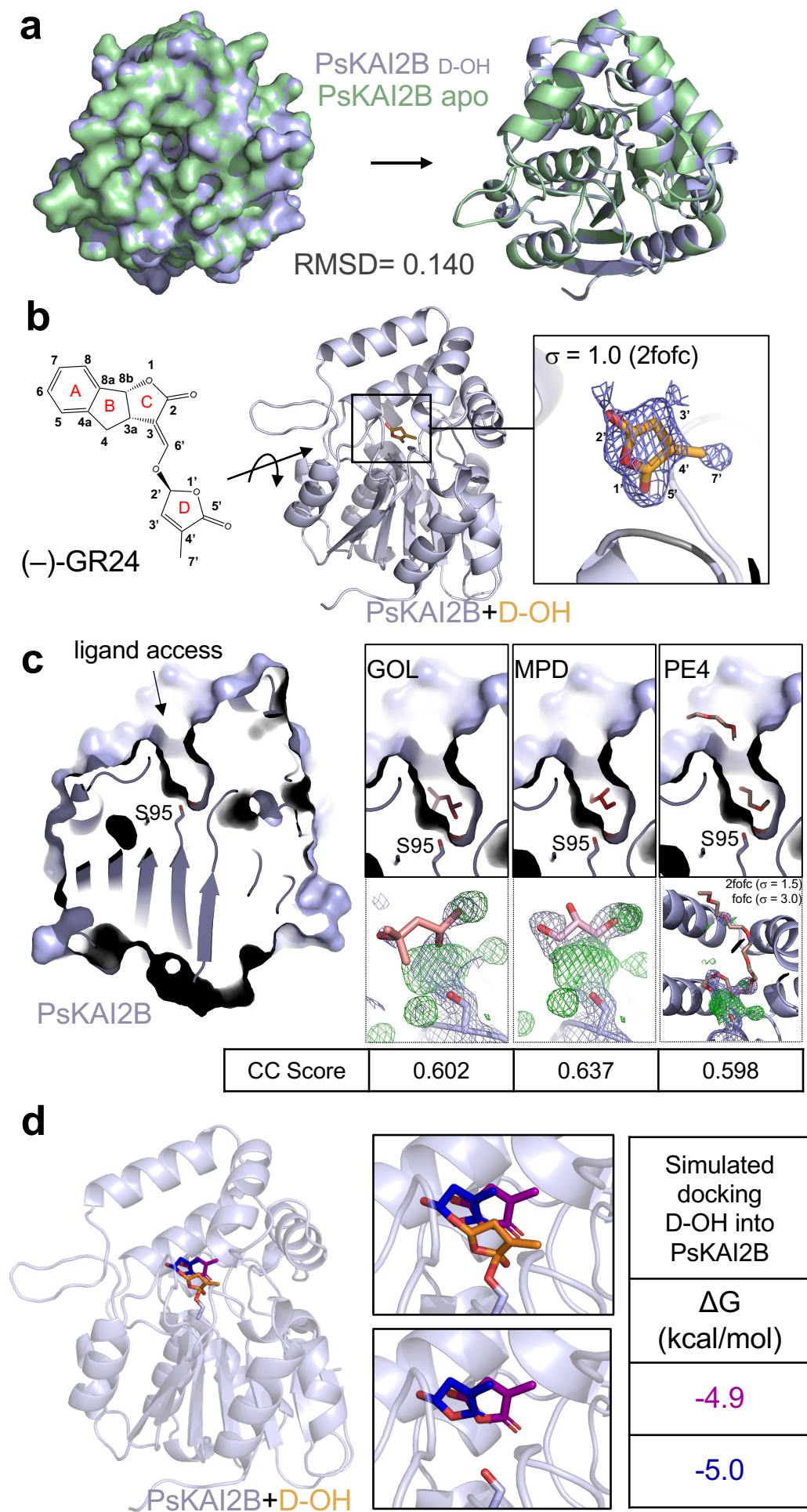
**Fig. S9. Intrinsic tryptophan fluorescence of PsKAI2s, AtKAI2 and RMS3 proteins in the presence of SL analogs.** Intrinsic tryptophan fluorescence of PsKAI2A (**a-d**), PsKAI2B (**e-h**), AtKAI2 (**i-l**) and RMS3 (**m-p**) proteins in the presence of SL analogs. Changes in intrinsic fluorescence emission spectra in the presence of various concentrations of (+)-GR24 (**a;e;i;m;q**), (-)-GR24 (**b;f;j;n;r**), (+)-2'-*epi*-GR24 (**c;g;k;o;s**), (-)-2'-*epi*-GR24 (**d;h;l;p;t**). Proteins (10  $\mu$ M) were incubated with increasing amounts of ligand (0–400  $\mu$ M, top line to bottom line, respectively). The observed relative changes in intrinsic fluorescence were plotted as a function of SL analog concentration and transformed to degree of saturation and used to determine the apparent  $K_D$  values relevant to **Figure 2i**. The plots represent the mean of two replicates and the experiments were repeated at least three times. The analysis was performed with GraphPad Prism 8.0 Software.



