PNAS

Supporting Information for

Experimental evidence for the functional importance and adaptive advantage of A-to-I RNA editing in fungi

Kaiyun Xin[1], Yang Zhang[1], Ligang Fan[1], Zhaomei Qi[1], Chanjing Feng[1], Qinhu Wang[1], Cong Jiang[1], Jin-Rong Xu[2], Huiquan Liu[1]*

* Corresponding author: Huiquan Liu
Email: liuhuiquan@nwsuaf.edu.cn

This PDF file includes:

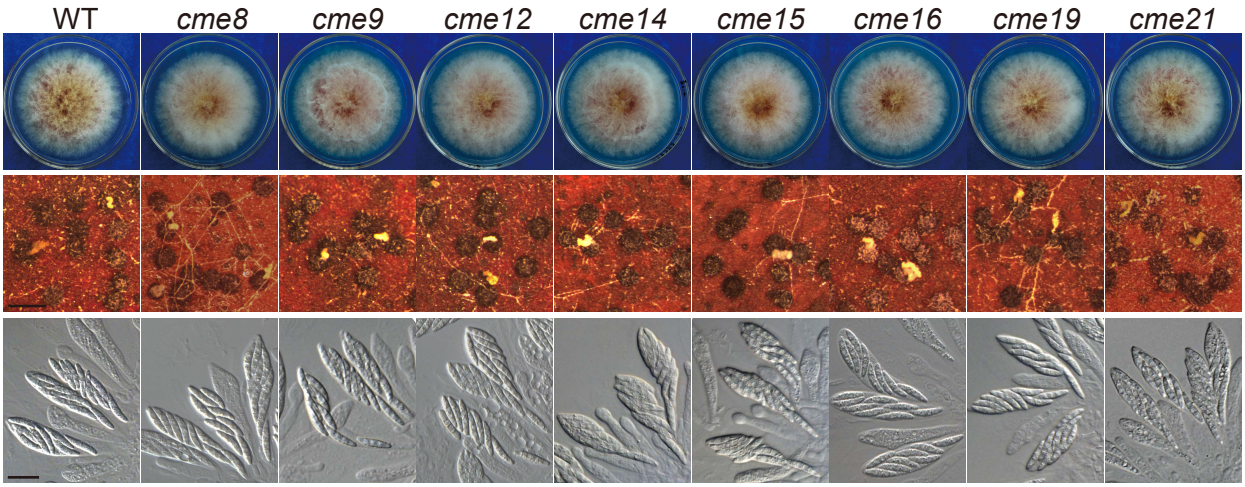

Fig. S1. Vegetative growth and sexual reproduction of deletion mutants of 8 *CME* genes. Three-day-old PDA cultures and 8-dpf mating cultures of PH-1 (WT) and the marked deletion mutants were examined for colony morphology, perithecium formation, and ascus/ascospore morphology. Bar = 0.5 mm (middle panel); Bar = 20 μm (bottom panel).

