

<i>U. virens</i>	.....MPSIAFSPINAQRESVR.IHRIITRE	NRSLWYQLTVLQQPERARACGSGMKANSRRFVDPFPVVELRIIEGSPVEAG	76
<i>M. oryzae</i>	.....MKASTD..HHVSGFPFVMIERVTRG	GRRLFYRIDVIQQPEKCRACGSGPKSSITRRFVDPFPVVELRIIEGSPVEAG	75
<i>M. anisopliae</i>	.....MAASSVQIFTHPSRDSVQCIRRVTRG	NRSLWYQLTVLQQPERARACGSGMKANSRRFVDPFPVVELRIIEGSPVEAG	78
<i>N. crassa</i>	MGACQVIAAASNGLCDDEPIASVKSIRTRS	GRRLWYSLRVVQEPFARACGSGPKSSACRRFVDPFPVVELRIIEGSPVEAG	82
<i>A. nidulans</i>	.....MATLAAPFPPLGSGNSNSVSRITRE	GRKITLYRLNMQPKRRARACGSGKSHITRRFVDPFPVVELNIFESDPHCHDS	78
<i>A. flavus</i>	.....MIVWTFPDASLTSD	PHSSDFELIIRQQPTRARVAGGKEK..ERRFVDPFPVQLRVREDGTLYAQ	63
<i>B. cinerea</i>	.....MAASIG.....PKSIFETITRQTKG	GRRLKYTLTVIQQPERARACGSGKSSADRRFVDPFPVQLRIYDETDPRQK	72
<i>U. virens</i>	R..DVTFDYNANFFLYASLEQARVMARGRVQNTAATNPFILTVGPVSGMAYLDRF..AEAGYIFFDLSVRHEGYRLSFSI		154
<i>M. oryzae</i>	R..DITFTYNANFFLYATLEKARPMARGRVITGFPVDAFPVLTGMPVSGMAYLDRF..IEAGYIFFDLSVRHEGYRLSFSI		153
<i>M. anisopliae</i>	R..DITFTYNANFFLYASLEQARTMARGRVQSAATNPFILTVGPVSGMAYLDRF..TEAGYIFFDLSVRHEGYRLSFSI		156
<i>N. crassa</i>	QERDVTFTYNANFFLYATLEHARVMAQGRQLQTPSANTFPVLTGMPVSGMAYLDRF..KLAGYIFFDLSVRHEGYRLSFSI		162
<i>A. nidulans</i>	NKTDITFTYNANFFLYATLEPERPIATGKLMTH..CGSPVLTGVPVAGVAYLDRF..NRAGYIFFDLSVRHEGYRLSFSI		156
<i>A. flavus</i>	H.....YLQSFYFYMCCSLFDPSSDDVPVPFSPS....TALTGTLVSSLRLKCDVNNDCGGFVFGDLSVKVVEGDFRLKFTL		135
<i>B. cinerea</i>	R..EITFTYNANFFLYATLEVARNIAQGRVQTSAPQAF..VLTGMPVSGMAYLDRF..NEAGYIFFDLSVRHEGYRLSFSI		149
<i>U. virens</i>	YEETKREKDFD..LEPAEP.....DLPPG.VDWRMEINTQFNVYSAKKFFGLMESTQLSKTVADQGCVRIRRDVR		223
<i>M. oryzae</i>	YEETKREKDFD..MEPDEFS.....SEPQGFYHMEIKSADFACFSAKKFFGLMESTQLSKTVADQGCVRIRRDVR		224
<i>M. anisopliae</i>	YEETKREKDFD..MEPANS.....DLPPG.VDWRMEINTQFNVYSAKKFFGLMESTQLSKTVADQGCVRIRRDVR		225
<i>N. crassa</i>	YEETKREKDFD..EENPAPP.....DGSPGSDFRMDIKSHDFVYSAKKFFGLMESTQLSKTVADQGCVRIRRDVR		234
