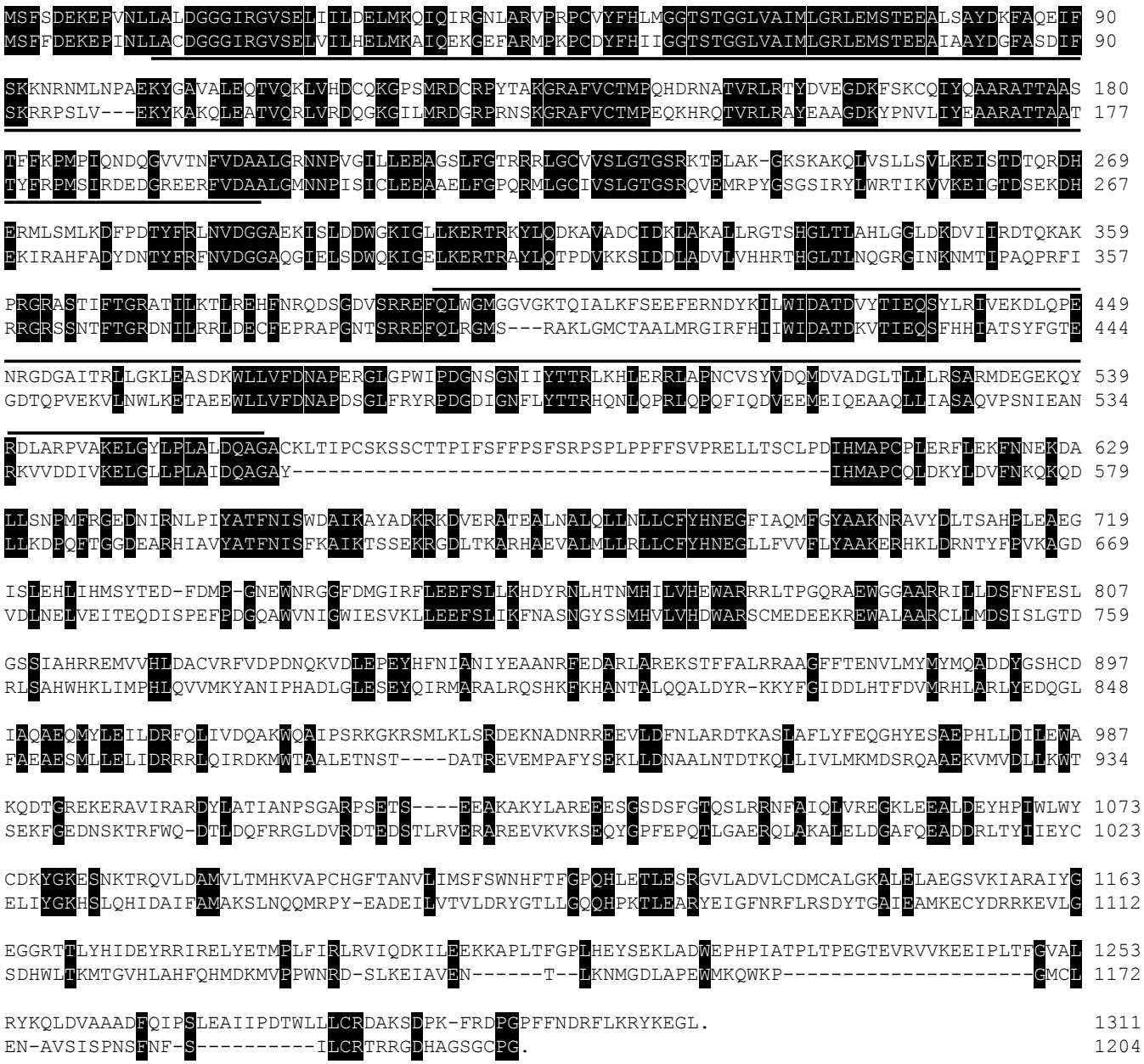

Figure S1 Amino acid alignment of candidate *vic2* alleles in strain EP155 (*vic2-2*) and in strain EP146 (*vic2-1*) performed using MegAlign in Lasergene (DNASTAR Inc. Madison WI). Note the high level of polymorphism that spans nearly all of the patatin-like protein (39% identity). A region in the N-terminal portion of the ORF (aa 13-212 in the EP155 sequence) containing a patatin-like phospholipase domain (PLA2; EC3.1.1.4) consisting of the esterase box GTSTG and anion binding element DGGGXRG (Scherer et al., 2010 Patatin-related phopholipase A: nomenclature, subfamilies and functions in plants. *Trends in Plant Science* **15**:693-700), and conserved in both the *vic2-1* and *vic2-2* alleles, is underlined. An NB-ARC, or P-loop NTPase domain, detected in the EP155 genome sequence but not in the EP146 sequence, is indicated by an overline extending from aa 396-561. Amino acid identity is indicated by the solid background, while dashes indicate deletion events.