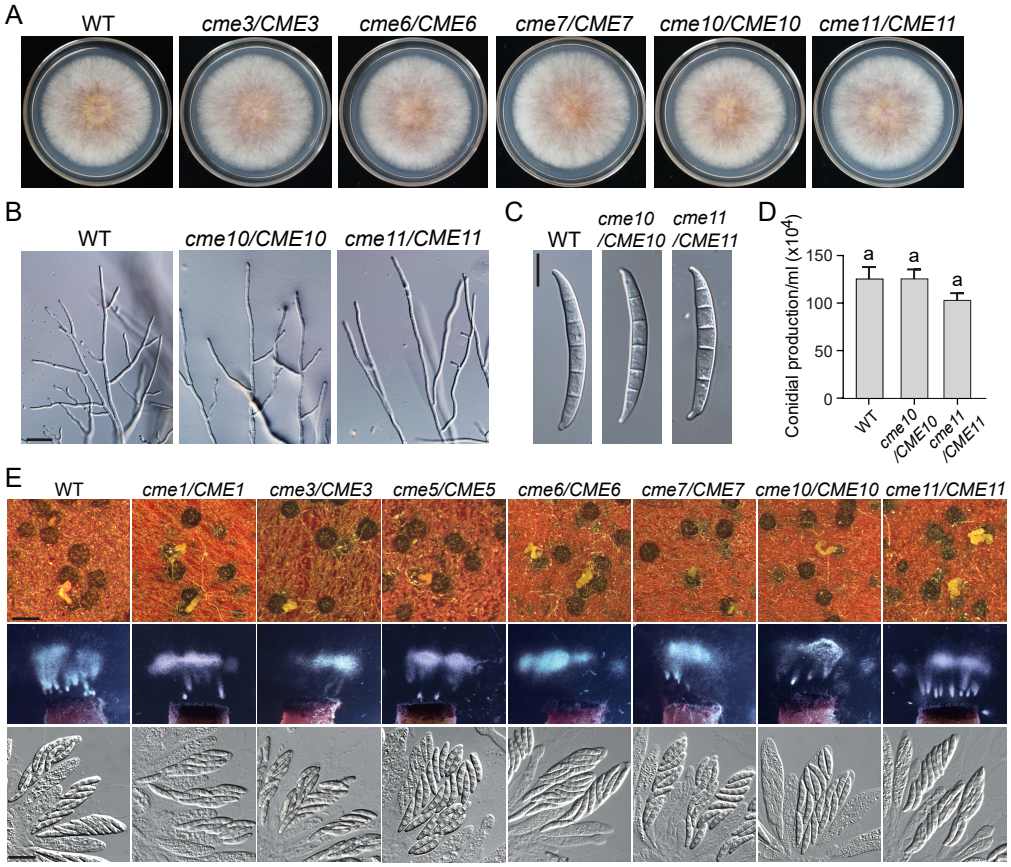

Fig. S2. Vegetative growth, conidiation, and sexual reproduction of complemented strains. (*A*-*B*) Three-day-old PDA cultures (*A*) and 48-h hyphal tips (*B*) of the marked strains. Bar = 20 μm. (*C*-*D*) Conidial production of the marked strains were examined by differential interference contrast microscopy. Bar = 10 μm. Mean and standard deviation were calculated with data from three independent repeats (n = 3). The same letters indicate no significant differences based on one-way ANOVA followed by Turkey's multiple range test ($P > 0.05$). (*E*) Mating cultures of the marked strains were examined for perithecium formation, ascospore discharge, and asci/ascospores morphology at 8-dpf. Bar = 0.5 mm (top panel); Bar = 20 μm (bottom panel).

