

Table S1 Oligonucleotide primers/adaptor used for cDNA cloning and reverse transcription (RT)-PCR detection of different mycoviruses and dsRNAs in this study

Primer Name	Sequence (5'→ 3')	Position ¹	Polarity ²
Full cDNA cloning			
HV-R (BcHV1)	CCAGGCAGGAAACAAGC	1680-1697	-
HV0-R (BcHV1)	CCCAATAGCGAAACCAAC	2723-2741	-
HV1-R (BcHV1)	TCAAAGGATGGTGCAAGG	4521-4539	-
HV2-F (BcHV1)	ACGCTGTCCAACGTATAAGAA	5139-5159	+
HV2-R (BcHV1)	CACCAGATTGAGCCCTTT	7950-7968	-
HV3-F (BcHV1)	TTAGCAAGGGAAGGAACT	8412-8430	+
HV4-F (BcHV1)	CCTGGAGGCTACGAAGAT	8966-8984	+
FV1-R (BcFV1)	GCAGGAGTTGCGATAAGG	1210-1228	-
FV2-F (BcFV1)	ACCTCAAGTGAAGAAAGC	5653-5672	+
FV3-F (BcFV1)	ACTGTGGAGGAAGAAGAGC	6655-6674	+
CV1-R (dsRNA-C)	GGCAGAGGAGGGTTGATG	1369-1386	-
CV2-F (dsRNA-C)	CGTTGAGGCTTCTACCA	2886-2903	+
DV1-R(dsRNA-D)	GCGGACCACGTCATTCAA	1904-1921	-
DV2-F(dsRNA-D)	ACTCACCGTTGCCCTCAA	2798-2815	+
EV1-R(dsRNA-E)	AACATTGAAGCGACTGGA	1927-1946	-
EV2-F(dsRNA-E)	AACCCAGAGGCATACGAG	2782-2799	+
110A (3'-adaptor)	TATCTTATCGGCGTGTCCCC	to 3'-end of dsRNA	+/-
RC110A (primer)	GGGGGACACGCCGATAAGATA	complementary to the adaptor 110A	-/+
RT-PCR detection of different mycoviruses and dsRNAs			
H-RT-F (BcHV1)	ACCGATTGGTTGAAGTGA	5514-5532	+
H-RT-R (BcHV1)	TTGCCATAGAAAGGTAGTAGTT	6212-6234	-
F-RT-F (BcFV1)	AGCAAGATGGCAAGAAAA	2586-2604	+

F-RT-R (BcFV1)	ACGTGCAGCACTAGCAGA	3318-3336	-
C-RT-F (dsRNA-C)	TTCGGGTGGCGATGT	1024-1038	+
C-RT-R (dsRNA-C)	TTGGCACTAAGGAAGTTATGT	1728-1748	-
RT-F-F (dsRNA-F)	CCTTATTGCAAGGCCAGCATGTCA	136-159	+
RT-F-R (dsRNA-F)	TGAACCTTCTCGAGCATTGCTCAT	783-807	-

to probe BcHV1 and BcFV1

Probe 1-F (BcHV1)	ACCGATTGGTTGAAGTGA	5514-5532	+
Probe 1-R (BcHV1)	TTGCCATAGAAAGGTAGTAGTT	6212-6234	-
Probe 2-F (BcFV1)	AGCAAGATGGCAAGAAAA	2586-2604	+
Probe 2-R (BcFV1)	ACGTGCAGCACTAGCAGA	3318-3336	-

RAPD primer

OPC-04	CCGCATCTAC
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¹ Positions for the PCR primers or the adaptor in the cDNA of BcHV1 and BcFV1 were labeled in

Fig. S1.

² Polarity refers to the positive strand (+) and the negative strand (-) of BcHV1 and BcFV1.

Table S2 The presence of *Botrytis cinerea* hypovirus 1 (BcHV1) and *Botrytis cinerea* fusarivirus 1 (BcFV1) in the population of *Botrytis cinerea* isolated from Hubei, Shandong, Hunan, Jiangxi and Jilin Province, China.

Location	Number of Strains (host, presence of mycoviruses)		
	Total	BcHV1+ ¹	BcFV1+
Wuhan, Hubei	5	1	0
Yichang, Hubei	7	1	1 ²
Suizhou, Hubei	5	0	0
Xiantao, Hubei	7	0	0
Qianjiang, Hubei	6	0	0
Xiaogan, Hubei	8	2	1
Xiangyang, Hubei	6	3	0
Jingmen, Hubei	5	3	3
Huangshi, Hubei	4	1	0
Shiyan, Hubei	3	1	0
Jingzhou, Hubei	7	1	0
Huanggang, Hubei	6	3	0
Enshi, Hubei	3	0	0
Xianning, Hubei	6	0	0
Ezhou, Hubei	6	0	0
Weifang, Shandong	7	7	5
Zibo, Shandong	1	1	1

Changsha, Hunan	2	1	0
Jiujiang, Jiangxi	1	1	0
Changchun, Jilin	2	2	2
Siping, Jilin	1	1	1
Total	98	29	14

¹ “+” represents the presence of BcHV1 or BcFV1 through the detection of RT-PCR with primer pairs listed in Table S1.