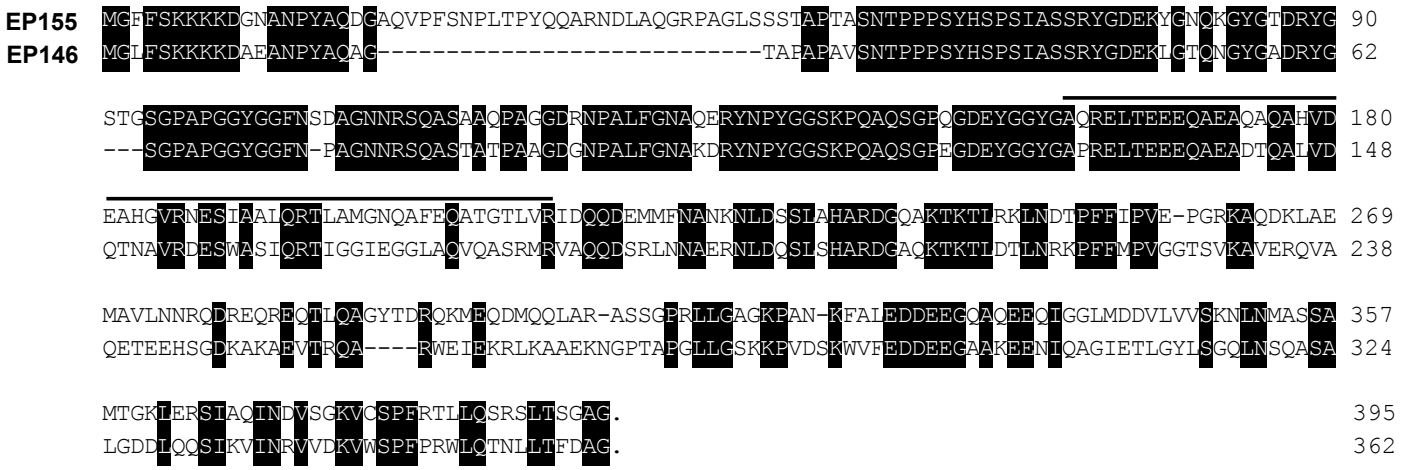

Figure S2 Amino acid alignment of the Sec9-like protein candidate *vic2a* alleles in strain EP155 (*vic2a-2*) and EP146 (*vic2a-1*). Note the large indel near the N-terminus and the high level of polymorphism in the C-terminal half of the coding region. A Pfam DUF3359 domain, found only in the EP155 allele, is indicated by a line above the EP155 sequence. Amino acid identity is indicated by the solid background, while dashes indicate indels.