<i>A. nidulans</i>	FEQIKDFDKATEGTPMPSFVPG.....KLSSPQFLEFRLEVISNFFIVYSAKKFFGLITSTPLSRMIAEQGCVRIRRDVR		234
<i>A. flavus</i>	FEMRFD.....MVTIHSIISDRFTVSPKSFPGMAESTFLSRFADQGVKLRIRKEPR		189
<i>B. cinerea</i>	YEETKREKDFD..IEPNDSSMRQMSAAAAAESSFDMRMLKSDQFTVYSAKKFFGLMESTQLSKTVADQGCVRIRRDVR		229
<i>U. virens</i>	MRKRDGKSGG...YD.....RREDDYRR..RTVPASEEPHGLHTRSLGNNNNNNNNNNNNNNDSSNGNSAQQRVFPYG...		291
<i>M. oryzae</i>	MRKRDGKSGG...FE.....RREDFRR..RTVPAPEDPHGIRNRSL.....TGDAPARN...		282
<i>M. anisopliae</i>	MRKRDGKSSA...FE.....RREDFRR..RTVPAPEDPHGIRNRSL.....SNSSEHVPFYA...		274
<i>N. crassa</i>	MRKRDGKSGGNDYE.....NGEEYRRARRATPDTAKQAYRQRSMG.....STERTFYSS...		289
<i>A. nidulans</i>	MRKRDGKRTED.YDYD.....NERGYNNRRFDQVAGSDAYANAPERFSTIS.....ISTNMDPYS...		288
<i>A. flavus</i>	TLIKRSVFRP.....EDYQPFIPFRSPDRSSIQMPG.....NTFGYVP...		227
<i>B. cinerea</i>	MRKRDGKSGG...DFG.....EKEDEYQGRATSPFFDYNIAQARQALASVHE.....DPQQRGSGEISFYHSFV		294
<i>U. virens</i>	.ADTPRRVSVDES...HHPPFFPGYN.AFPFSRSHLSFGGN...HANHATPQYAY...RQYPAPFPQTS.....T		349
<i>M. oryzae</i>	.PEPCRRPSAADYRAPFPFPFPFPFQSPQVATS.HLTFGGAPYGSHSQYQPTSSSSSSQSVSVFQSPAY...SSHAQQ		360
<i>M. anisopliae</i>	.PEPHRRPSAVDAY...HAPFPFGYDSAPFSPRSCHLSFG.....EHTAQYANP..RHYAPFPPLNGH.....V		333
<i>N. crassa</i>	.ISDPQRRPSMADY.....PPQYAAQTFPTSGG.HLGFLLG..NTHHQYPAQFPFPQS.FAQPHSVFSPVYPTVSQAFYQ		358
<i>A. nidulans</i>	..YPSRRPSAVEYQGPIAQFYQRPMASTFAESSTFIAPFIEMP...GPVALPSTPSPASAHAPAPSPVLAAPPLHTPS		364
<i>A. flavus</i>	..AAAAASRDY.....GYAGFVVRQRTSIDFG.....NRGMYDDGRMRQMEAYFQTAA.....M		276
<i>B. cinerea</i>	VNTPTPTSPISPTP...NAPLPPQNLQWIPNGPYNAAAPS...IQPPHPPFPFPSPYQSPATHHNQSP...STQFR		365
<i>U. virens</i>	HLPTTHYSS.ALSSPVIKSEHS...YFQRG..RSLSTCSPSCSPCVKRESSYEQVACTYH	REP	422
<i>M. oryzae</i>	HYGQAPQFPFTTYFERRLSDHRSSQPNHMQSPHCHSYSHRSSPQRER.EMPDSRRPSAPSHSHLS.ARPMTQCSANEIR		440
<i>M. anisopliae</i>	QLPVAAPYSSFGQATNYVKEQSNY..GYPTHT..RNPSTCSPSPVES.VKHLSYDRAPGNVYSP..SPSRYSSTSRDS		407