² The strain of *B. cinerea* infected by BcFV1 is not the same strain infected by BcHV1 in Yichang, Hubei.

Table S3 Oligonucleotide primers/adaptor used for quantitative reverse transcription (qRT)-PCR detection of infection formation related gene in *Botrytis cinerea* strains

Gene	Primer	Sequence (5'→ 3')	Accession no.	References ¹
Name				
Bciqg1	Bciqg1-F	TTGCCCGGAACCTTGTCA	XM_001555933	(Marschall and
	Bciqg1-R	GCAAGAACCTTGGCACGTT		Tudzynski, 2016)
Bcmsb2	Bcmsb2-F	CTTGGATCAGCCACTTCGGT	XM_001550277	(Leroch et al., 2015)
	Bcmsb2-R	GTAGCCGAGGCAGATAACACC		
Bcpdi1	Bcpdi1-F	CGATATCCTCGCTGGCCTT	XM_001559009	(Marschall and
	Bcpdi1-R	GCTACGGCTACCGTTGTAGG		Tudzynski, 2017)
Bcsac1	Bcsac1-F	TGCCACTCTGCTCCTGATT	AJ276473	(Jurick and Rollins,
	Bcsac1-R	ACCTCCCCACCTCTGTTTTC		2007)
Bcrgb1	Bcrgb1-F	AAAAATCATAGACCGTTCCGC	XM_001546540	(Erental et al., 2007)
	Bcrgb1-R	CATCGTAGAGAACCTGGTAGCC		
Bccaf1	Bccaf1-F	CGGGCTCTGGCTTGT	XM_001556300	(Xiao et al., 2014)
	Bccaf1-R	TCCGCTTGGTCCCATAACG		
Bcactin	Bcact-F	TCCAAACCGCCAGTCAATCC	AJ000335	(Liu et al., 2011)
	Bcact-R	GATACCACCGCTCTCAAGACC		

¹ Xiao, X.Q.; Xie, J.T.; Cheng, J.C.; Li, G.Q.; Yi, X.H.; Jiang, D.H.; Fu, Y.P. Novel Secretory Protein Ss-Caf1 of the Plant-Pathogenic Fungus *Sclerotinia sclerotiorum* Is Required for Host Penetration and Normal Sclerotial Development. *Mol Plant Microbe Interact* 2014, 27, 40–55.

Leroch, M.; Mueller, N.; Hinsenkamp, I.; Hand, M. The signalling mucin Msb2 regulates surface sensing and host penetration via BMP1 MAP kinase signalling in *Botrytis cinerea*. *Molecular plant pathology* 2015, 16, 787–798

Marschall, R.; Tudzynski, P. BCIQG1, a fungal IQGAP homolog, interacts with NADPH oxidase, MAP kinase and

calcium signaling proteins and regulates virulence and development in *Botrytis cinerea*. Mol Microbiol 2016, 101, 281–298.

Marschall, R.; Tudzynski, P. The Protein Disulfide Isomerase of *Botrytis cinerea*: An ER Protein Involved in Protein Folding and Redox Homeostasis Influences NADPH Oxidase Signaling Processes. Front Microbiol 2017, 8, 1–15.

Jurick, W.M.; Rollins, J.A. Deletion of the adenylatecyclase (*sac1*) gene affects multiple developmental pathways and pathogenicity in *Sclerotinia sclerotiorum*. Fungal Genet Biol 2007, 44, 521–530.

Erental, A.; Harel, A.; Yarden, O. Type 2A phosphoprotein phosphatase is required for asexual development and pathogenesis of *Sclerotinia sclerotiorum*. Mol Plant Microbe Interact 2007, 20, 944–954.

Liu, W.; Soulié, M.C.; Perrino, C.; Fillinger, S. The osmosensing signal transduction pathway from *Botrytis cinerea* regulates cell wall integrity and MAP kinase pathways control melanin biosynthesis with influence of light. Fungal Genet Biol 2011, 48, 377–387

Table S4. Sequence identities of *Botrytis cinerea* hypovirus 1 (BcHV1) to other viruses through multiple alignments of the polyprotein sequences and the amino acid (aa) residue sequences of different domains