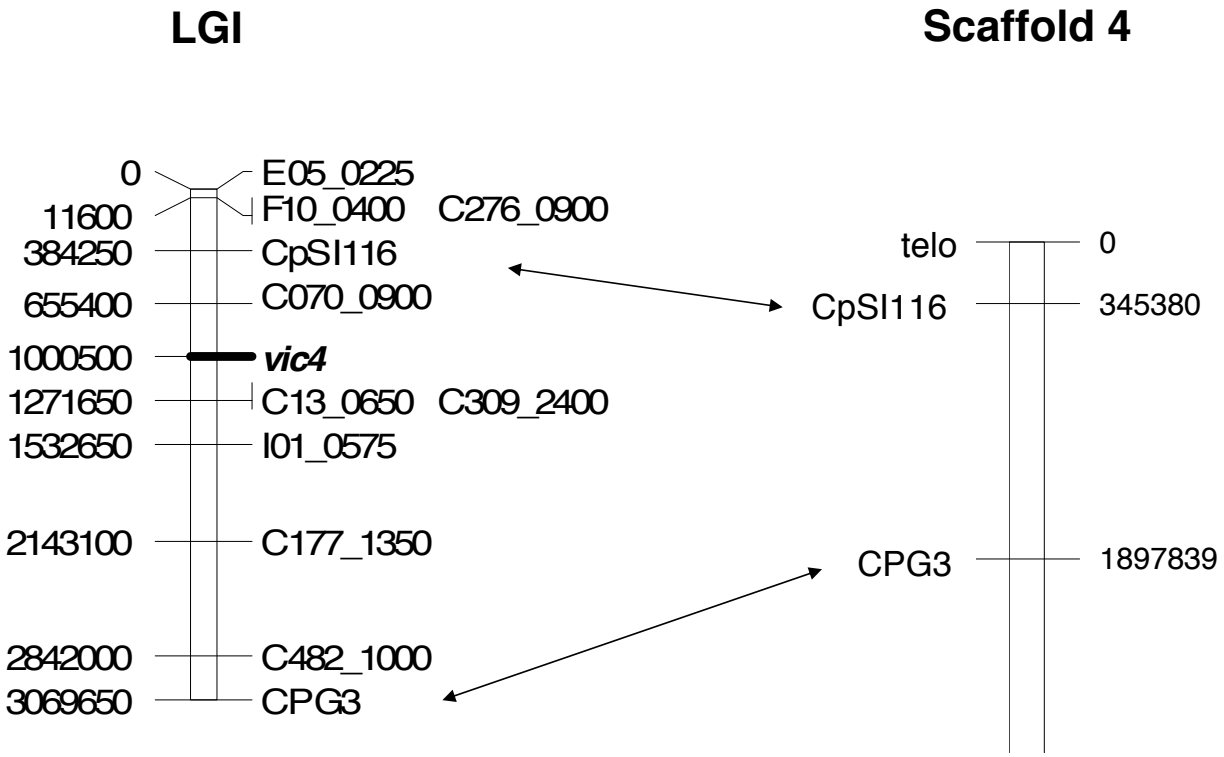

Figure S3 Alignment of linkage group LGI of the *C. parasitica* genetic linkage map generated by Kubisiak TL, Milgroom MG (2006 *Fungal Genetics and Biology* **43**: 453-463) that contains the position of the *vic4* genetic locus with Scaffold 4 of version 2 of the *C. parasitica* genome sequence. The estimated physical distance (bp) of each marker from the end of the linkage group (shown on the left side of the linkage map) was calculated using the estimate of 1cM = 14.5 kbp (Kubisiak and Milgroom, 2006 *Fungal Genetics and Biology* **43**: 453-463). Markers linking the physical and genetic maps are connected by double arrows.

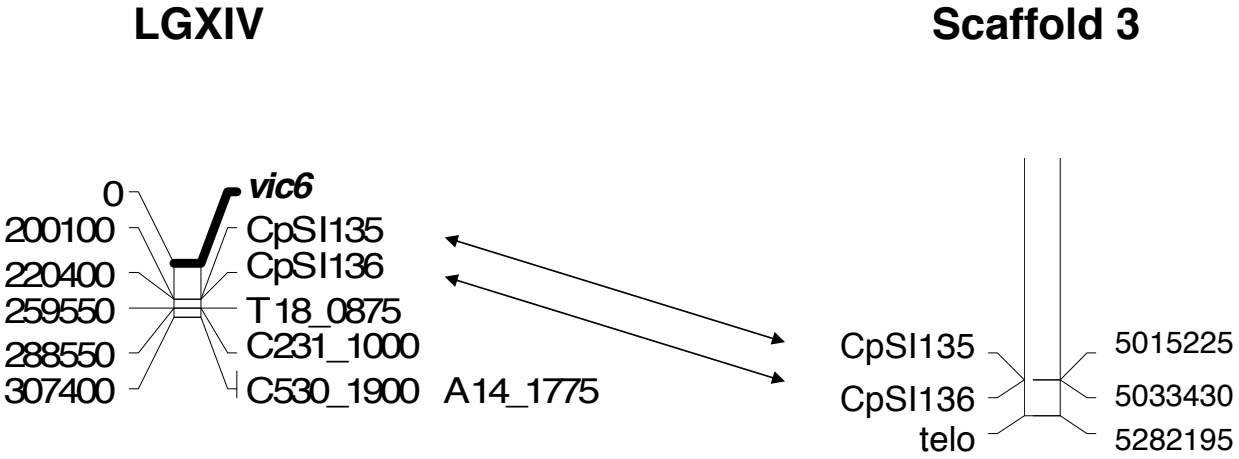

Figure S4 Alignment of linkage group LGXIV of the *C. parasitica* genetic linkage map generated by Kubisiak and Milgroom (2006 *Fungal Genetics and Biology* **43**: 453-463) that contains the position of the *vic6* genetic locus with Scaffold 3 of version 2 of the *C. parasitica* genome sequence. The estimated physical distance (bp) of each marker from the end of the linkage group (shown on the left side of the linkage map) was calculated using the estimate of 1cM = 14.5 kbp (Kubisiak and Milgroom, 2006 *Fungal Genetics and Biology* **43**: 453-463). Markers linking the physical and genetic maps are connected by double arrows.