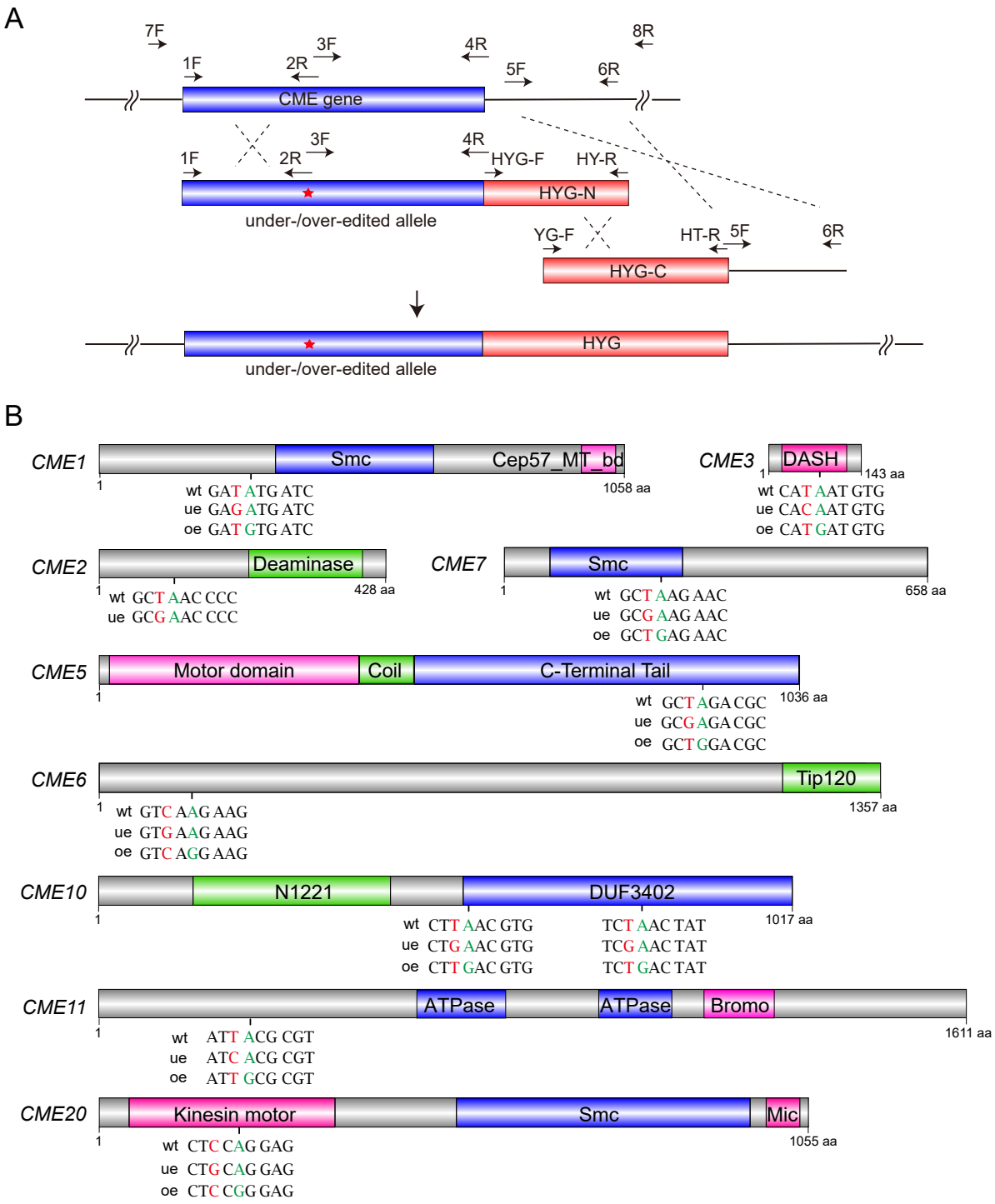

Fig. S3. Schematic representation of site-directed mutagenesis strategies for CME sites. (*A*) Graphical representation of the mutagenesis strategy used for CME sites. The hygromycin-resistance gene (HYG) is the selectable marker. Allelic fragments with desired mutations (marked with a red star) were generated by overlapping PCR. Locations of PCR primers (F, forward and R, reverse) are indicated. (*B*) Conserved domains and sequences of edited codons and 5' and 3' nearest-neighboring codons of *CME* genes. The editing and mutated sites are marked in green and red, respectively, for wild type (wt), under-edited (ue), and over-edited (oe) alleles. Abbreviations for domain names: Smc, chromosome segregation ATPase; Cep57_MT_bd, centrosome microtubule-binding domain of Cep57; DASH, DASH complex subunit Dad2; Deaminase, cytosine deaminases domain; Motor domain, Kinesin motor domain of KIP3-like subgroup; Coil, coiled-coil domain; C-terminal Tail, the C-terminal tail domain of KIP3-like subgroup; Tip120, TATA-binding protein interacting domain; N1221, N1221-like protein; DUF3402, a domain of unknown function; Mic, microtub_bind domain; ATPase, AAA+-type ATPase; Bromo, the bromodomain of TBP7_like subfamily; Kinesin motor, kinesin motor domain of BimC/Eg5 spindle pole proteins.