<i>N. crassa</i>	HQPSYFPFPFPFHQPIEQSEHHTSR.TYAPINPASRHDHSIQSTKQYTLPLLEAVSPTQPHHCHESI.APHRLPVTLPL		438
<i>A. nidulans</i>	YQSHLSFGATCTQYPAQLSHIF.QQTITFTHEYSRPSISHSRNQSISEYEPSSMGVSGQTRLSAER.PSYGQSPQTTSLP		444
<i>A. flavus</i>	YANQFGAYFTTFIMQGYETHTG....VPDYMSYGIIPSAQVQMQDPAHSRSSQATMQSLGMVNPPTPTGDSGTGM		352
<i>B. cinerea</i>	QQFQGFPPAFPIGYERRSSYSQFRFFTFNSQQQSYESDYRRMSFGYQIPASSQGGQFIAFAVQNFAYNQSQSMPTYSRNPF		447
<i>U. virens</i>	QRNYSLASLFPQFPRQTC.A.SSLAPLKIAALVS...FSPFLPIEA.....QTEFLPFPFAL		479
<i>M. oryzae</i>	RFSYNRPFPFPFDVLVADT.QATPHLPIRWR...FNMNLSFPESE.....HQEALQFLQAFPLHYESQTHQQQLGG		510
<i>M. anisopliae</i>	HQSYFPTFLLEQ.FPRRTSQ.TSLAPLKIAALVS...FSP.LPPIEA.....QTEFVFSFPIIF.....TG		462
<i>N. crassa</i>	QVDRFSSASHNQHMVMSFN.MAAPFYFRAYSVS...MSGGLTSAGG.....YNQLFPFPFPFPF.....QVAG		497
<i>A. nidulans</i>	FLRHSLEFSVNSRSTFPMNITSLPFIQSLSELP...STTSQPSAIG...SSPANFEGFRLWEIN.....SML		507
<i>A. flavus</i>	MFQGIARQQYQASSTILPFLQSRNFAQGANGAT...ARGYFDQTS.....QAATFILFSQF.....LG		408
<i>B. cinerea</i>	AYSTSFQDSVALAFLRAAEQFLAMSFLASVTSISRGTQNSAPMPSHYNKLRSGSYQYAPIEAEAF.....KST		518
<i>U. virens</i>	GRKRRH.....SVFTHISLFVYNCQRRHDETCRRIQGMTDPDPDQGLYTRADG.....KVEFVQFNQF		536
<i>M. oryzae</i>	RKRTHSYEHSYSYGSYSHNHSBGYGTFSARFPAKNNRRRPEDVEERYTVIETMVVDREPEQFPFRFLKYVWVQCRAEFPF		592
<i>M. anisopliae</i>	GRKRRH.....DVEMHG...LQNGRQQTQYGRFGLSPETLEGRYSRADG.....KVDVIQFNRY		516
<i>N. crassa</i>	SKRAHD.....QTFRADFEMRRYQDGAERESVDDKEPFLCTFYRRADG.....SVECKQADIG		552
<i>A. nidulans</i>	SKRTYEESFG.....HDDRPLYNGMRPDSSESYFGGMQRPSYERSLLDGPDMQAYKRANG.....RMVSKPATMR		573
<i>A. flavus</i>	TNEADR.....YGSAPGQAFFHFGSSNGTFR.....IDIEENTRKQEQMIYRRANG.....NIQNRKAPGL		435
<i>B. cinerea</i>	NKRSEF.....DVFSTPTESLNGRPFSAIG.IDIEENTRKQEQMIYRRANG.....NIQNRKAPGL		574
<i>U. virens</i>	SDVFA		541
<i>M. oryzae</i>	RE...		594
<i>M. anisopliae</i>	Q...		517
<i>N. crassa</i>	GY...		554
<i>A. nidulans</i>	....		573
<i>A. flavus</i>	....		435
<i>B. cinerea</i>	N...		575