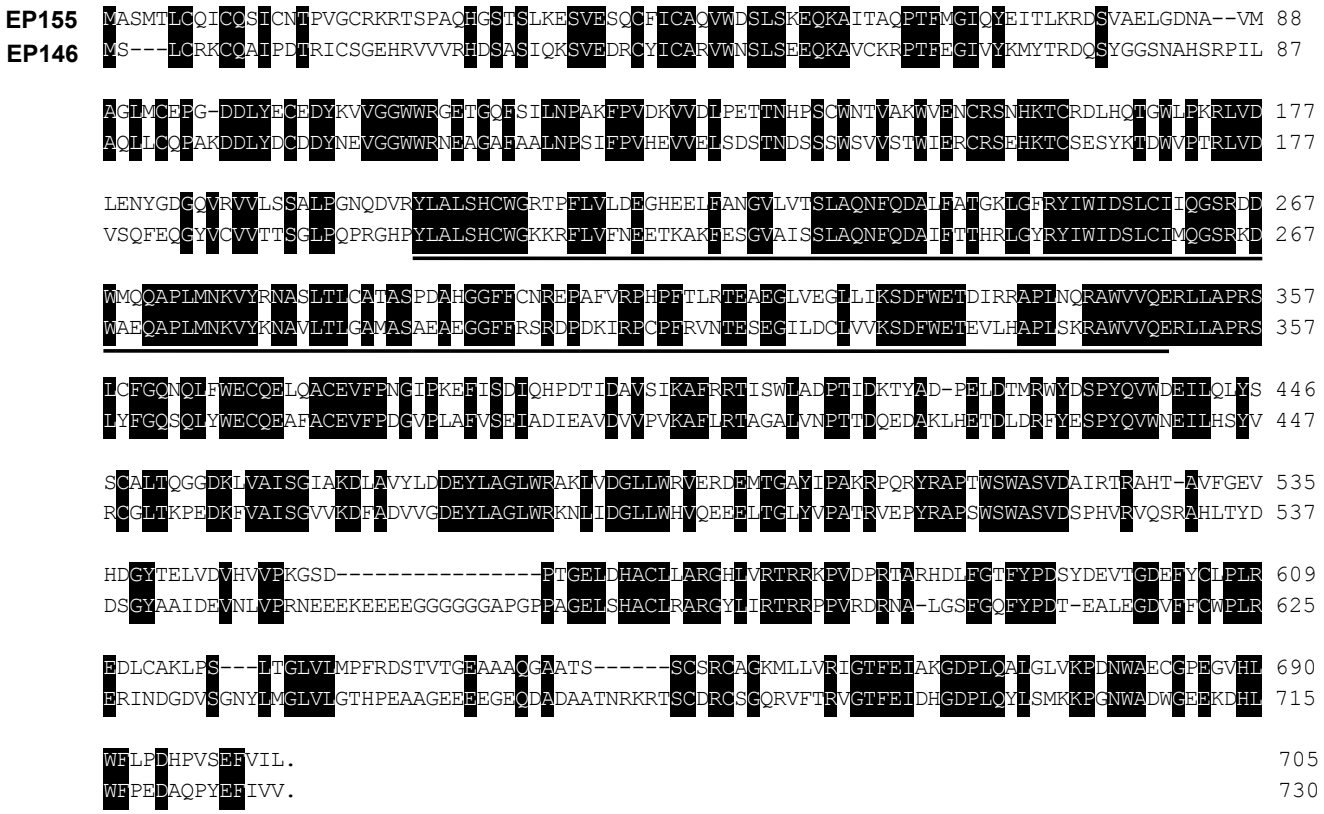

Figure S5 Amino acid alignment of *vic6* alleles in strain EP155 (*vic6-2*) and in strain EP146 (*vic6-1*). Note the high level of polymorphism that spans nearly all of the predicted protein (53% identity). The conserved HET domain found in this candidate *vic* gene is underlined. Amino acid identity is indicated by the solid background, while dashes indicate indels.

