Supplementary Materials:

Uvpks1, Involved in Ustilaginoidin Biosynthesis, Contributes to Metabolic Profile, Development, Stress Responses, and Pathogenicity in *Villosiclava virens*

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Construction of *Uvpks1* Gene Deletion and Complemented Strains

The Δ*Uvpks1* strain was generated through CRISPR/Cas9-mediated homologous recombination as previously described [10,20,22]. Briefly, the primers uvpks1-1F/ and uvpks1-2R were used for the amplification of the upstream through PCR, primers uvpks1-3F/ uvpks1-4R for the downstream, primers GenF/ GenR for the *G418*, then these three fragments were fused through double-joint PCR, thus obtaining the gene knock-out cassette. This cassette and the pCAS9-tRp vector contained sgRNA were transformed into the protoplasts of the wide-type strain *Villosicalva virens* (*Ustilaginoidea virens*) P1. The primers Com-uvpks1-F/ Com-uvpks1-R were used for the amplification of the CDS sequence of *uvpks1*, which subsequently cloned into PCBHT vector containing RP27 promoter and the selected marker Hyg via ClonExpress Ultra One Step Cloning Kit (Vazyme). This vector was introduced into the Δ*Uvpks1* protoplasts for complementary. The YTD medium with 700 μg/mL geneticin or 200 μg/mL hygromycin were used for screening knock-out transformants or complementary transformants, respectively.

All the right gene knock-out and complementary transformants were confirmed by PCR. For knock-out transformants, the primers uvpks1-5F/ and uvpks1-6R were used to test target gene, primers uvpks1-7F/ G855R and G856F/ uvpks1-8R were used to test *G418*. Primers Com testF/ Com testR were used to test complementary transformants. All the primers used are listed in Table S1. The strategies for *Uvpks1* knockout are shown in Figure S1. The selection of Δ*Uvpks1* knockout trans-formants by PCR is shown in Figure S2. The selection of Δ*Uvpks1*-C complementary transformants by PCR is shown in Figure S3.

Table S1. All the primers used in this study.

Primer Name	Sequence
uvpks1-1F	ttcttacggcgatgctggtg
uvpks1-2R	cagatacggcagagaaatcgcaacctcaaagcccgatggcacaacc
uvpks1-3F	ctgtttgccgacatcctgg
uvpks1-4R	gtttagattccaagtgtctactgctggcgcatctggaccattacagcaac
uvpks1-5F	tccaagaaccaggtctacatca
uvpks1-6R	ccgtcatctcgccaaact
uvpks1-7F	cgaggccatcttggacacg
uvpks1-8R	tatggcgtctgactgtaatgg
GenF	GAGGTTGCGATTTCTCTGCCGTATCTG
GenR	GCCAGCAGTAGACACTTGGAATCTAAAC
G855R	TGTTGGGTTTGAGCTAGGTGGG
G856F	GAATGGTCAAATCAAACTGCTAGATAT
sgRNA 1F	acctCACGGCAGGAGGGTCTTTAT
sgRNA 1R	aaacATAAAGACCCTCCTGCCGTG
Com-uvpks1-F	caaaaattcgagctcggtaccATGGCGAACGTGTTCCAAAT
Com-uvpks1-R	gttgagtggaatgatggatccTTAGATACCCGCGCCGGTAG
Com testF	ACCAAAACGATGCAGCGATC
Com testR	ACATGCTGAAGAACCTCGAG

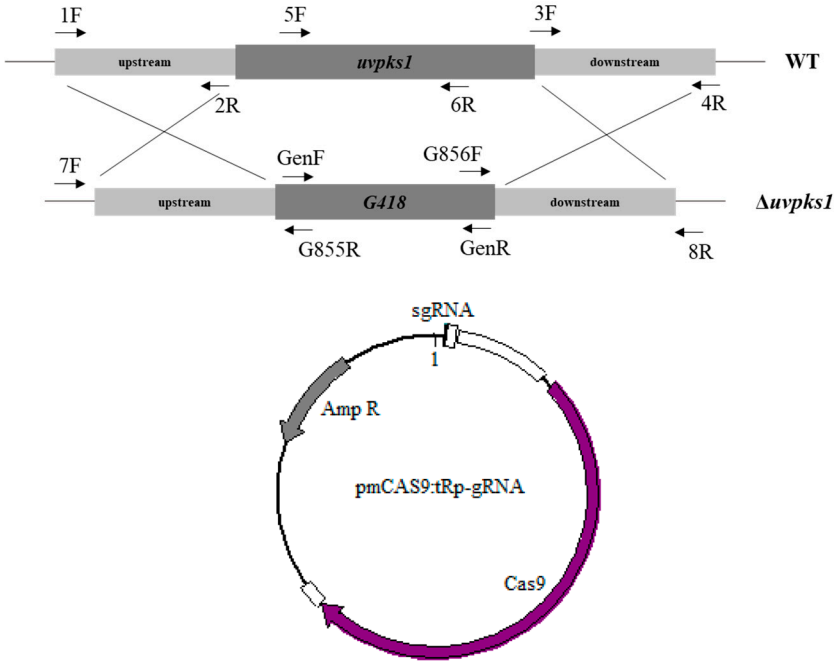


Figure S1. Strategies for *Uvpks1* knockout.

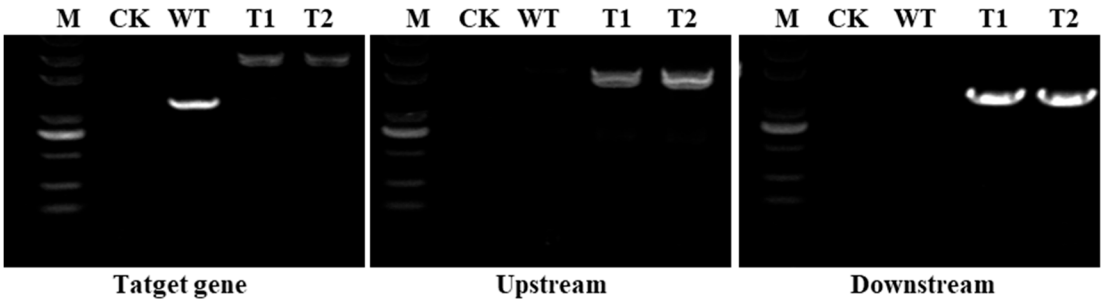


Figure S2. Selection of Δ*Uvpks1* knockout transformants by PCR.

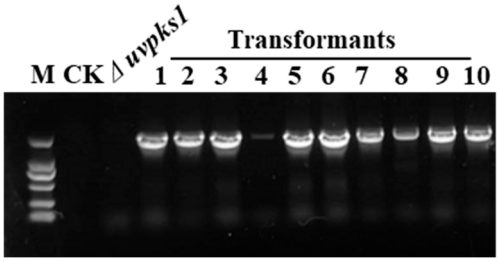


Figure S3. Selection of Δ*Uvpks1*-C complementary transformants by PCR.