

Supplementary Figure S1. Callose-containing encasements around *Bh* haustoria in barley induced by tetraconazole. (A) *Bh* colony and conidia chains developed on barley 4 days after inoculation. (B,C) Tetraconazole-induced encasements around *Bh* haustoria in barley epidermal cells. Inoculation was made 2 h after treatment. Images taken 4 dpi. UV-fluorescence microscopy after aniline blue treatment. Size bars, 100  $\mu$ m in A and B, 10  $\mu$ m in C.



Supplementary Figure S2. Barley *Hv*MON1 complements the function of Arabidopsis *At*MON1. (A) Transformation of Col-0 *mon1-1* with *35S::*Hv*MON1* rescues it from its lethality phenotype. Size bars, 1 cm, (B) CSLM subcellular localization of GFP-*Hv*MON1 expressed in *mon1-1* leaf cells epidermal. (C) Wortmannin-induced ring-shaped structures of GFP-*Hv*MON1 and GFP-*At*MON1 resolved by CSLM. Roots were treated with 33 µM wortmannin for 1 hour. Size bars, 10 µm in B and 10 µm C.



Supplementary Figure S3. Secondary hyphal lenght of Go on No-O and mon1-2. Lenght of secondary hyphae developed from penetrated appressoria at 2 days after inoculation. Error bars, SE. \*\*\*, P<0.001 assessed by Student's T-tests. n=3.

## Supplementary Table S1. Primers used in this work.

Name	Information	Sequence (5'-3')
attbHvMON1-F	Hv <i>MON1</i>	AAAAAGCAGGCTACATGGATCCGGCCCCCGAT
attbHvMON1-R	Cloning	AGAAAGCTGGGTCTCACCAGGCAATGGTGCT
attBCSEP0162-F	CSEP0162 Cloning	AAAAAGCAGGCTACATGGCCCAATATTCTAGACATATTAA
attBCSEP0162-R		AGAAAGCTGGGTCTCCCGAGCCAACTGCG
attbAtMON1-F	At <i>MON1</i> Cloning	AAAAAGCAGGCTACATGGCGACTTCAGATTCG
attbAtMON1-R		AGAAAGCTGGGTCTCACCAAGAGAAAGGACTAGCT
pGB9-FP	pDEST-AS2-1	AGTGCGACATCATCG
pGB9-RP	sequencing	CGTTTTAAAACCTAAGAGTCAC
pACT2-FP	pDEST-ACT2	GATGATGAAGATACCCCAC
pACT2-RP	sequencing	CAGTTGAAGTGAACTTGC
attbHvMON1i-F	Hv <i>MON1</i> RNAi Cloning	AAAAAGCAGGCTACCACCACTTCTAGGTGGCACA
attbHvMON1i-R		AGAAAGCTGGGTCGGCAAGCATATGGGTGAAAA
qRT-VIGS-Mi-F1		CCGCACAAAACACAATTCAG
qRT-VIGS-Mi-R1	qRT-PCR for Hv <i>MON1</i> RNAi	CCCTGATCCACTGGCATACT
HvUBC2-F		GGATCCAGGGCACCTCAC
HvUBC2-R		CGTCCAAGCTTTTTGAGGAC
Bgh-GPD-F	Blumeria hordei quantification	TGGCAATGCGTGTTCCTACT
Bgh-GPD-R		CATTTCCGGCGGCAATCTTT
qPCR-HvM-F		TTCTGATGAGGCAATTGGTG
qPCR-HvM-R		TTCTTATGGGCAAGGCAAAC
BS10	VIGS vector	GGTGCTTGATGCTTTGGATAAGG
BS32	sequencing	TGGTCTTCCCTTGGGGGAC
At3g21215-RBD-F	Golovinomyces	GAATCCACCCATACCACCAG
At3g21215-RBD-R		GAGGAGGAGGATGGTGATGA
GoATPase1-F	quantification	TCGCCGCTATATTTGGAGTC
GoATPase1-R	quantineation	CTGGGTCAGATGGTTCACCT
mon1-mutant-LP	mon1 mutant	CGGTTTGCCTGAGTTACTCAG
mon1-mutant-RP		AAAAGCCCAACAATATGGGTC
DS5-3	<i>mon1-2</i> (No-0)	TACCTCGGGTTCGAAATCGAT
LBb1.3	<i>mon1-1</i> (Col-0)	ATTTTGCCGATTTCGGAAC
eds1-F760	eds1-2 mutant	ACACAAGGGTGATGCGAGACA
eds1-R1458		GGCTTGTATTCATCTTCTATCC
eds1-R2333		GTGGAAACCAAATTTGACATTAG
ndr1-F3129		GGTTGTGAAATCAAGAATTAATGTGGA
ndr1-F4443	<i>ndr1-1</i> mutant	TTGCCTAATGGATCGGCTG
ndr1-R4928		GTTCCTTGATTTGAAACCCAACA

Name	Information	Reference
pDEST-ACT2-GW	Yeast two-hybrid	Robertson. 2004
pDEST-AS2-GW		
pB4GWnG		
pB4GWcCG		Kamizaki at al. 2016
pB4nGGW	BIFC	Kamigaki et al. 2016
pB4cCGGW		
p35S-mCherry-GW	Protoplast	
pUbi-GW-YFP	transformation and	Kwaaitaal et al. 2010
pUbi-GW-nos	particle bombardment	
pIPKTA30N-GW		Nowara et al. 2010
pCaBS-γ-GW	VIGS	pCaBS-γ (Yuan et al. 2011) were adapted
		to Gateway compatible
pUBN10-GFP-GW	Arabidopsis	Grefen et al. 2010
	overexpression	

## Supplementary Table S2. Gateway destination vectors used in this work.

## References

**Grefen C, Donald N, Hashimoto K, Kudla J, Schumacher K, Blatt MR** 2010). A ubiquitin-10 promoter-based vector set for fluorescent protein tagging facilitates temporal stability and native protein distribution in transient and stable expression studies. Plant Journal 64: 355–365

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Nowara D, Gay A, Lacomme C, Shaw J, Ridout C, Douchkov D, Hensel G, Kumlehn J, Schweizer P. 2010. HIGS: host-induced gene silencing in the obligate biotrophic fungal pathogen Blumeria graminis. The Plant Cell **22**, 3130–3141.

**Robertson M**. 2004. Two transcription factors are negative regulators of gibberellin response in the HvSPYsignaling pathway in barley aleurone. Plant Physiology **136**, 2747–2761.

Yuan C, Li C, Yan L, Jackson AO, Liu Z, Han C, Yu J, Li D. 2011. A high throughput barley stripe mosaic virus vector for virus induced gene silencing in monocots and dicots. PLoS ONE 6, e26468.

Supplementary Table S3. Overview of *Hv*MON1/CSEP0162 bifluorescence complementation (BiFC) results.

Combination	Fluorescence signal
HvMON1-nGFP + CSEP0162-cCFP	No
HvMON1-nGFP + cCFP-CSEP0162	No
nGFP- <i>Hv</i> MON1 + cCFP-CSEP0162	No
nGFP- <i>Hv</i> MON1 + CSEP0162-cCFP	Yes
HvMON1-cCFP + CSEP0162-nGFP	No
HvMON1-cCFP + nGFP-CSEP0162	No
cCFP- <i>Hv</i> MON1 + nGFP-CSEP0162	No
cCFP- <i>Hv</i> MON1 + CSEP0162-nGFP	No