Family	Virus	Acronym	aa identity					Accession no.
			Full	Prot	UGT	RdRp	Hel	
<i>Hypoviridae</i>	Sclerotinia sclerotiorum hypovirus 1	SsHV1	67.4	88.57	63.34	85.94	71.48	NC_015939.1
	Cryphonectria hypovirus 3	CHV3	58.31	26.02	69.7	82.03	40.74	NC_000960.1
	Phomopsis longicolla hypovirus	PlHV1	57.84	11.32	67.44	85.16	69.63	NC_024685.1
	Valsa ceratosperma hypovirus 1	VcHV1	56.48	32.46	69.37	78.13	66.30	NC_017099.1
	Cryphonectria hypovirus 4	CHV4	44.55	27.05	57.61	75.78	54.24	NC_006431.1
	Cryphonectria hypovirus 1	CHV1	5.43	11.43	-	11.03	21.60	NC_001492.1
	Cryphonectria hypovirus 2	CHV2	6.14	10.28	-	10.31	21.33	NC_003534.1
	Fusarium graminearum hypovirus 1	FgHV1	4.86	12.26	-	12.1	19.51	NC_023680.1
	Fusarium graminearum hypovirus 2	FgHV2	4.36	9.43	-	11.22	20	NC_026813.1
	Macrophomina phaseolina hypovirus	MpHV1	3.90	-	-	15.38	22.34	KP900893.1
<i>Fusariviridae</i>	Sclerotinia sclerotiorum hypovirus 2	SsHV2	3.55	13.89	-	15.08	14.42	KJ561218.1
	Sclerotinia sclerotiorum fusarivirus 1	SsFV1	8.83	-	-	29.39	18.61	NC_027208.1
	Penicillium roqueforti ssRNA	PrRV1	8.18	-	-	26.74	20.34	NC_024699.1
	Rosellinia necatrix fusarivirus 1	RnFV1	8.58	-	-	26.36	21.02	NC_024485.1
	Fusarium graminearum dsRNA	FgV1	8.33	-	-	24.62	21.45	NC_006937
	Fusarium poae fusarivirus 1	FpFV1	7.9	-	-	20.59	19.86	NC_030868.1
	Pleospora typhicola fusarivirus 1	PtFV1	8.7	-	-	21.97	19.73	NC_028470.1
	Penicillium aurantiogriseum	PaFV1	8.3	-	-	22.95	19.79	NC_028467.1
	Alternaria brassicicola fusarivirus 1	AbFV1	8.91	-	-	21.73	20.61	NC_029056.1
	Nigrospora oryzae fusarivirus 1	NoFV1	8.09	-	-	21.78	21.43	KU980909.1
	Macrophomina phaseolina	MpSRV1	8.17	-	-	28.85	21.18	KP900890.1

¹ The full sequence means the larger ORF for alphahypoviruses and fusariviruses which contain the RdRp or Hel domain.

Table S5 Sequence identities of *Botrytis cinerea* fusarivirus 1 (BcFV1) to other viruses through multiple alignments of the polyprotein sequences and the amino acid (aa) residue sequences of different domains

Family	Virus	Acronym	aa identity			Accession no.
			Full sequence ¹	RdRp	Hel	
Fusariviridae	Alternaria brassicicola fusarivirus 1	AbFV1	25.58	47.17	32.44	NC_029056.1
	Sclerotinia sclerotiorum fusarivirus 1	SsFV1	24.30	46.98	33.22	NC_027208.1
	Pleospora typhicola fusarivirus 1	PtFV1	23.84	44.59	29.61	NC_028470.1
	Fusarium poae fusarivirus 1	FpFV1	23.83	44.13	29.63	NC_030868.1
	Fusarium graminearum dsRNA mycovirus-1	FgV1	23.72	46.31	28.96	NC_006937
	Penicillium roqueforti ssRNA mycovirus 1	PrRV1	23.51	43.63	27.61	NC_024699.1
	Penicillium aurantiogriseum fusarivirus 1	PaFV1	22.77	38.85	30.43	NC_028467.1
	Rosellinia necatrix fusarivirus 1	RnFV1	22.73	40.95	27.09	NC_024485.1
	Nigrospora oryzae fusarivirus 1	NoFV1	22.55	37.76	31.94	KU980909.1
	Macrophomina phaseolina single-stranded RNA virus 1	MpSRV1	22.26	38.54	27.85	KP900890.1
Hypoviridae	Cryphonectria hypovirus 3	CHV3	7.35	25.8	23.57	NC_000960.1
	Cryphonectria hypovirus 4	CHV4	7.29	27.39	22.37	NC_006431.1

	Sclerotinia sclerotiorum hypovirus 1	SsHV1	7.10	28.8	23.91	NC_015939.1
	Valsa ceratosperma hypovirus 1	VcHV1	7.46	27.07	22.90	NC_017099.1
	Phomopsis longicolla hypovirus	PlHV1	7.77	26.43	23.75	NC_024685.1
	Cryphonectria hypovirus 1	CHV1	6.73	14.07	12.62	NC_001492.1
	Cryphonectria hypovirus 2	CHV2	6.1	14.14	14.10	NP_613266
	Fusarium graminearum hypovirus 1	FgHV1	5.34	16.01	15.91	NC_023680.1
	Fusarium graminearum hypovirus 2	FgHV2	5.47	13.49	17.18	NC_026813.1
	Macrophomina phaseolina hypovirus	MpHV1	4.79	14.36	16.83	KP900893.1
	1					
	Sclerotinia sclerotiorum hypovirus 2	SsHV2	4.74	12.62	14.06	KJ561218.1
Potyviridae	Barley mild mosaic virus	BmMV	9.86	22.33	16.17	AJ242725.1
	Wheat yellow mosaic virus	WyMV	4.43	20.19	-	AB627812.1
Poxviridae	Pseudocowpox virus	PCPV	6.68	-	19.88	GQ329669.1
	Bovine papular stomatitis virus	BPSV	7.29	-	20.43	NC_005337.1
Iflaviridae	Opsiphantes invirae iflavivirus 1	Oily-1	5.59	18.04	-	KR534892.1
Secoviridae	Strawberry mottle virus	SMoV	6.60	17.39	-	NP_599086.1

¹The full sequence means the larger ORF for alphahypoviruses and fusariviruses which contain the RdRp or Hel domain.