**Fig. S10. Structural divergence analysis of legume KAI2A and KAI2B.** (a) Structural alignment of PsKAI2A and PsKAI2B shown in pink and light green respectively. Calculated RMSD of aligned structures is shown. Residues differentiating all legume KAI2A from KAI2B are shown on each structure as sticks and labeled with residue number. Catalytic triad is shown in red. (b) Residues 102, 140, 160, 190, 191, 214, 218, and 225, L190, and L218 are highlighted as divergent legume KAI2 residues, conserved among all legume KAI2A or KAI2B sequences (with the exception of D140 in GmKAI2A 2) as shown in reduced Multiple Sequence Alignment from Fig. S1. (c) *In silico* docking analysis of intact (-)-GR24 with solvent-accessible pocket shown for each structure and corresponding docking scores reported in kcal/mol.

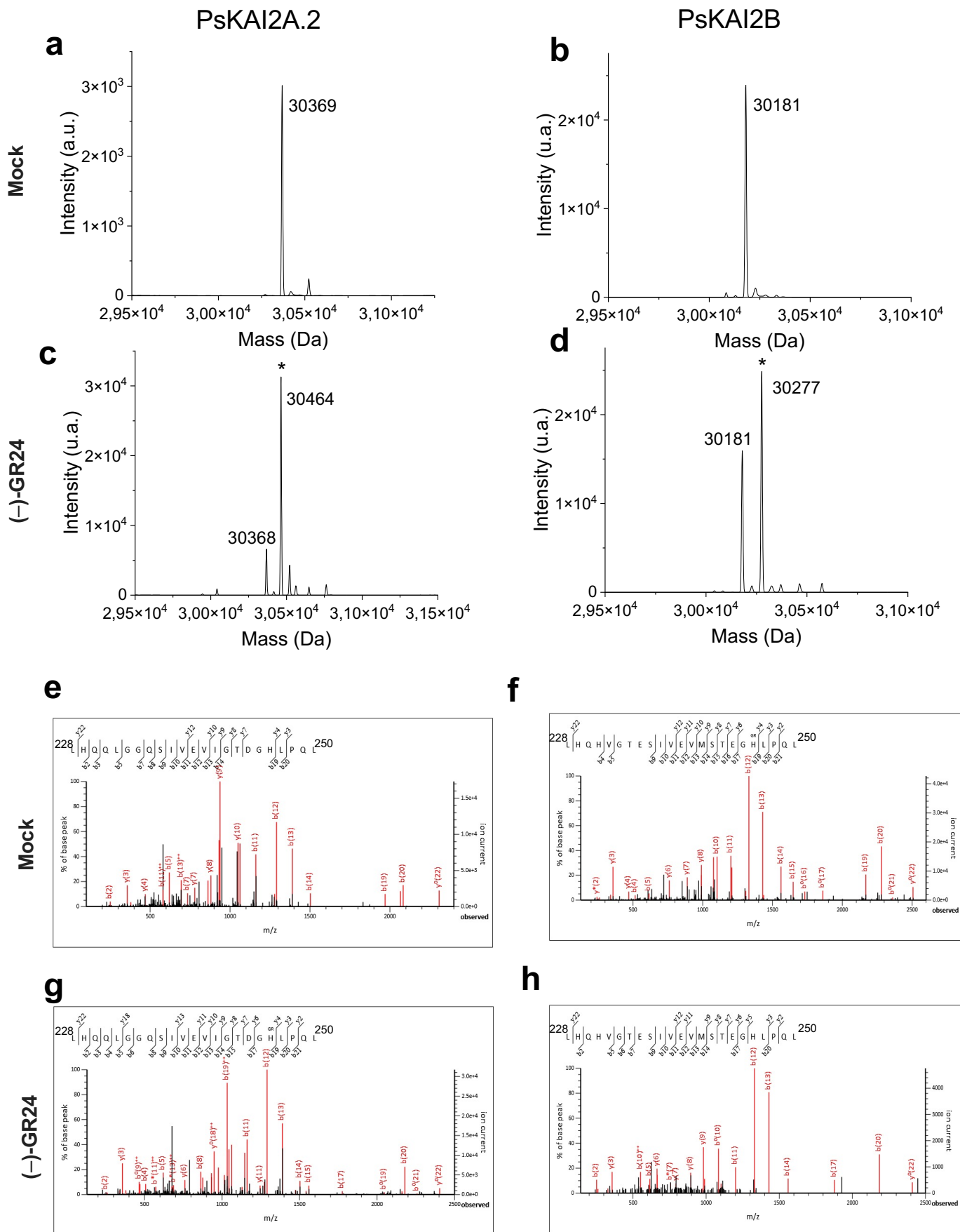


**Fig. S11. Biochemical and structural analysis of the interaction between wildtype and residue 160 and 190 swap mutant PsKAI2 proteins and (-)-GR24 by DSF.** The melting temperature curves of 10  $\mu$ M PsKAI2A.2 (a), PsKAI2A.2<sup>L160M, S190L</sup> swap (b), PsKAI2B (c), and PsKAI2B<sup>M160L, L190S</sup> swap (d), with (+)-GR24 or (-)-GR24 at the effective concentration of 62.5  $\mu$ M are shown as assessed by DSF. Each line represents the average protein melt curve for three technical replicates and the experiment was carried out twice. (e) pocket surface representation of PsKAI2A model, PsKAI2B apo structure, and modelled PsKAI2A.2<sup>L160M, S190L</sup> and PsKAI2B<sup>M160L, L190S</sup> swap mutants.



**Fig. S12**

**Fig. S12. Structural interrogation of the ligand bound PsKAI2B crystal structure.