Velvet domain IPR037525 (amino acid 47-358)  
Predicted NLS (amino acid 56-68)  
Predicted PEST domain (amino acid 406-420)

**Figure S1. Comparison of VeA-like proteins of *U. virens* and different fungi.**

Deduced VeA-like proteins of *Magnaporthe oryzae*, *Metarhizium anisopliae*, *Neurospora crassa*, *Aspergillus nidulans*, *Aspergillus flavus*, and *Botrytis cinerea*, similar to VeA of *U. virens* were aligned by DNASTAR using ClustalW multiple sequence alignment. The velvet domain predicted by InterProScan for the UvVEA protein is depicted in light blue according to IPR037525. The predicted NLS is shown in purple and the PEST motif in green.

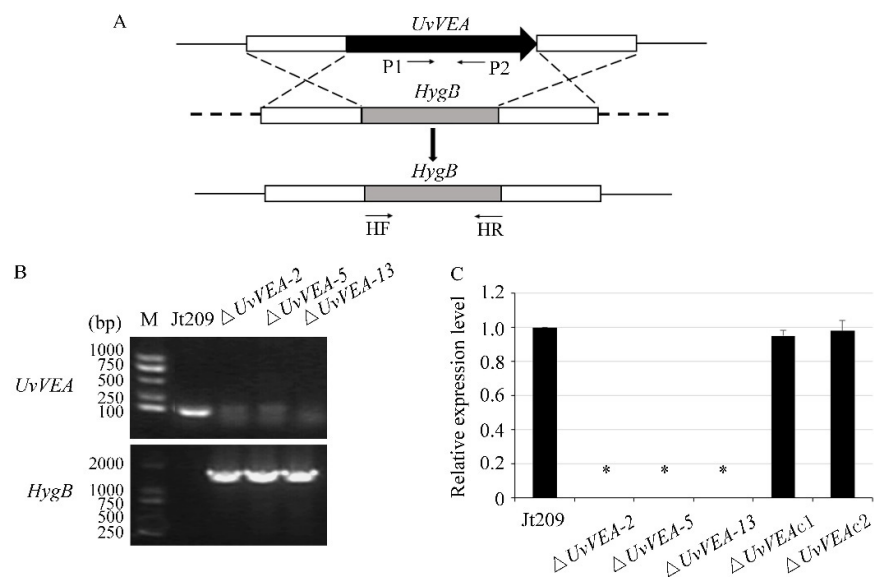
<i>U. virens</i>	.....MFPSSAHL.....	9
<i>M. oryzae</i>	.....MNPGYSTASQPGHNFSALPHDVHHAIPAIGHFGS...YHAS...MPQLP...SIPFSMPF...HYS	56
<i>M. anisopliae</i>	MGSTYPPFSDMSQQLPLPGGSYPPFGSTAASSHQTTGHSYGYSHSHHHQHQQLFPSPQRPFS.....	63
<i>N. crassa</i>	MQPYTSNLPHQLPFDHGYHHDPHATFYPPFRHHQLQQHQQLFSPMGPFSAFPFHHTNFPYQDPTMSGASASSVPFHAFPPGPGSSH	82
<i>A. nidulans</i>	.....MYAVEDRAHSG..HHFPFLSMDR.....	21
<i>A. flavus</i>	.....MYAIEERAHFP..PPFPFLSMDR.....	20
<i>B. cinerea</i>	.....MNTIAYASPPRDMHFRSTMPSLHDTTYRSGFP..HQGAFYSMPQTM.....ASQHSII...SAY	54
<i>U. virens</i>	RLDEASSDS..PGSLASANS....GKFPVG.NQAQLLAPFSKIDFVSGRQYHLD.....MCG...GDKDRR	74
<i>M. oryzae</i>	QYGMMPQDQPPAFSSSTTEGGAETKPSADAVARTQKKLQPHVG.EQDGRKYRLDVVQCFKRRAR.....MCG...FGDKDRR	128
<i>M. anisopliae</i>	QLSAHDVHHQRFPGSQSQSPFHHSHFNQFP.HQLSQHVQSHFSHFLSQHQLFSSQMPPFSRSRQDDTQFASAGGVSTFAGK	144
<i>N. crassa</i>	GDGSGHAGQLQSTFPSSHQQLHERAPLTAERMQQEQLNKPYSGTISQGRMYSLVQVQCFKRRAR.....MCG...FGDKDRR	155
<i>A. nidulans</i>	.....IPFPSTMYFSSAGFSAMVSP..AGQCFEPESLST.VHDGRINSLQVQCFKRRAR.....MCG...FGDKDRR	81
<i>A. flavus</i>	.....IPFPSSSYPTFGSGGGMVSAGIFSSSHLAPLST.VHDGRINSLQVQCFKRRAR.....MCG...FGDKDRR	82
<i>B. cinerea</i>	EQYNNLSLVSRPPFPEHLPSSSEAVSSFSIG..LPGQEPSPRSI.TVDGRKYTLVQVQCFKRRAR.....MCG...FGDKDRR	124
<i>U. virens</i>	FITPPFCVRLIITDVATGREIDCNDIDHSMFVLNVDLNSDGSKEVNLVRSSAGSF..SASTPHPYTTLNGGDTAMVPCNQ	154
<i>M. oryzae</i>	FITPPFCIRLIVIDAVTGKEIDCNEIHTMYVLNVDLNSDAQVEVNLVRHTSATPSISSTTPASFAQIDSTPPAFAQIPGT	210
<i>M. anisopliae</i>	PLGNPQQLSFPFSKIDDVIGRKYHDIDHSMFVLNVDLNNEDGTKEVNLVRSSSTGSPSISSTTPYSYTTLNGGDMGAFYTH	226
<i>N. crassa</i>	FITPPFCVRLIIRDAKTGKEIDCNEIYSMFICNVSLYTEDALKEVNLVRHTSTPSISSTTPASYSLEQTTPAYSILP	237
<i>A. nidulans</i>	FITPPFCIRLIVKDAQTQKEVDINSLDSSFYVVMADLNNADGTHEVNLVRHSATSPSISTAMSSSYFPPF.....	151
<i>A. flavus</i>	FITPPFCIRLIVKDAQTQKEIDINEIDTSFYVLMVDLNNAEGTNEVNLVRHSATSPSISTAMSSSYFPPF.....	152
