Table S8. Selected predicted off-targets against members of the PpKAI2L gene family clade i.i-iii for the

analysis of off-target activity.

On-target gene	Predicted off-targets sequence ^{a,b}	Off-target annotation ^c
PpKAI2L-F	AGA <mark>G</mark> G <mark>CG</mark> TTTCCACACCGTC <u>CGG</u>	OffT1*: 5' UTR of Pp3c15_18460 locus
	A <mark>A</mark> ACGTATTTCCACA <mark>T</mark> C <mark>C</mark> T <mark>G</mark> <i>TGG</i>	OffT2*: Exon of Pp3c16_12790
PpKAI2L-J	ACGTT <mark>CC</mark> GAGCC <mark>C</mark> TCAGG <mark>C</mark> A <u><i>AGG</i></u>	OffT2*: 3' UTR of Pp3c15_11920 locus
	ACGTTG <mark>A</mark> GAGCC <mark>G</mark> T <mark>G</mark> A <mark>T</mark> GTA <u>GGG</u>	OffT5*: Exon of Pp3c15_4920
PpKAI2L-K	A <mark>G</mark> GTGGC <mark>C</mark> AA <mark>G</mark> TACATGGT <mark>G<i>AGG</i></mark>	OffT1*: Exon of Pp3c5_23900
	AA <mark>A</mark> TGG <mark>A</mark> GCATTACAT <mark>A</mark> GTA <u><i>TGG</i></u>	OffT2*: 3' UTR of Pp3c2_2130 locus
	AA <mark>A</mark> TGGCGAA <mark>A</mark> T <mark>C</mark> CAT <mark>T</mark> GTA <u><i>AGG</i></u>	OffT3*: Exon of Pp3c15 5520

a: 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),
b: Mismatch of off-target sequence with respect to the crRNA sequence is shown in red,
c: Identifier and annotation of genomic elements from Phytozome V11 - *P. patens* genome V3.3

^{*}Off-targets for which the activity of the sgRNA was measured by sequencing PRC fragments of the surrounding predicted site in eight mutants