** (a) Structural alignment of PsKAI2B apo (light green) and PsKAI2B in complex with (-)-GR24 D- OH (gray/blue). Calculated RMSD value is shown. Similar orientation of the superposition is shown in surface (right) and cartoon (left) representations. (b) Chemical structure of intact (-)- GR24 molecule with numbered carbons. Electron density mesh fit with D-OH ring of (-)-GR24. Protein structure is shown in blue/gray and ligand in orange. The electron density of the ligand is derived from 2mFoDFc (2fofc) map contoured at 1.0s. (c) LigandFit examination of crystallization and purification conditions reported here. Glycerol denoted: GOL, (+/-)-2-Methyl- 2,4-pentanediol denoted: MPD. Polyethylene glycol, PEG, denoted: PE4. Ligand access pocket is shown together with the ligand placed via LigandFit fitting software (top), and the obtained electron density maps following 3 cycles of Phenix refine. The electron densities shown are derived from 2mFoDFc map (2fofc, blue mesh) contoured at 1.5s and mFoDFc map (fofc, green mesh) contoured at 3s. Correlation Coefficient (CC) scores were calculated via LigandFit. (d) *In silico* analysis of D-OH ligand docking is shown in orange (found in the structure) and predicted orientations in navy and magenta with corresponding docking scores reported in kcal/mol.



**Figure S13. Mass spectrometry characterization of covalent PsKAI2-ligand complexes.** A Deconvoluted electrospray mass spectra of PsKAI2A.2 and PsKAI2B before (a-b) and after (c-d) adding of ligand (-)-GR24 are shown respectively on upper and lower panels. Peaks with an asterisk correspond to PsKAI2 covalently bound to a (-)-GR24 ligand (PsKAI2-ligand). A mass increment of 96 Da is measured for two PsKAI2-ligand complexes. (e-h) Ligand-modified H246 amino-acids were identified by nanoLC-MSMS analyses after chymotrypsin proteolysis. Fragmentation spectra of unmodified and ligand-modified peptides are shown. Labeled peaks correspond to b and y fragments of the triple charged precursor ion. Histidine 246 residue modified by ligand is marked with GR on the sequence displayed on the top.