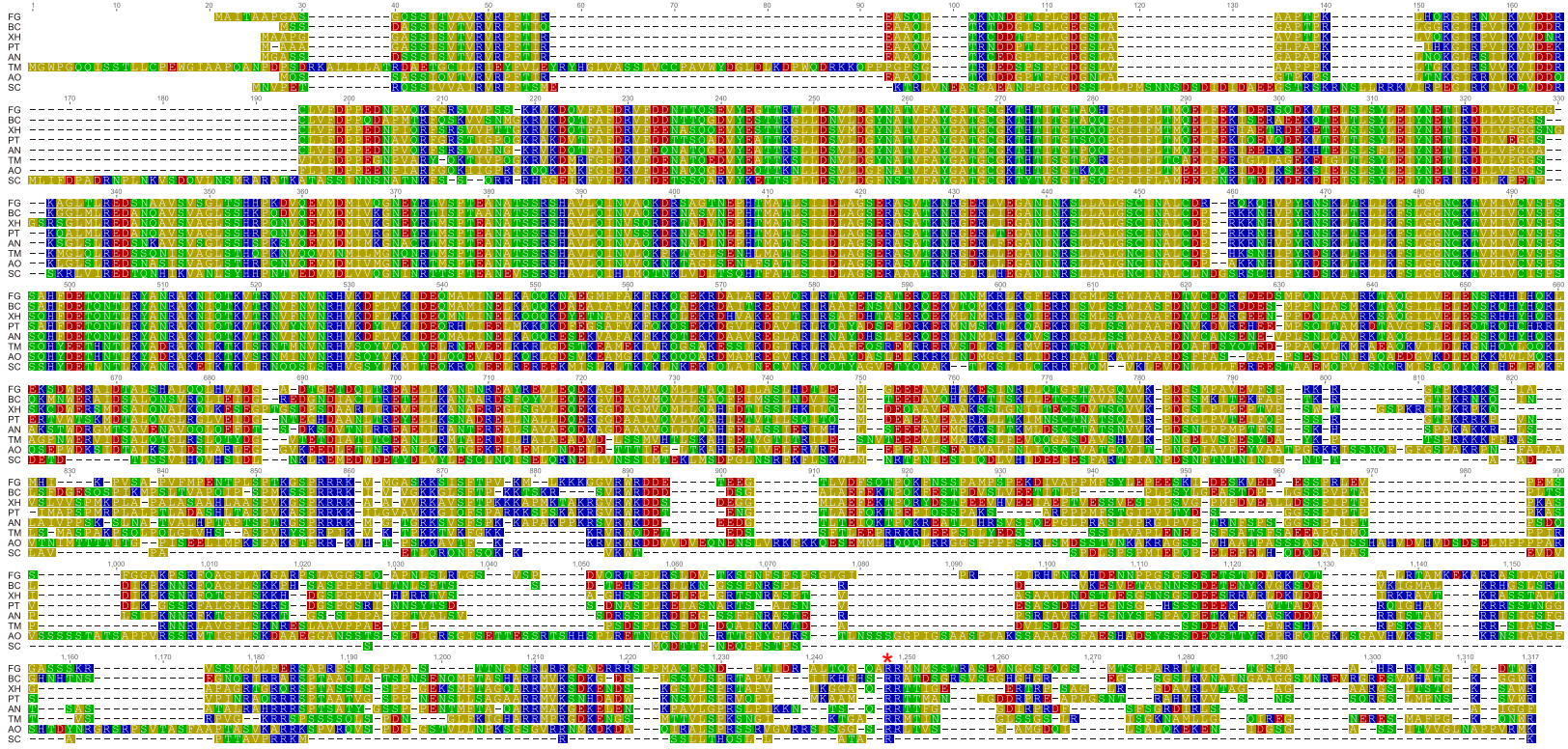

Fig. S4. Multiple sequence alignment of Cme5 and its orthologs in representative ascomycetes. Residues are colored according to their polarity. The CME site is marked with an asterisk (*). Abbreviations: FG, *Fusarium graminearum*; BC, *Botrytis cinerea*; XH, *Xylona heveae*; PT, *Pyrenophora teres*; AN, *Aspergillus nidulans*; TM, *Tuber magnatum*; AO, *Arthrobotrys oligospora*, SC, *Saccharomyces cerevisiae*.

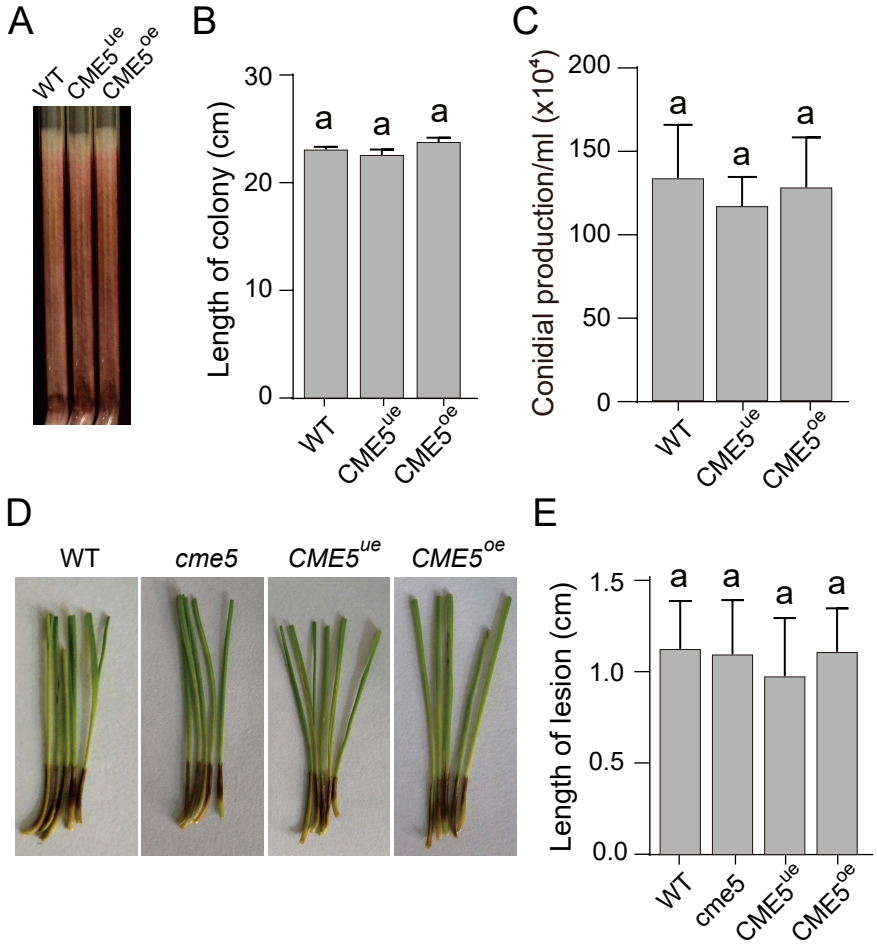

Fig. S5. Growth rate, conidial production, and virulence of the under-edited and over-edited mutants of *CME5.* (*A*) Fifteen-day-old PDA cultures of PH-1 (WT) and the under-edited (*CME5*^{ue}) and over-edited (*CME5*^{oe}) mutants in race tubes. (*B*) Length of the colony in race tubes. (*C*) Conidial production of the marked strains in 5-day-old CMC cultures. (*D*) Wheat coleoptiles inoculated with conidia of the marked strains were photographed at 7 days post-inoculation. (*E*) The length of lesion on infected wheat coleoptiles. Mean and standard deviation were calculated with data from three independent repeats (n = 3) for (*B*) and (*C*) and at least five independent repeats (n＞5) for (*E*). The same letters indicate no significant differences based on one-way ANOVA followed by Turkey's multiple range test (P＞0.05).