Table S6. Data used to generate histogram**Figure 1**

Strain	Lesion diameter (mm)	Growth rate (mm/d)
HBtom-372	0.55 ± 1.79	2.03 ± 0.78
B05.10	18 ± 1.5	15.08 ± 0.38

Figure 7

Strain	Growth rate	Lesion diameter (mm)
	(mm/d)	Wounded
HBtom-372	2.63 ± 0.87 d	1.3 ± 2.91 c
Z26	4.38 ± 1.25 c	7.1 ± 1.78 b
Z1	4.81 ± 1.65 c	1.1 ± 1.75 c
Z3	9.38 ± 1.51 b	1 ± 2.24 c
Z33	15.75 ± 0.96 a	4 ± 3.76 bc
HBtom-459	14.56 ± 2.08 a	19.7 ± 1.51 a

Figure 8

Strain	Lesion diameter (mm)	Lesion diameter (mm)	Number of
	Intact	Wounded	infection cushion
HBtom-372	0.6 ± 1.87 efg ¹	2.85 ± 4.06 de	0 ± 0 d
Z26	4.65 ± 3.54 d	10.45 ± 0.99 c	14.67 ± 1.15 cd
Z1	0.35 ± 1.56 fg	2.55 ± 3.66 def	10.67 ± 5.03 cd
Z3	0 ± 0 g	0.7 ± 2.17 efg	20 ± 4 c
Z33	3.75 ± 5.39 d	17.35 ± 1.84 ab	55.33 ± 3.06 b
HBtom-459	16.45 ± 1.27 b	19.35 ± 2.25 a	195.33 ± 24.44 a