**Table S1. List of the mutations identified during TILLING and mutant alleles used in the study.**

<b><i>PsKAI2A</i> TILLING mutants</b>				
<b>Mutant allele</b>	<b>Base position<sup>1</sup></b>	<b>Protein position</b>	<b>Type of mutation</b>	<b>Protein location of the mutated amino acid</b>
<i>Pskai2a-1</i>	G266A	C89Y	Missense	
2	C333T	L111L	Silent	
<i>Pskai2a-2</i>	G368A	R123K	Missense	This R is conserved across KAI2s, located at the left base of the V lid in the back of a loop
4	G368A	R123K	Missense	
<i>Pskai2a-3</i>	G379A	D127N	Missense	This D is conserved across KAI2s, located at the left base of the V lid in the middle of a loop
6	C390T	Y130Y	Silent	
<i>Pskai2a-4</i>	G395A	G132E	Missense	This G is conserved across KAI2s, located on the same left loop region as <i>Pskai2a-2</i> and <i>Pskai2a-3</i> , but are pointing more inwards so possibly closer to the entrance of the pocket
<i>Pskai2a-5</i>	G398A	G133E	Missense	see <i>Pskai2a-4</i>
9	G409A	E137K	Missense	
10	G412A	D138N	Missense	
11	G417A	L139L	Silent	
12	G417A	L139L	Silent	
13	G454A	A152T	Missense	
14	C465T	Y155Y	Silent	
15	G467A	G156E	Missense	
<i>Pskai2a-6</i>	G487A	G163R	Missense	This G is conserved across KAI2s, located at the right base of the V lid in a loop region, also pointing slightly inwards
17	G561A	L187L	Silent	
18	G676A	E226K	Missense	
19	G676A	E226K	Missense	
20	G715A	E239K	Missense	

<b><i>PsKAI2B</i> TILLING mutants</b>				
<b>Mutant allele</b>	<b>Base position<sup>1</sup></b>	<b>Protein position</b>	<b>Type of mutation</b>	<b>Protein location of the mutated amino acid</b>
1	Before ATG	-		
2	44	T15I	Missense	
<i>Pskai2b-1</i>	34	V12I	Missense	This V is conserved across all KAI2s, located on the back of the protein pointing outwards not near lid or known binding interfaces
<i>Pskai2b-2</i>	254	R85K	Missense	This R is not conserved, AtKAI2 has a K at this position, located on the back of the protein pointing outwards not near lid or known binding interfaces
5	120	L40L	Silent	
6	121	L41L	Silent	
7	201	Y67Y	Silent	
<i>Pskai2b-3</i>	488	G163E	Missense	see <i>Pskai2a-6</i>
9	508	A170T	Missense	
10	655	A219T	Missense	
11	658	V220I	Missense	
12	664	V222I	Missense	
13	674	A225V	Missense	
14	540	N180N	Silent	
15	558	A186A	Silent	
16	663	P221P	Silent	

<sup>1</sup> base position from the ATG

**Table S2. Data collection, phasing and refinement statistics**

	PsKAI2B (apo form, with glycerol)	(-)-GR24 D-OH - bound PsKAI2B
<b>Data collection</b>		
Space group	C2	C2
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	87.59, 71.14, 49.06	87.08, 71.82, 48.79
$\alpha$ , $\beta$ , $\gamma$ (°)	90, 117, 90	90, 117.3, 90
Resolution (Å)	43.47-1.61 (1.66-1.61)*	43.36-2.00 (2.07-2.00)
<i>R</i> <sub>sym</sub>	0.080 (0.589)	0.082 (0.316)
<i>I</i> / $\sigma$ <i>I</i>	31.01 (1.52)	35.13 (4.11)
Completeness (%)	99.2 (84.5)	98.73 (87.53)
Redundancy	6.4 (3.2)	6.1 (4.5)
<b>Refinement</b>		
Resolution (Å)	1.61	2.00
No. reflections	34306	17837
<i>R</i> <sub>work</sub> / <i>R</i> <sub>free</sub> (%)	15.9/17.7	16.9/21.1
No. atoms	2395	2298
Protein	2110	2110
Ligand/ion	6	8
Water	279	180
<i>B</i> -factors		
Protein	19.92	26.5
Ligand/ion	33.73	24.60
Water	32.07	32.22
R.m.s. deviations		
Bond lengths (Å)	0.009	0.013
Bond angles (°)	0.88	1.03
Ramachandran favored (%)	98.51	98.88
Ramachandran allowed (%)	1.49	1.12
Ramachandran outliers (%)	0	0
PDB ID	7K2Z	7K38