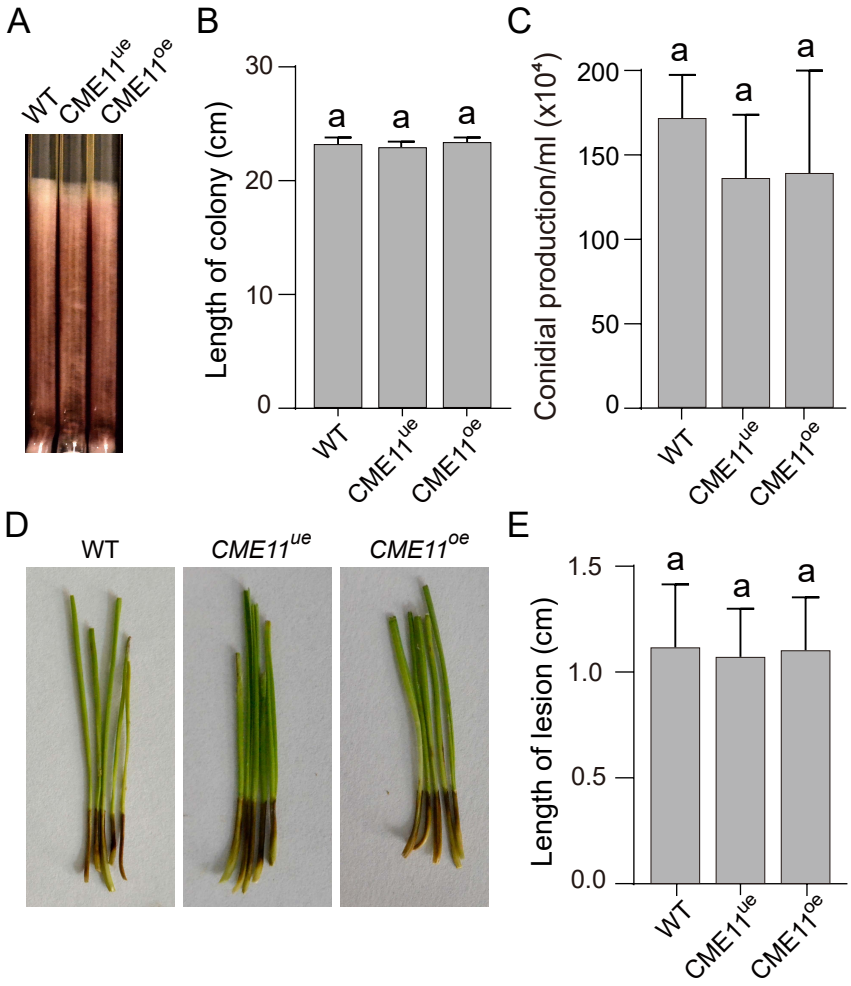

Fig. S6. Growth rate, conidial production, and virulence of the under-edited and over-edited mutants of *CME11.* (*A*) Fifteen-day-old PDA cultures of the wild-type PH-1 (WT) and the under-edited (*CME11*^{ue}) and over-edited (*CME11*^{oe}) mutants in race tubes. (*B*) Length of the colony in race tubes. (*C*) Conidial production of the marked strains in 5-day-old CMC cultures. (*D*) Wheat coleoptiles inoculated with conidia of the marked strains were photographed at 7 days post-inoculation. (*E*) The length of lesion on infected wheat coleoptiles. Mean and standard deviation were calculated with data from three independent repeats (n = 3) for (*B*) and (*C*) and at least five independent repeats (n＞5) for (*E*). The same letters indicate no significant differences based on one-way ANOVA followed by Turkey's multiple range test (P＞0.05).

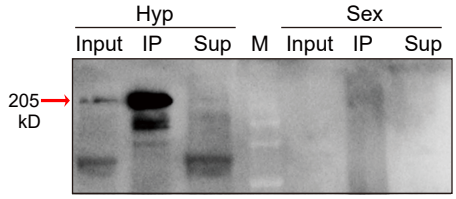

Fig. S7. Western blots of total proteins (Input), proteins eluted from anti-GFP affinity beads (IP), and supernatant solution (Sup) from 16-h hyphae (Hyp) or 7-dpf perithecia (Sex) of the transformant expressing *CME11*-GFP were detected by the anti-GFP antibody. The expected size (205 kD) of the Cme11-GFP protein is indicated.