Figure S1

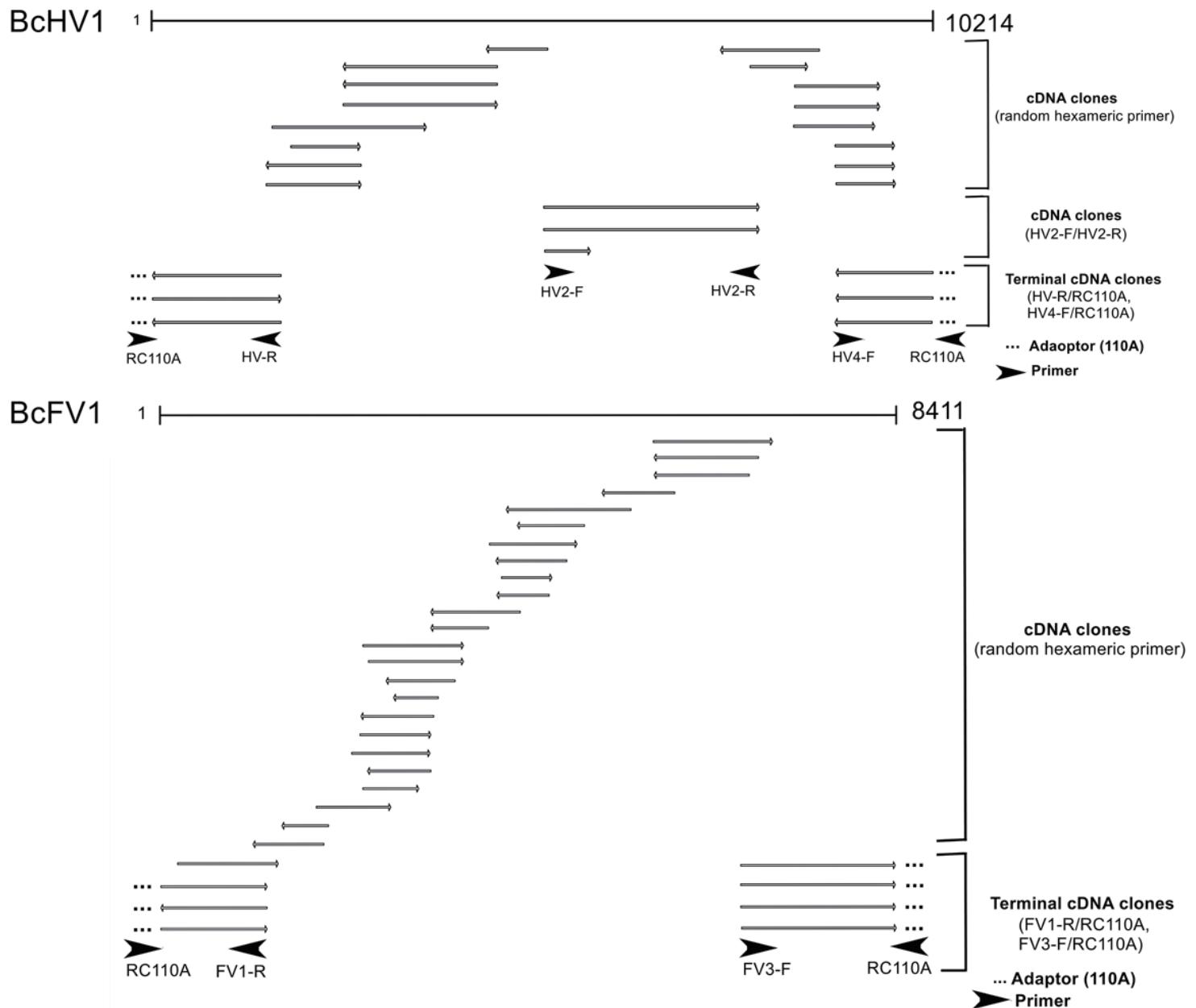


Figure S2

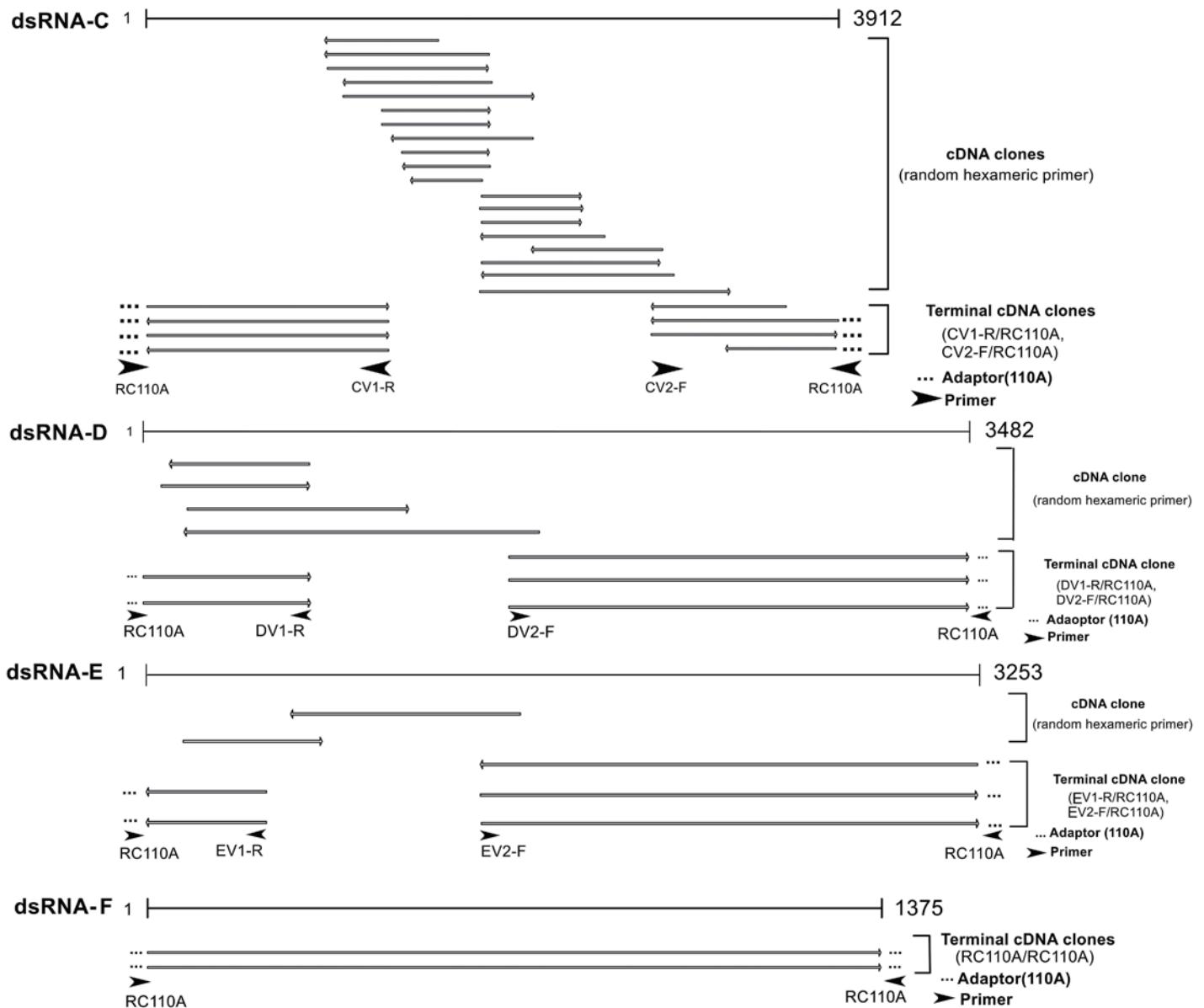


Figure S3

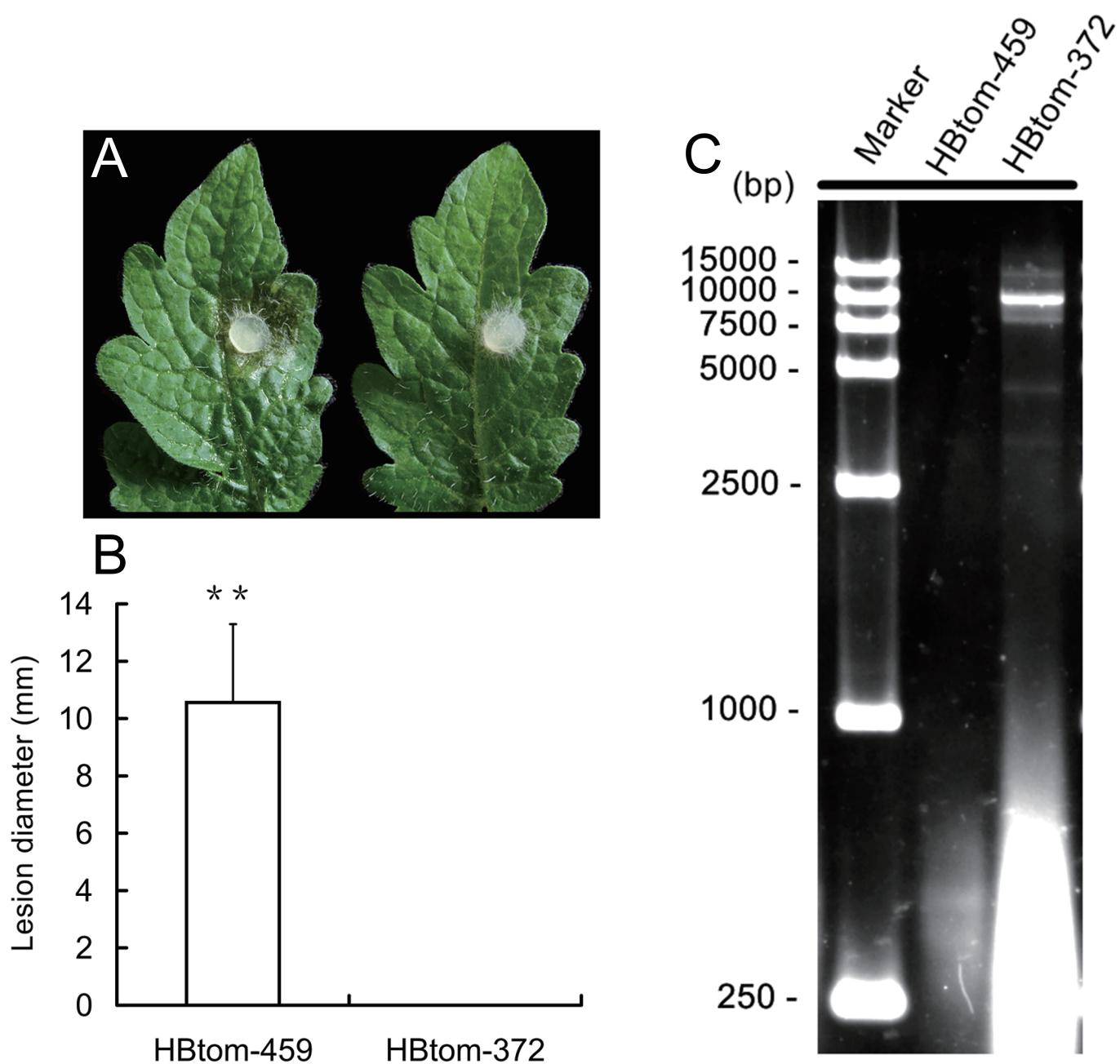


Figure S4