<i>B. cinerea</i>	FITPPFCVKLSITEIATGKEVDVNNIEHGMFVLNVDLNSADGERFVNLVRHSQTSFSPISATVPSYTIQGGAAAYSLLP	206
<i>U. virens</i>	IASPSRSDST..YNPTHGVCYV..QEYQMQASFAQTSFYPRNGSYGFPQQ..YYPCHQ..PYQADGAMSALFSHA..NATTYGRSG	229
<i>M. oryzae</i>	...NREMPSYPSQAYAPSVTPFAQGGGQCAISFVGGPHYGMVAN..TYGGQG...GYTYASPTDMHHPQLGSGVQVYG...P	283
<i>M. anisopliae</i>	VLPFSNRDQS..YNTFPTVGYG..QDYQIQGGYGQAFSPYPSNGSYGFPQQ..YFEHHQ...SYRPEGNVTELPSSQS...NVSEYTRNG	301
<i>N. crassa</i>	...NRDVG..YGHQGMQS....YQGNPGMNPFYDMQTSFYHAGFVNGNPFYGAFAFHYQYQPLFPQPGFYGAFRGFYDH..P	308
<i>A. nidulans</i>	...HFTSSDYP..ASYQT..NFGYQFVGG..P..VGQFVGYAGVGN..YGGST..QLQYQYAY..PNPQAQ..YYQPMY....	212
<i>A. flavus</i>	...QTLSEFTY...AQYPPQ..NAY.....GQPVVPYQMNSY..YFQGNF..QLQYQYFYGASPQTS..YYQPYVP..TG	208
<i>B. cinerea</i>	QSQREFTSPTYGSAPFPQG...AGFSFPFGP..PQVSAYSQQQPGQQSG..YGGNF..NYPFPNGYQVAFQQSNYYYYPQFSQSI	281
<i>U. virens</i>	SSGSTGYVQHNSLGRMSMVGGQ.....PQGMFTRNLIGSLAASAFRLSDTELRIGIWFVLQDLSVRTEGFFRLRFSEV	303
<i>M. oryzae</i>	RIENGAP..DMG...MHQRMALQGT...HQAAPPQGMFTRNLIGSLASAFRLNDPQEKIGIWFVLQDLSVRTEGIFRLRFSEV	359
<i>M. anisopliae</i>	STGFTGYVQDHNFMARMAMVGGQ.....PQGMFTRNLIGSLAASAFRLSDTELRIGIWFVLQDLSVRTEGFFRLRFSEV	375
<i>N. crassa</i>	TQYGMHGGVGGFLQHRMSVSSSTSG..QGQGPQGMFTRNLIGSLVCSAFRLTDTNDRIGIWFVMQDLSVRTEGVFRLQFSEV	389
<i>A. nidulans</i>	GGMAQFQMPAAQFVTPG.....PQGMFTRNLIGSLASAFRLYDTELRIGIWFVLQDLSVRTEGIFRLRFSEV	280
<i>A. flavus</i>	GHMFPQANISPAQFVSTG.....PQGMFTRNLIGSLASAFRLTDPDNKIGVWFILQDLSVRTEGVFRLRFSEV	276
<i>B. cinerea</i>	PSHNNODEYFSRFFTPODLGIGRIPISTNFPQGMFTRNLIGSLASAFRLTDPDDRIGIWFVLQDLSVRTEGDFRLRFSEV	363
<i>U. virens</i>	NVGQPD...GAPRDGSAPKVNKSRAPIACCFSEIFNVYSAKKFFGVCESTPLSKTFATQGIKIPIRK...ANVK.....VG	375
<i>M. oryzae</i>	NVGAPTRTPNGGFANQTSILNTGKAFILASCFSDAFQVYSAKKFFGVCESTPLSKCFAGQGIKIPIRK...EGGG.....KNND	436
<i>M. anisopliae</i>	NVGPPD...GTPRDSGAFKINKNRAPILACCFSEIFNVYSAKKFFGVCESTPLSKTFATQGIKIPIRK...SNVK.....GG	447
<i>N. crassa</i>	NVGLPTLPSSGSSGSGTGINGQS..KAPILASVSEFPQVFSAKKFFGVCESTPLSKCFATQGIKIPIRK...GANS.....KNE	465
<i>A. nidulans</i>	NVGKSV...SDLQSDIAEVINKGTAPILASTFSEFPQVFSAKKFFGVCESTPLSKVFANQGIKIPIRK...GVKGGSGRGRHSD	360
<i>A. flavus</i>	NVGTQS...SDSPNGGVS..VINHGSAPVLASVSEFPQVFSAKKFFGVCESTPLSKCFALQGIKIPIRK...GVKKG...SRGRNND	353
<i>B. cinerea</i>	NVGVES...AFONNANSSCSVNTGKAFVLASCFSEVFKVYSAKKFFGVCESTPLSKCFATQGIKIPIRK...GKDGFGRGKGDGS	443
<i>U. virens</i>	DDDDYGD..	382
<i>M. oryzae</i>	DDDDY...	441
<i>M. anisopliae</i>	DDDDYGD..	454
<i>N. crassa</i>	DDDDY...	470
<i>A. nidulans</i>	EDDGLDNE	368
<i>A. flavus</i>	DDGDDYD	361
<i>B. cinerea</i>	RGDDDDY	451