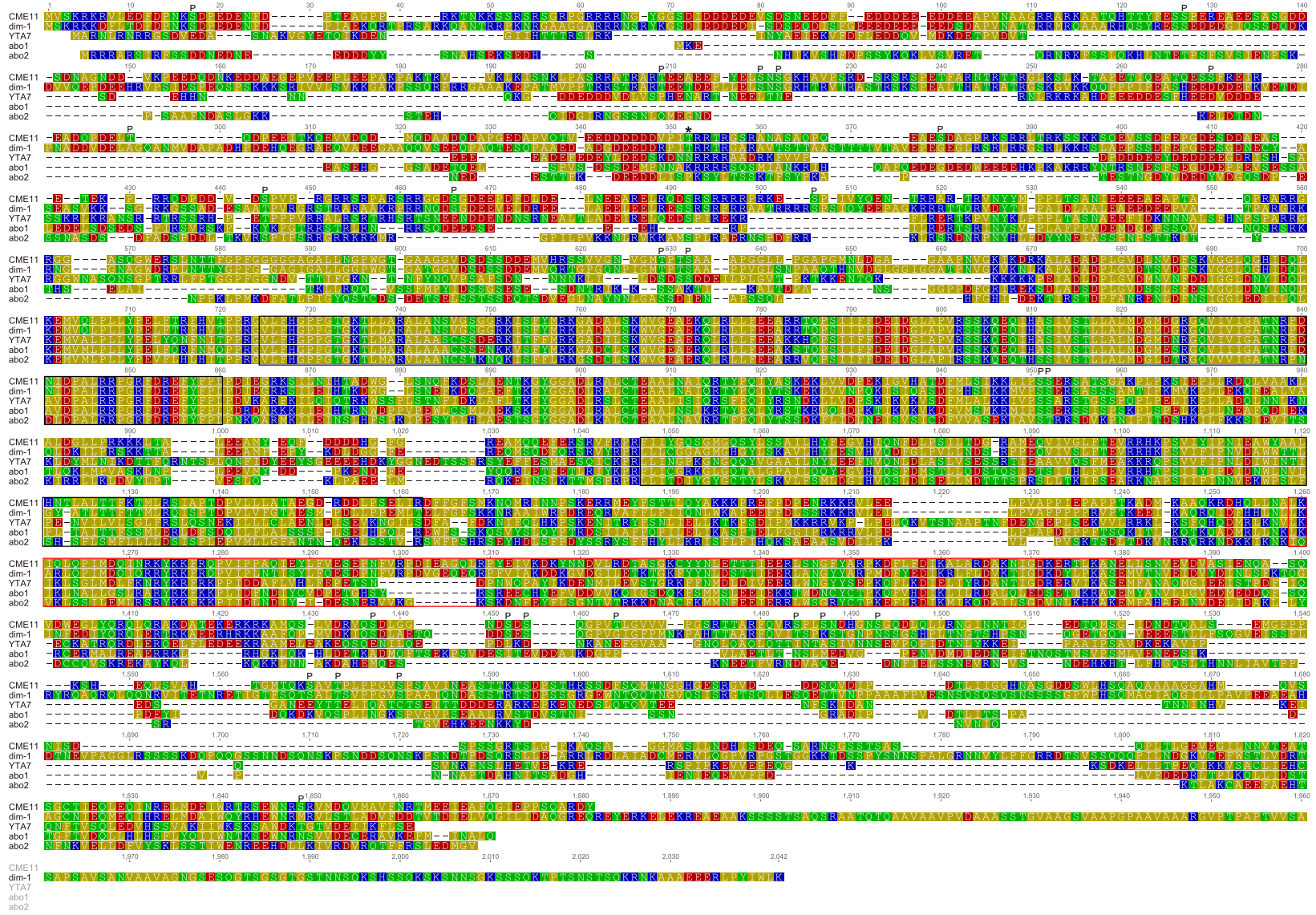

Fig. S8. Multiple sequence alignment of Cme11 and its orthologs in yeasts and *N. crassa*. Residues are colored according to their polarity. Identified phosphorylation sites of Cme11 during vegetative growth in *F. graminearum* are marked as a "P" letter above the alignment. The CME site is marked with an asterisk (*).

Table S1. Information of 21 *CME* genes containing 22 CME sites with editing levels of ⩾ 50% in both *F. graminearum* and *N. crassa*.

F. graminearum				***N. crassa***				***S. cerevisiae***	***S. pombe***
Gene name	Gene ID	Editing site in CDS	Editing level (%)*	Gene name	Gene ID	Editing site in CDS	Editing level (%)*	Gene name	Gene name
CME-1	FG1G04370	967	91	*kpr-14*	NCU03225	1033	91	-	-
CME-2	FG1G22250	289	65	-	NCU01725	229	84	*TAD3*	*tad3*
CME-3	FG1G23600	244	54	*dash-3*	NCU01841	229	50	*DAD2*	*dad2*
CME-4	FG1G28230	310	52	*gpr-2*	NCU04626	344	53	-	-
CME-5	FG3G02660	2956	71	*kin-8*	NCU06144	3670	77	*KIP3*	*klp5*, *klp6*
CME-6	FG3G17740	683	67	-	NCU08875	647	67	-	*knd1*
CME-7	FG3G19720	670	76	-	NCU09481	757	55	-	-
CME-8	FG3G21370	1202	55	*cac-1*	NCU04198	1238	54	-	-
CME-9	FG3G21560	865	69	-	NCU03709	211	84	*HMX1*	-
CME-10	FG4G11660	1903	83	*ham-2*	NCU03727	1984	82	*FAR11*	*far11*
CME-10	FG4G11660	2194	57	*ham-2*	NCU03727	2266	84	*FAR11*	*far11*
CME-11	FG4G12740	910	50	*dim-1*	NCU06484	1027	63	*YTA7*	*abo1*, *abo2*
CME-12	FG2G11430	794	50	-	NCU01998	809	52	-	-
CME-13	FG2G12190	700	82	*gh81-1*	NCU07076	484	65	*DSE4*, *ACF2*	*eng1*, *eng2*
CME-14	FG4G25980	181	80	*ldh-4*	NCU00780	226	78	-	-
CME-15	FG4G25530	703	98	-	NCU09929	718	83	-	-
CME-16	FG4G24350	469	70	*cly-1*	NCU06361	463	65	-	-
CME-17	FG1G41810	916	59	-	NCU03994	1291	80	-	-
CME-18	FG1G41840	604	57	-	NCU05809	784	89	-	-
CME-19	FG3G06430	250	93	-	NCU01182	349	64	-	-
CME-20	FG1G10370	470	91	*kin-5*	NCU00927	842	69	*CIN8, KIP1*	*cut7*
CME-21	FG1G23580	2374	67	-	NCU01840	2488	67	-	-