Prot

Figure S5

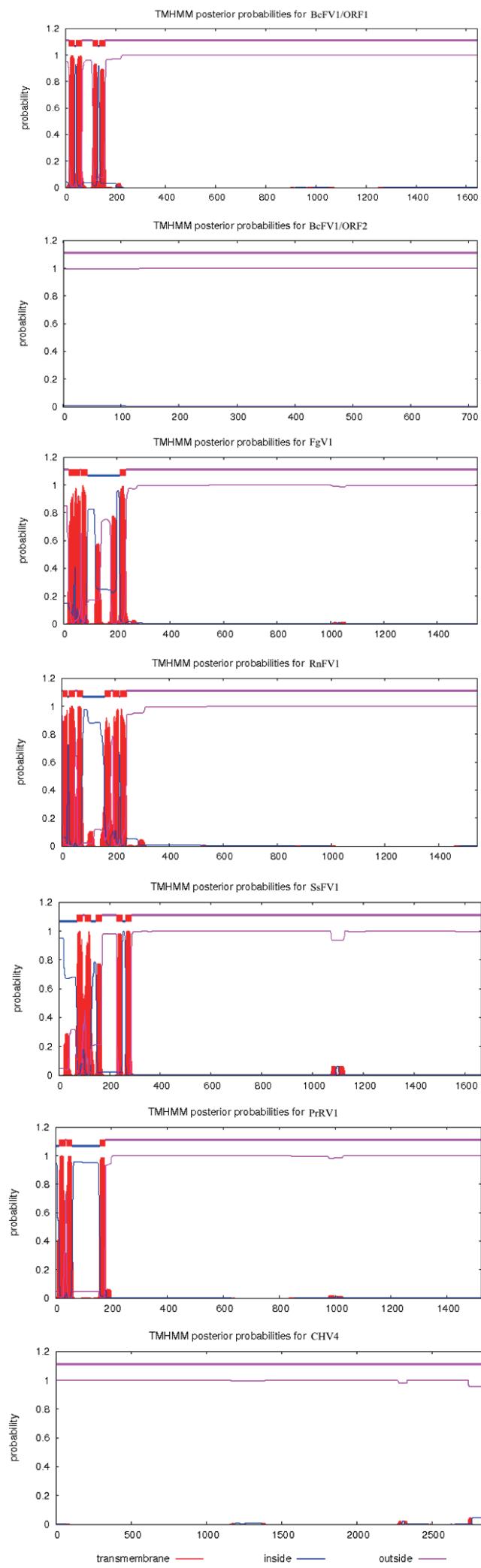


Figure S6

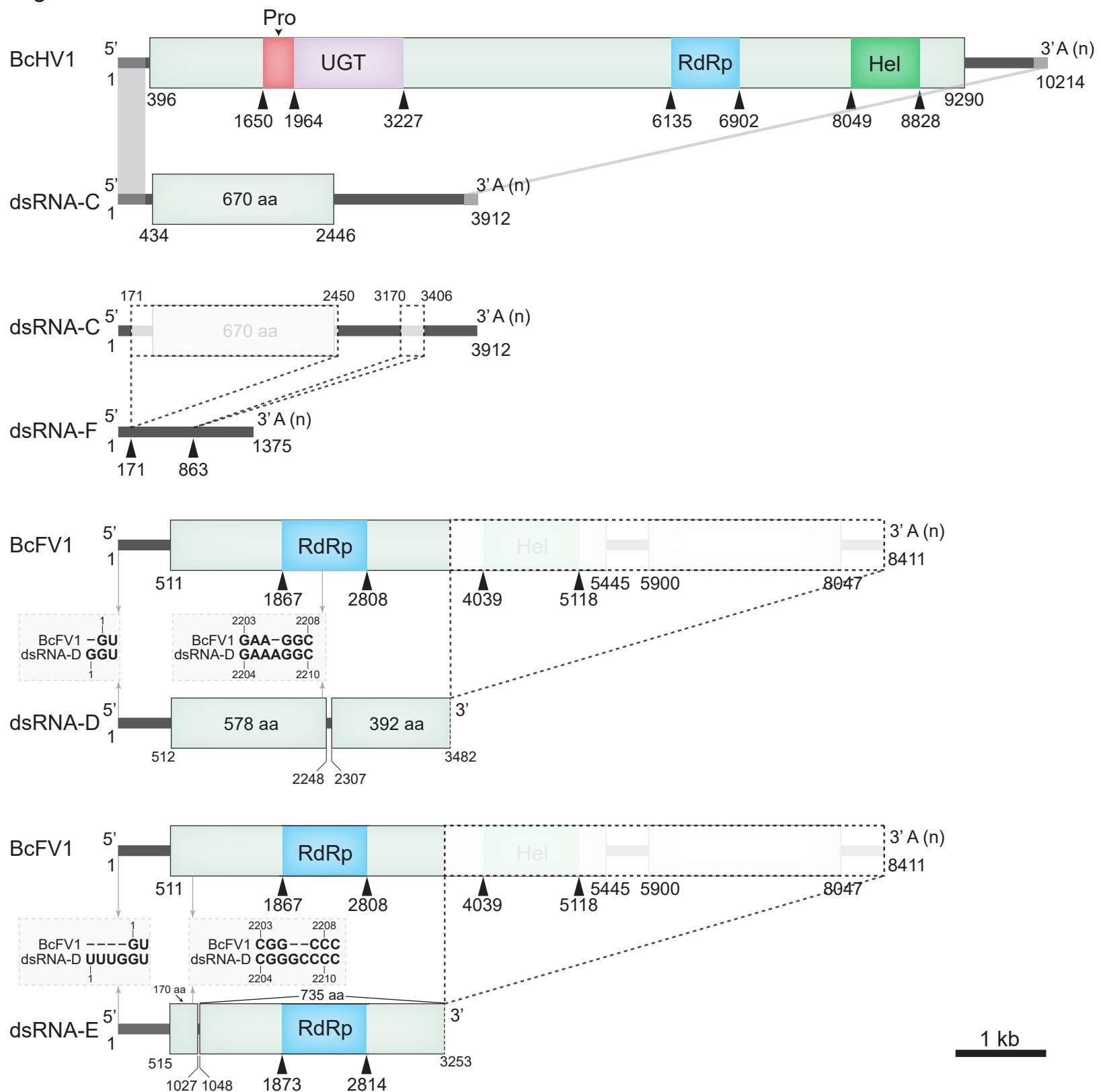


Figure S7

HBtom-372



HBtom-372



HBtom-459