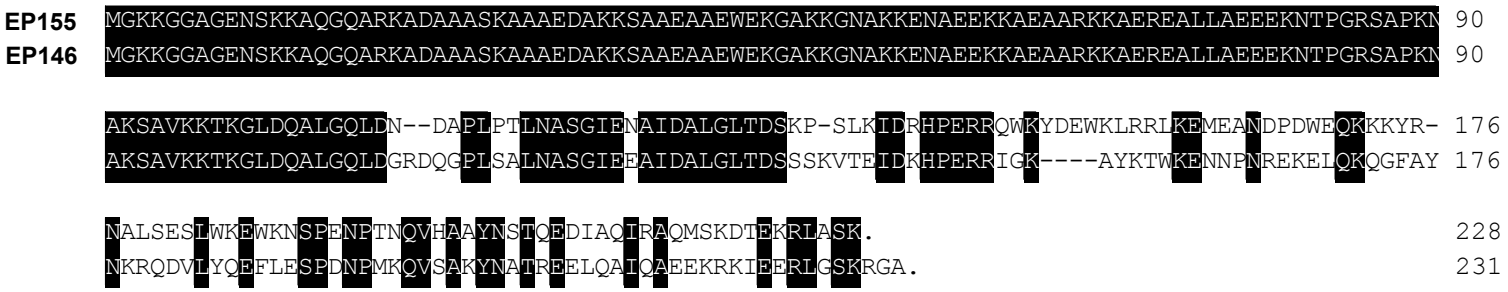

Figure S6 Amino acid alignment of alleles *pix6-1* and *pix6-2* in strains EP146 and EP155, respectively. Note the highly conserved N-terminal region and highly polymorphic C-terminal region. Amino acid identity is indicated by the solid background, while dashes indicate indels.

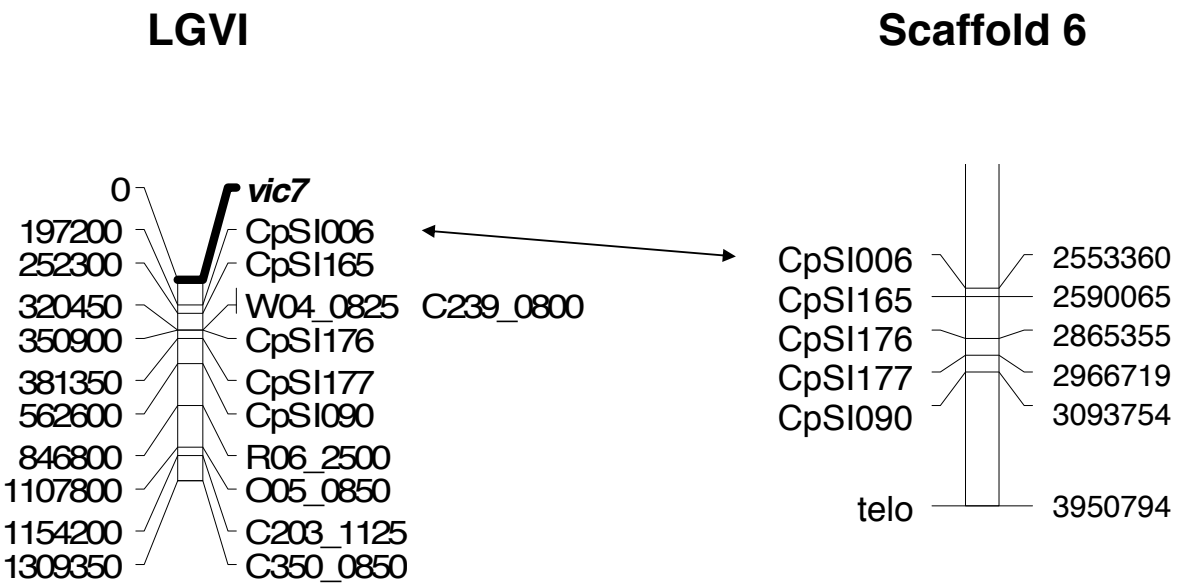

Figure S7 Alignment of linkage group LGVI of the *C. parasitica* genetic linkage map generated by Kubisiak and Milgroom (2006 *Fungal Genetics and Biology* **43**: 453-463) that contains the position of the *vic7* genetic locus with Scaffold 6 of version 2 of the *C. parasitica* genome sequence. The estimated physical distance (bp) of each marker from the end of the linkage group (shown on the left side of the linkage map) was calculated using the estimate of 1cM = 14.5 kbp (Kubisiak and Milgroom, 2006 *Fungal Genetics and Biology* **43**: 453-463)). Markers linking the physical and genetic maps are connected by double arrows.