Velvet domain IPR037525 (amino acid 47-358)

Predicted NLS (amino acid 56-68)

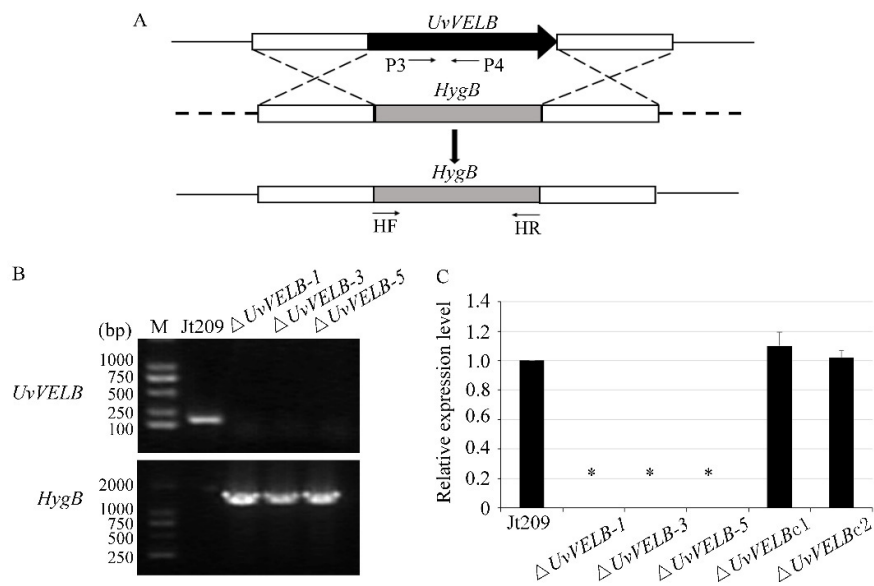
**Figure S2. Comparison of Velb-like proteins of *U. virens* and different fungi.**

Deduced VeA-like proteins of *Magnaporthe oryzae*, *Metarhizium anisopliae*, *Neurospora crassa*, *Aspergillus nidulans*, *Aspergillus flavus*, and *Botrytis cinerea*, similar to Velb of *U. virens* were aligned by DNASTAR using ClustalW multiple sequence alignment. The velvet domain predicted by InterProScan for the UvVELB protein is depicted in light blue according to IPR037525. The predicted NLS is shown in purple.



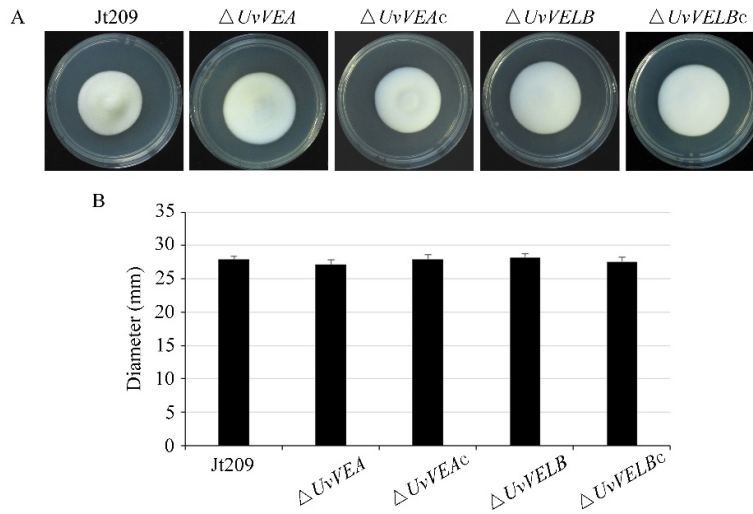
**Figure S3. Deletion of the *UvVEA* gene in *U. virens*.**