HBtom-459

Figure S8

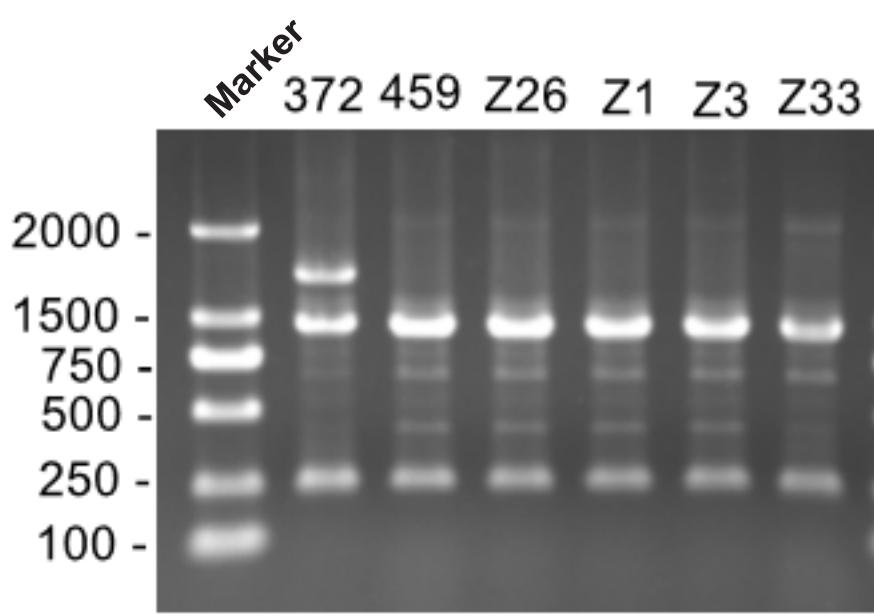


Figure S9

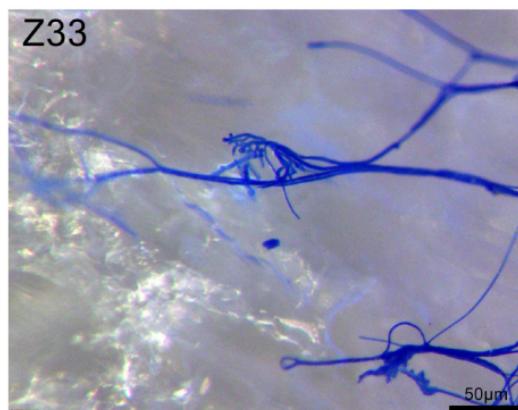
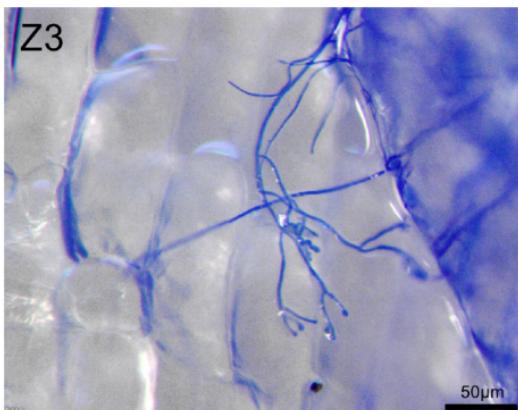