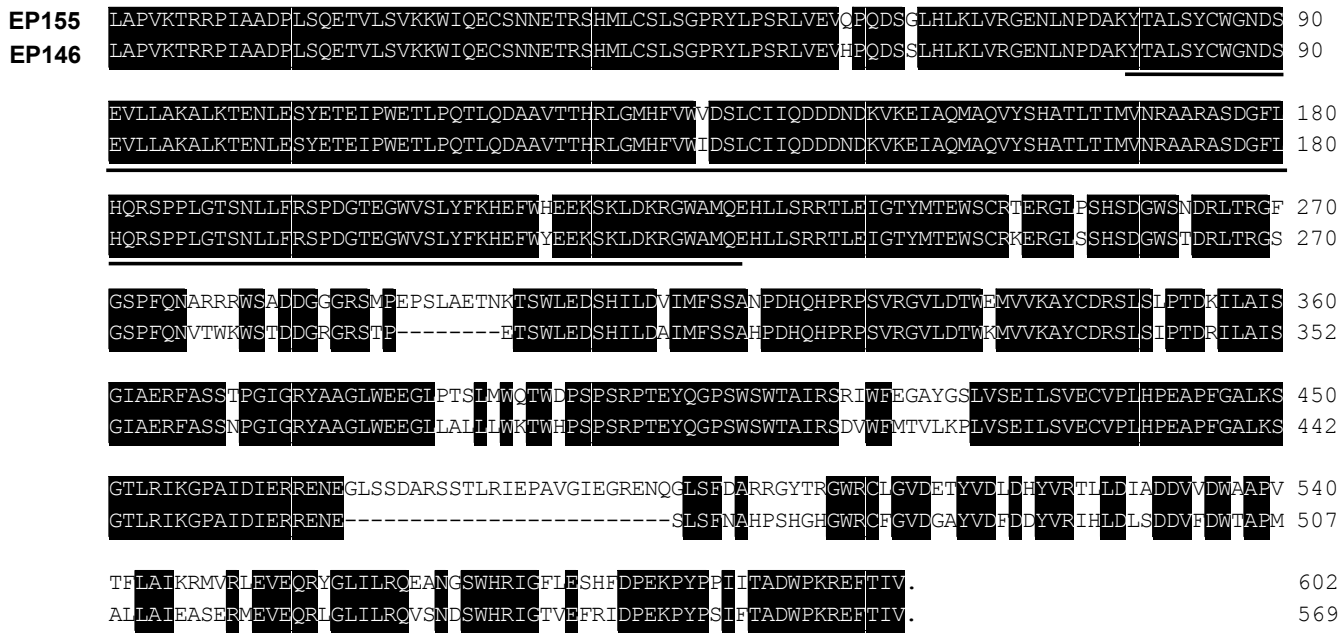

Figure S8 Amino acid alignment of candidate *vic7* alleles in strain EP155 (*vic7-2*) and in strain EP146 (*vic7-1*). The alignment was performed using MegAlign in Lasergene (DNASTAR Inc., Madison WI) with manually annotated amino acid sequences. Note that, in contrast to *vic2, vic4,* and *vic6*, the *vic7* alleles are quite similar to each other, except in the C-terminal region. The conserved HET domain found in this candidate *vic* gene is underlined.