(A) Illustration of targeted deletion of *UvVEA* utilizing CRISPR/Cas9 and homologous replacement. (B) PCR analysis of *UvVEA* deletion mutants  $\Delta UvVEA-2$ ,  $\Delta UvVEA-5$ , and  $\Delta UvVEA-13$ . The wild-type strain Jt209 was included as control. (C) Results of qRT-PCR to validate the expression of *UvVEA* gene in deletion mutants ( $\Delta UvVEA-2$ ,  $\Delta UvVEA-5$ , and  $\Delta UvVEA-13$ ), the wild-type strain Jt209 and the complemented transformants.



**Figure S4. Deletion of the *UvVELB* gene in *U. vires*.**

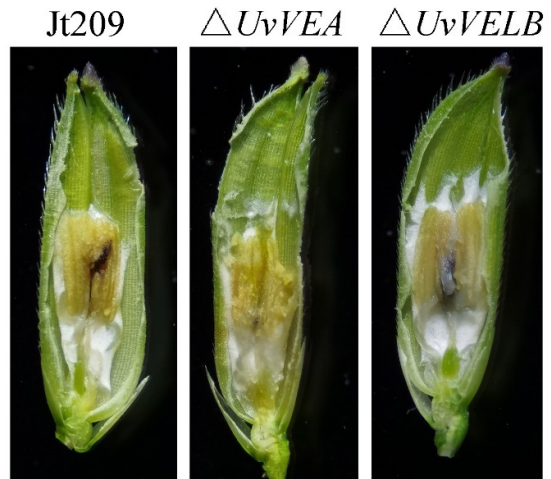
(A) Illustration of targeted deletion of *UvVELB* utilizing CRISPR/Cas9 and homologous replacement. (B) PCR analysis of *UvVELB* deletion mutants  $\Delta UvVELB-1$ ,  $\Delta UvVELB-3$ , and  $\Delta UvVELB-5$ . The wild-type strain Jt209 was included as control. (C) Results of qRT-PCR to validate the expression of *UvVELB* gene in deletion mutants ( $\Delta UvVELB-1$ ,  $\Delta UvVELB-3$ , and  $\Delta UvVELB-5$ ), the wild-type strain Jt209 and the complemented transformants.



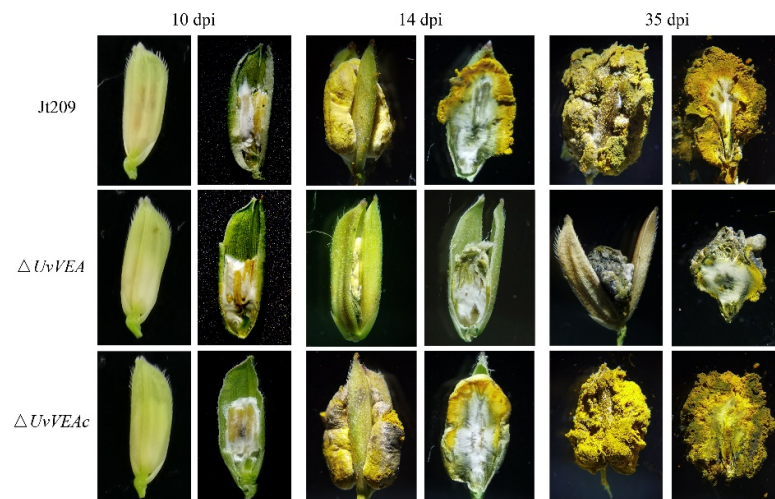
**Figure S5. Hyphal growth of the *UvVEA* or *UvVELB* gene deletion mutants.**

(A) Comparison of colony morphology and mycelial growth between the wild type (Jt209) and mutant lines. Colonies from the WT and mutant lines were incubated on YT media for 12 days at 28°C. (B) Colony diameter of the WT and mutants on YT plates.