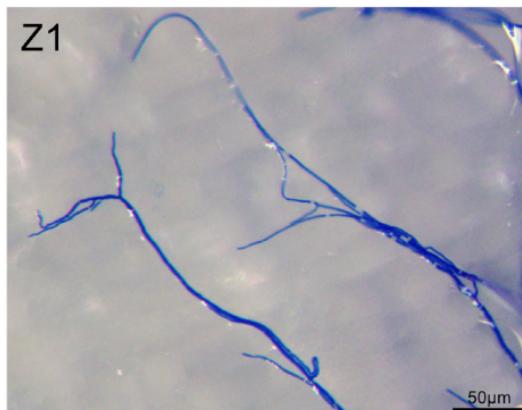
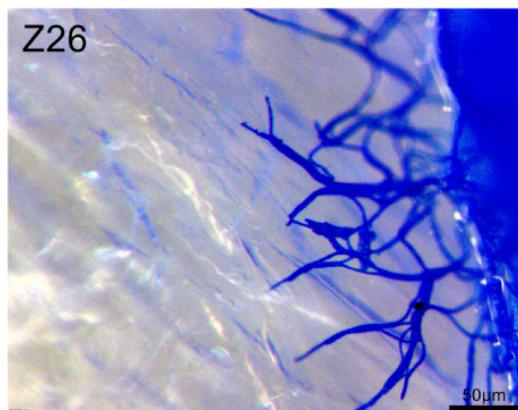
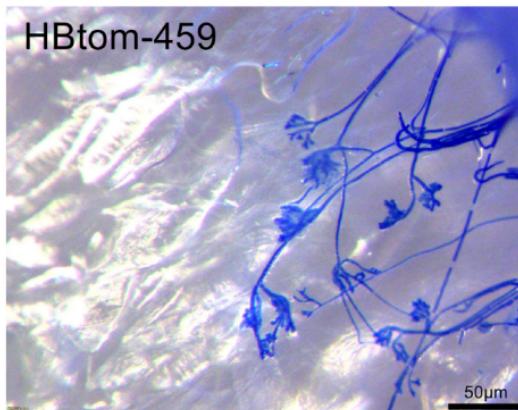
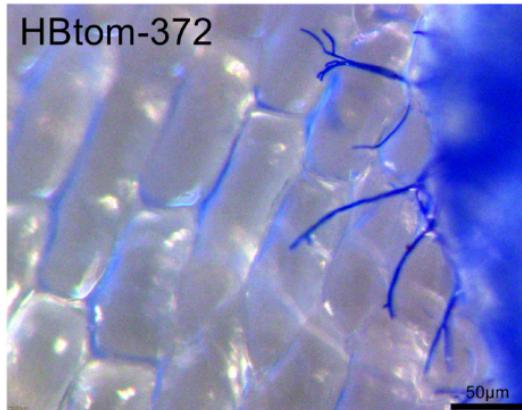


Figure S10

