

Table S1. Features of selected CRISPR RNA (crRNA) against members of the *PpKAI2L* gene family clade i.

On-target gene ^a	crRNA sequence and SS (%) ^b	Predicted off-targets sequence ^{b,c}	Off-target annotation ^a
Pp3c2_19340 (<i>PpKAI2L-A</i>)	GTTGTCCTCGGCCATGGTTT <u>TGG</u> (99)	<u>C</u> TTG <u>A</u> CT <u>G</u> TCCATGGTTT <u>TGG</u>	OffT1*: Intergenic between Pp3c22_8523 - Pp3c22_8540
		GT <u>A</u> GCCT <u>A</u> AGCCATGG <u>G</u> TT <u>TGG</u>	OffT2*: Exon of Pp3c10_1460 (PpKAI2L-F)
		GT <u>A</u> GT <u>G</u> CTCAGCCATGG <u>A</u> TT <u>TGG</u>	OffT3*: Exon of Pp3c1_18010 (PpKAI2L-K)
		GT <u>A</u> GCCTC <u>A</u> TCCATGG <u>G</u> TT <u>CGG</u>	OffT4*: Exon of Pp3c19_19860
		GT <u>G</u> GT <u>A</u> CT <u>A</u> GGCCATGG <u>G</u> TT <u>TGG</u>	OffT5: Exon of Pp3c12_8770 (PpKAI2L-I)
		GT <u>G</u> GTCC <u>A</u> CGGCCATGG <u>C</u> G <u>TGG</u>	OffT6: 5'UTR of Pp3c20_10270 locus
		<u>I</u> TTT <u>T</u> CCCTCGGC <u>A</u> TTGGTTT <u>TGG</u>	OffT7: Intergenic between Pp3c22_14060 - Pp3c22_14160
		GTTGT <u>I</u> CT <u>I</u> GGCGATGG <u>G</u> TT <u>GGG</u>	OffT8: 3'UTR of Pp3c1_24440
		GTTG <u>G</u> CCCTCGGC <u>T</u> TG <u>T</u> GT <u>CGG</u>	OffT9: Exon of Pp3c19_4690
		<u>C</u> TTGTCCTCGG <u>G</u> CCGTGG <u>C</u> TT <u>CGG</u>	OffT10: nf
		GTTGTCCTCG <u>A</u> CGTTGGTT <u>C</u> T <u>TGG</u>	OffT11: Exon of Pp3c12_14550
		GTTGTCCT <u>I</u> GGCTTGGTTT <u>GGG</u>	OffT12: Intergenic between Pp3c20_19870 - Pp3c20_19940
Pp3c14_6110 (<i>PpKAI2L-B</i>)	GTGTCCGGGACCGACTCTGT <u>TGG</u> (100)	GTGT <u>A</u> CGGGTCCGACGGT <u>G</u> T <u>CGG</u>	OffT1: Exon of Pp3c5_25610
		GTGT <u>A</u> CGGGTCCGACGGT <u>G</u> T <u>CGG</u>	OffT2: Exon of Pp3c5_25430
		<u>C</u> TGTCCGGT <u>A</u> CCCGCTCTGT <u>AGG</u>	OffT3*: Exon of Pp3c11_17570
		GTGTCC <u>A</u> GGACG <u>G</u> ACA <u>C</u> T <u>TGGG</u>	OffT4: Exon of Pp3c1_4090
Pp3c25_5350 (<i>PpKAI2L-C</i>)	GCTAAAATACTCCGGGTCGGT <u>TGG</u> (99)	GCT <u>G</u> AAGTACTCGGGTCT <u>G</u> T <u>TGG</u>	OffT1*: Exon of Pp3c6_10610 (PpKAI2L-D)
		GCTAAAAT <u>A</u> CTCCGGATCT <u>G</u> T <u>TGG</u>	OffT2*: Exon of Pp3c2_19340 (PpKAI2L-A)
		GCCAAAAT <u>C</u> TCCG <u>I</u> GT <u>CGC</u> <u>AGG</u>	OffT3*: Exon of Pp3c20_4150
Pp3c6_10610 (<i>PpKAI2L-D</i>)	TCGGCACGGATCAATCTGT <u>TGG</u> (98)	<u>T</u> GGCACAGATCAAT <u>C</u> CGT <u>G</u> T <u>TGG</u>	OffT1*: Exon of Pp3c25_5350 (PpKAI2L-C)
		TCGGCACGGATCA <u>G</u> T <u>C</u> G <u>TGG</u>	OffT2*: Exon of Pp3c5_16420 (PpKAI2L-E)
		TCG <u>A</u> CA <u>T</u> GGGTCAAGCTGT <u>G</u> <u>AGG</u>	OffT3*: Exon of Pp3c2_19340 (PpKAI2L-A)
		TCCGCACGGGGCAAT <u>G</u> TGT <u>G</u> <u>AGG</u>	OffT4: 3'UTR of Pp3c7_22220
		<u>T</u> GGCACCGAC <u>C</u> AG <u>I</u> CTGT <u>G</u> T <u>TGG</u>	OffT5: Exon of Pp3c6_29320
Pp3c5_16420 (<i>PpKAI2L-E</i>)	TGGACATTCGGTGGCCGGT <u>ATGG</u> (99)	TG <u>A</u> AAATTCTGTGGCCGGT <u>A</u> <u>AGA</u>	OffT1*: 5'UTR of Pp3c26_10400
		TGGACATTCGGTGGGGGGT <u>ATGG</u>	OffT2*: Exon of Pp3c16_8770
		TGG <u>C</u> ATTCTGTG <u>T</u> CTGGT <u>ATGG</u>	OffT3*: Exon of Pp3c6_10610 (PpKAI2L-D)
		TGGACA <u>C</u> TCCGGT <u>C</u> AGG <u>C</u> <u>ATGG</u>	OffT4*: Exon of Pp3c25_5350 (PpKAI2L-C)

^a: Identifier and annotation of genomic elements from Phytozome V11 - *P. patens* genome V3.3, nf: not found,

^b: Protospacer adjacent motif (PAM) sequence preceded by crRNA sequence is shown in italics and underlined. SS; Specificity score data from CRISPOR (Hsu *et al.*, 2013),

^c: Mismatch of off-target sequence with respect to the crRNA sequence is shown in red,

*Off-targets for which the activity of the sgRNA was measured by sequencing PRC fragments of the surrounding predicted site in 10 mutants.