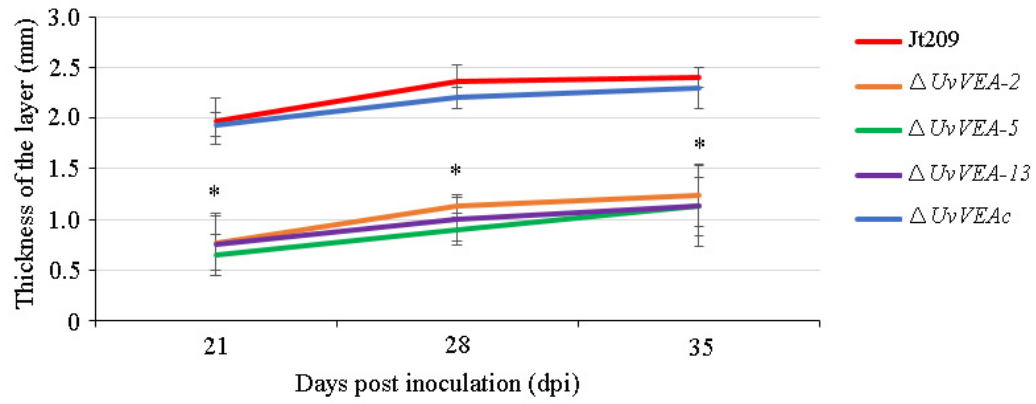


**Figure S6. Mycelial extension of the wide-type strain, the  $\Delta UvVEA$  and  $\Delta UvVELB$  mutants inside the spikelets at 10 dpi.**



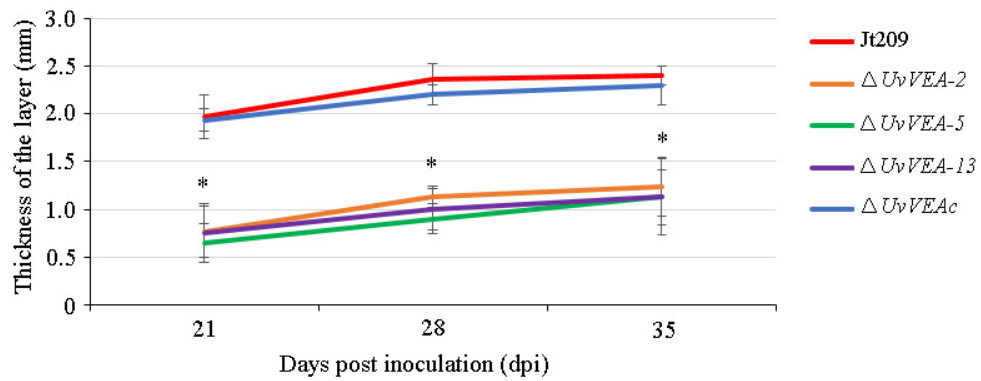
**Figure S7. Process of infection in inoculated rice spikelets.**

Infection progress of rice spikelets by the wide-type strain and  $\Delta UvVEA$  at 10, 14 and 35 dpi, respectively.



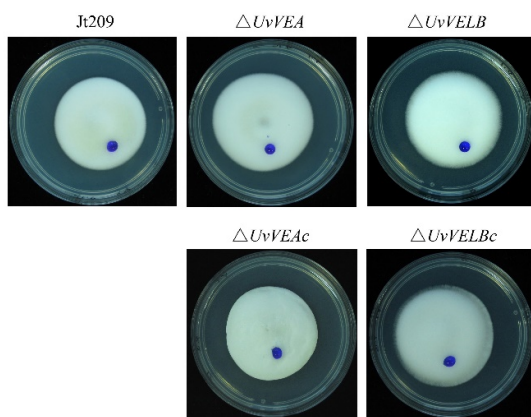
**Figure S8.** The chlamydospore layer was thinner for false smut balls in the mutants than in Jt209.





**Figure S8. The thickness of the yellow hypha and chlamydospore layer of rice false smut balls.**

The rice false smut balls were cut from the middle with a scalpel. The thickness of the yellow hypha and chlamydospore layer of balls were measured with a ruler at its longitudinal section, at 21, 28 and 35 dpi, respectively. Line bars in each column denote standard deviation of three experiments. Asterisks represent significant differences relative to Jt209 (one-way ANOVA,  $\alpha=0.05$ )



**Figure S9. Role of velvet complex proteins in colony hydrophobicity.**

The indicated strains were grown in YT plates for 12 days at 28°C in the dark. Twenty  $\mu$ l of water stained with bromophenol blue were applied on the colony surface and images were taken after 2 hours, incubation at room temperature. During this time, the drop remains intact on hydrophobic colonies.