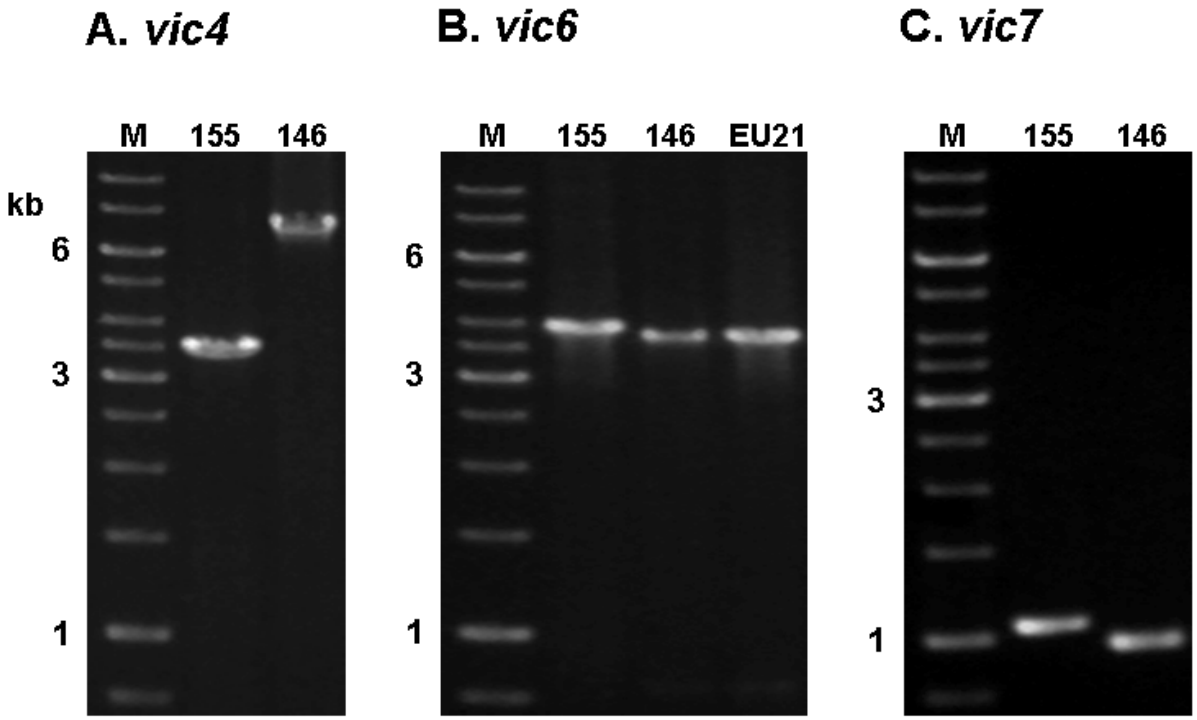

Figure S9 Agarose gel migration of candidate *vic* allele-specific PCR fragments. The relative migration positions of PCR products for *vic4-2* (3,456 bp from strain EP155 DNA) and *vic4-1* (6,884 bp strain EP146 DNA) are shown in Panel A. The candidate *vic6* allele-specific PCR product *vic6-1* (3,780 from strain EP146 and *vic* tester strain EU-21 DNAs) migrates slightly faster than the *vic6-2* PCR product (3,948 bp from EP155 DNA) as indicated in Panel B. Confirmatory nucleotide sequence analysis of PCR fragments was performed when differentiation based on relative migration was in doubt. The migration positions for candidate *vic7* allele-specific PCR products for *vic7-2* (1,053 bp from EP155 DNA) and *vic7-1* (954 bp from EP146 DNA) are shown in Panel C. Nucleotide sequence differences were also used to distinguish the candidate *vic2-1* and *vic2-2* alleles and the adjacent sec9-like gene alleles *vic2-1a* and *vic2-2a*. The lanes marked M contained the 1 Kbp DNA ladder size markers (Fermentas, Glen Burnie, MD).

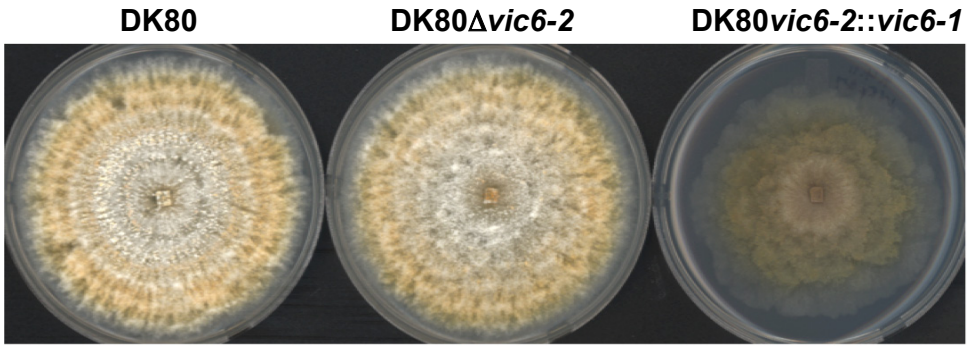

Figure S10 Colony morphology for disruption strain DK80 *Δvic6-2* (middle panel) and replacement strain DK80 *vic6-2*::*vic6-1* (right panel) in which the disrupted *vic6-2* allele was replaced with an intact *vic6-1* gene. The *vic6-1* replacement strain DK80 *vic6-2*::*vic6-1* exhibited abnormal colony morphology, irregular margins, reduced aerial hyphae, reduced biomass production and reduced conidiation. All candidate *vic* gene disruption mutants examined in this study showed normal colony morphology.