*Editing levels in *F. graminearum* were from our previous study (1) calculated with RNA-seq data of perithecia (Sex) samples. Editing levels in *N. crassa* were the largest editing levels calculated with RNA-seq data of 3- to 6-dpf perithecia in our previous study (2).

Table S2. RNA-seq data used in this study.

Species	Sample	Accession number
F. graminearum	Conidia (Coni) of PH-1	SRR2182470, SRR2182494, SRR12677105
F. graminearum	Hyphae (Hyp) of PH-1	SRR2182495, SRR2182497, SRR12677098
F. graminearum	Perithecia (Sex) of PH-1	SRR2182501, SRR2182499
F. graminearum	1-dpf perithecia of PH-1	SRS16391626
F. graminearum	2-dpf perithecia of PH-1	SRS16391627
F. graminearum	3-dpf perithecia of PH-1	SRS16391628
F. graminearum	4-dpf perithecia of PH-1	SRS16391629
F. graminearum	5-dpf perithecia of PH-1	SRS16391630
F. graminearum	6-dpf perithecia of PH-1	SRS16391631
F. graminearum	7-dpf perithecia of PH-1	SRS16391632
F. graminearum	8-dpf perithecia of PH-1	SRS16391633

Table S3. Strains used in this study.

Strains	Brief description	Reference
PH-1	The wild-type strain	(3)
cme1-13, 16, TK12, TK13	*CME1* deletion mutants of PH-1	This study
cme3-1, 2, TK9, TK11	*CME3* deletion mutants of PH-1	This study
cme5-1, 5, TK9, TK11	*CME5* deletion mutants of PH-1	This study
cme6-1, 2, TK19, TK20	*CME6* deletion mutants of PH-1	This study
cme7-3, 4, TK9, TK10	*CME7* deletion mutants of PH-1	This study
cme8-1, 2	*CME8* deletion mutants of PH-1	This study
cme9-1, 6, 8	*CME9* deletion mutants of PH-1	This study
cme10-4, 5, TK1, TK3	*CME10* deletion mutants of PH-1	This study
cme11-1, 2, TK1, TK2	*CME11* deletion mutants of PH-1	This study
cme12-6, 8	*CME12* deletion mutants of PH-1	This study
cme14-10, 11	*CME14* deletion mutants of PH-1	This study
cme15-8, 12	*CME15* deletion mutants of PH-1	This study
cme16-1, 7	*CME16* deletion mutants of PH-1	This study
cme19-8, 12	*CME19* deletion mutants of PH-1	This study
cme21-8, 7	*CME21* deletion mutants of PH-1	This study
cme1/CME1-6, 4	Complemented strains of *cme1* deletion mutants	This study
cme3/CME3-3	Complemented strains of *cme3* deletion mutants	This study
cme5/CME5-1, 3	Complemented strains of *cme5* deletion mutants	This study
cme6/CME6-5, 12	Complemented strains of *cme6* deletion mutants	This study
cme7/CME7-9	Complemented strains of *cme7* deletion mutants	This study
cme10/CME10-1, 2	Complemented strains of *cme10* deletion mutants	This study
cme11/CME11-1, 6	Complemented strains of *cme11* deletion mutants	This study
CME1^{ue}-1, 4	Under-edited mutants of *CME1*	This study
CME2^{ue}-3, 9	Under-edited mutants of *CME2*	This study
CME3^{ue}-2, 7	Under-edited mutants of *CME3*	This study
CME5^{ue}-4, 12	Under-edited mutants of *CME5*	This study
CME6^{ue}-2, 4	Under-edited mutants of *CME6*	This study
CME7^{ue}-1, 3	Under-edited mutants of *CME7*	This study
CME10^{ue1}-2, 5	Under-edited mutants of the first CME site of *CME10*	This study
CME10^{ue2}-2, 15	Under-edited mutants of the second CME site of *CME10*	This study
CME10^{ue1,2}-2, 6	Under-edited mutants of double CME sites of *CME10*	This study
CME11^{ue}-20, 18	Under-edited mutants of *CME11*	This study
CME20^{ue}-7, 10	Under-edited mutants of *CME20*	This study
CME1^{oe}-5, 6	Over-edited mutants of *CME1*	This study
CME3^{oe}-1, 2	Over-edited mutants of *CME3*	This study
CME5^{oe}-25, 26	Over-edited mutants of *CME5*	This study
CME6^{oe}-5, 6	Over-edited mutants of *CME6*	This study
CME7^{oe}-2, 4	Over-edited mutants of *CME7*	This study
CME10^{oe1}-7, 8	Over-edited mutants of the first CME site of *CME10*	This study
CME10^{oe2}-5, 6	Over-edited mutants of the second CME site of *CME10*	This study
CME10^{oe1,2}-7, 8	Over-edited mutants of double CME sites of *CME10*	This study
CME11^{oe}-10, 5	Over-edited mutants of *CME11*	This study
CME20^{oe}-1, 3	Over-edited mutants of *CME20*	This study
CME11-CK-12	The control check strain containing the same selectable marker inserted at the *CME11* locus but without mutations at *CME11* coding regions	This study
CME11$^{ue\text{-}oe}$-6,10	Mutants expressed both under-edited and over-edited alleles of *CME11*	This study
CME11-pDL2-5	*CME11-GFP* ectopic expression strain	This study