**Table S3. List of primers used in this study**

GENE	Primer name	Sequence	Observation
<b>Primers for qRT-PCR analysis</b>			
<i>PsACTIN</i>	PsACTIN_F1	GTGTCTGGATTGGAGGAT	Used in figure 1
	PsACTIN_R1	GGCCACGCTCATCATATT	
<i>PsTUB</i>	ST206-F	CAGAACAAGAAGACTCGTCATACT	Used in figure 2
	ST207-R	AGCCTTCCTCCTGAACATA	
PsKAI2B Psat4g083040	PsKAI2B_F1	GCCCTAAGCGTGTACAAAC	Used in figure 2
	PsKAI2B_R1	ACTCGGTGCCGACGTGT	
	PsKAI2B_F2	GCCCTAAGCGTGTACAAAC	Used in figure 1
	PsKAI2B_R2	ACTCGGTGCCGACGTGT	
<i>PsKAI2A</i> Psat2g169960	PsKAI2A_F1	CTTTGATAGTGTGCGAGGACGA	Used in figure 2
	PsKAI2A_R1	GACCACCCAATTGTTGATGT	
	PsKAI2A_F2	CTTTGATAGTGTGCGAGGACGA	Used in figure 1
	PsKAI2A_R2	GACCACCCAATTGTTGATGT	
<i>PsDLK2</i> Psat1g039520	ST208-F	AGGCTTGTCTCTTGGTGC	Used in figure 2
	ST209-R	ATCTGAACTTGCAAATCCTCCC	
<b>Primers for TILLING</b>			
<i>PsKAI2A</i> Psat2g169960	PsKAI2A_N1F	GGAAACACTTTGTACCACATCTCG	Nested 1 PCR
	PsKAI2A_N1R	TCCTTCATCTTGTGCCTTACTAGC	
	PsKAI2A_N2Ftag	<b>ACGACGTTGTA</b> AAACGACGATAACATG GGTGCTGG	Nested 2 PCR Specific primers (black nucleotides) have a M13 tag (red nucleotide) in 5' end.
	PsKAI2A_N2Rtag	<b>TAACAATTTACACAGG</b> CAATGGGCT ATAATGGG	
PsKAI2B Psat4g083040	PsKAI2B_Fw1	<b>TTCCCTACACGACGCTCTTCCGATCTCA</b> GCCTACAGTTATCAACAAACC	Specific primers (black nucleotides) have a Illumina adaptator (red nucleotide) in 5' end.
	PsKAI2B_Rv1	<b>AGTTCAGACGTTGCTCTTCCGATCTA</b> TTTTGGCAAACAAATCAGG	
	PsKAI2B_Fw2	<b>TTCCCTACACGACGCTCTTCCGATCTCG</b> ACAGTAATTACTTTGGAGG	
	PsKAI2B_Rv2	<b>AGTTCAGACGTTGCTCTTCCGATCTTC</b> ACCGAATAACAACATCC	
<b>Primers for splicing variant identification</b>			
<i>PsKAI2A</i> Psat2g169960	<i>PsKAI2A</i> _F100	AACTCACACAGTCGGTGAAT	
	<i>PsKAI2A</i> _F898	ACCCACAATATATAGTTGTTG	
	<i>PsKAI2A</i> _R1233	ATGCCACTTCCTTCATCTTG	
<b>Primers for protein preparation and purification</b>			
<i>PsKAI2A</i> Psat2g169960	PsKAI2A_F	aaaaccttacttccaatcgATGGGGATAGTGGAA GAAG	
	PsKAI2A.1_R	ccaccteatctccggTTACAAATCTGCCTCAA GTTTC	
	PsKAI2A.2_R	ccaccteatctccggTTACCTTATTGGCTCAA TATTAAGTTG	
PsKAI2B Psat4g083040	PsKAI2B_F	aaaaccttacttccaatcgATGGGAATAGTGGAA GAAGC	
	PsKAI2B_R	ccaccteatctccggTCAAGCTACAATATCA TAACGAATG	
<b>Primers for generation of Arabidopsis transgenic lines</b>			
<i>PsKAI2A</i> Psat2g169960	PsKAI2A_attb1	GGGGACAAGTTTGTACAAAAAAGCAG GCTtcATGGGGATAGTGAAGAAGCA	
	PsKAI2A.1_attb2	ggggaccactttgtacaagaaagctgggtcCAAATCTG CCTCAAGTTTCA	
	PsKAI2A.2_attb2	ggggaccactttgtacaagaaagctgggtcCCTTATTG GCTCAATATTAA	
PsKAI2B Psat4g083040	PsKAI2B_attb1	GGGGACAAGTTTGTACAAAAAAGCAG GCTtcATGGGAATAGTGAAGAAGC	
	PsKAI2B_attb2	ggggaccactttgtacaagaaagctgggtcAGCTACAA TATCATAACGAA	
AtKAI2 At4g37470	AtKAI2_attb1	ggggacaagtttgtacaaaaagcaggcttcATGGGTG TGGTAGAAGAAGC	
	AtKAI2_attb2	ggggacaagtttgtacaaaaagcaggcttcATGGGTG TGGTAGAAGAAGC	