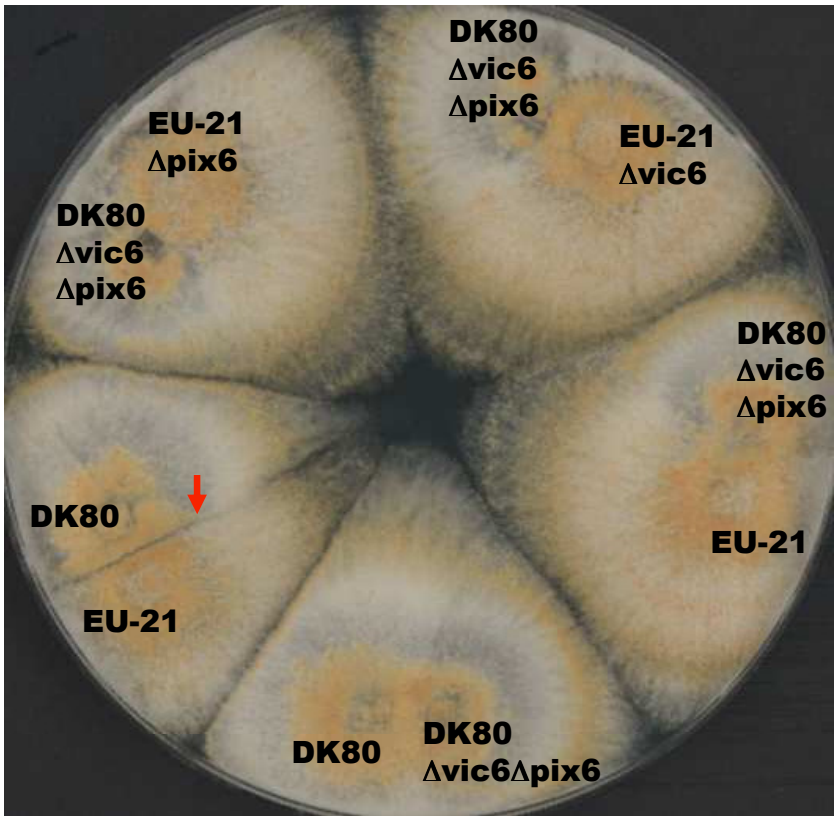

Figure S11 Mycelial incompatibility assay for strain DK80 disrupted in *pix6-2* and *vic6-2*. Barrage formation (arrow) resulting from an incompatible reaction between strains DK80 and EU-21 that differ only at the *vic6* locus is shown in at the lower left. While barrage formation is retained when only *vic6* or *pix6* alleles are independently disrupted (Table 5), the double disruption mutant DK80 *Δpix6-2 Δvic6-2* is compatible with strain EU-21 with no evidence of barrage formation (middle right) and no restriction to virus transmission in either directions (Table 5).

Table S1 Single Sequence Repeat (SSR) linkage markers used in this study

Linkage Marker	*vic*	Forward primer	Reverse primer	Scaffold map positions*
CpSI002	*vic2*	TTGGATAGACCCAGGTGTCC	GAGGTCTTCGAGGGCGTAG	7:1522713-1523193
Co16_1800	*vic2*	TGGCGGGATATGAAATAT	TGTTGGAGCGCCTTGCGGA	7:1762655-1763779
CpSI116	*vic4*	TGTCAAAGTTGACCACCACC	ATCAGCGTGTCCATACCACA	4:345343-345591
CPG3	*vic4*	CGTAAGGCAGAGGCAGAGAC	TCCCTATGCCCAAGACACTC	4:1897980-1898174
CpSI135	*vic6*	TACTCTTCGTGTCCCTTCGG	GGCAGAACAGTGACCGAAAT	3:5015099-5015384
CpSI136	*vic6*	AAGCTGTACAGTCAACGCGA	ACCTGGAATGGAGACACAGG	3:5033364-5033641
CpSI006	*vic7*	ATGTCGAGTTTACCCGATGG	GAGATGTGTGGAATGCAACG	6:2553319-2553459

* The map positions define the fragment containing the SSR region that would be amplified from *C. parasitica* genomic DNA. (KUBISIAK T. L., C. DUTECH and M. G. MILGROOM, 2007 Fifty-three polymorphic microsatellite loci in the chestnut blight fungus, *Cryphonectria parasitica*. Mol. Ecol. Notes **7**: 428-432)

Table S2 Oligonucleotide primers used in PCR reactions*

Gene	Primer	Nucleotide sequence
vic2	ptnF1	5′-TGCGGCACCTGCATGTACATA
	ptnR1	5′-CGTCATACAGGCGAACTGGAT
vic2a	sec9F1	5′-TACTCCTTCCCAAGCTCCCG
	sec9R1	5′-GCTCAACGTATGTGGTTCAGCAT
vic4	vic4F1	5′-CCATGCATGTGAGGCTTCTCA
	vic4R1	5′-CTTGATCGTGGAGTTCAGTCG
vic6	vic6F1	5′-GACCAGGCTCTTGGGCAGCT
	vic6R1	5′-CGAGACCCTTTGTTTCTAAGGTCT
pix6	vic6upF1	5′-GTGCAGGTCCAGCTGACTTG
	vic6up155R1	5′-TGTACAGCGTGGCCACTGAC
	vic5up146R1	5′-AGGCCTTTGAGGATGGGGTT
vic7	vic7F1	5′-CGTACACTTGAGATTGGGACTTA
	vic7R1	5′-ATAGGGCTTCTCGGGATCGA

*A Phire Plant Direct PCR kit (F-130) (New England BioLabs, Ipswich, MA) was employed in a 50 μl reaction volume with following parameters: denaturation at 98^0 C for 2 min followed by 30 cycles consisting of denaturation at 98^0 C for 5 sec, annealing at 64^0 C for 5 sec, extension at 72^0 C for 2.5 min, and then final extension at 72^0 C for additional one minute. The resulting PCR products were sequenced after purification with a QIAquick PCR purification kit (Qiagen, Valencia, CA).