Table S4. Phenotypes of deletion mutants of 21 *CME* genes in *F. graminearum*.

Gene ID	Gene name	Deletion[a]	Mycelial growth[b]	Conidiation[b]	Perithecia	Ascus formation[b]	Ascospore formation[b]	Reference
FG1G04370	*CME1*	Y	97.6%	99.6%	Reduced cirrhi	44.5%	4.0%	This study
FG1G22250	*CME2*	N	N/A	N/A	N/A	N/A	N/A	This study
FG1G23600	*CME3*	Y	92.1%	111.4%	Reduced cirrhi	60.8%	20.9%	This study
FG1G28230	*CME4*	Y	100.3%	92.2%	Normal	N/A	N/A	(4)
FG3G02660	*CME5*	Y	97.7%	90.7%	Reduced cirrhi	49.7%	5.9%	This study
FG3G17740	*CME6*	Y	81.1%	102.3%	Smaller & no cirrhi	No elongated asci	N/A	This study
FG3G19720	*CME7*	Y	64.0%	89.9%	Reduced cirrhi	34.9%	0.1%	This study
FG3G21370	*CME8*	Y	92.6%	Normal	Normal	Normal	Normal	This study
FG3G21560	*CME9*	Y	98.7%	Normal	Normal	Normal	Normal	This study
FG4G11660	*CME10*	Y	31.2%	12.0%	No perithecia	N/A	N/A	This study
FG4G12740	*CME11*	Y	30.5%	1.9%	No perithecia	N/A	N/A	This study
FG2G11430	*CME12*	Y	97.5%	Normal	Normal	Normal	Normal	This study
FG2G12190	*CME13*	Y	76.7%	Normal	Normal	Normal	Normal	(5)
FG4G25980	*CME14*	Y	99.9%	Normal	Normal	Normal	Normal	This study
FG4G25530	*CME15*	Y	98.0%	Normal	Normal	Normal	Normal	This study
FG4G24350	*CME16*	Y	95.5%	Normal	Normal	Normal	Normal	This study
FG1G41810	*CME17*	Y	0-30%	Normal	Normal	Normal	Normal	(6)
FG1G41840	*CME18*	Y	91-100%	Normal	Normal	Normal	Normal	(6)
FG3G06430	*CME19*	Y	100.1%	Normal	Normal	Normal	Normal	This study
FG1G10370	*CME20*	N	N/A	N/A	N/A	N/A	N/A	This study
FG1G23580	*CME21*	Y	100.6%	Normal	Normal	Normal	Normal	This study

[a] Y and N represent deleted genes and undeleted genes, respectively.
[b] Percentage of average growth rates on PDA, conidial production in CMC, number of asci per perithecium, asci with eight ascospores for each mutant relative to the wild-type PH-1. Normal, no visible defects; N/A, not applicable.

SI References

1. H. Liu *et al.*, Genome-wide A-to-I RNA editing in fungi independent of ADAR enzymes. *Genome Res* **26**, 499-509 (2016).
2. H. Liu *et al.*, A-to-I RNA editing is developmentally regulated and generally adaptive for sexual reproduction in *Neurospora crassa*. *Proc Natl Acad Sci U S A* **114**, E7756-E7765 (2017).
3. C. A. Cuomo *et al.*, The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. *Science* **317**, 1400-1402 (2007).
4. C. Jiang *et al.*, An expanded subfamily of G-protein-coupled receptor genes in *Fusarium graminearum* required for wheat infection. *Nat Microbiol* **4**, 1582-1591 (2019).
5. C. Hao *et al.*, The meiosis-specific APC activator FgAMA1 is dispensable for meiosis but important for ascosporogenesis in *Fusarium graminearum*. *Molecular microbiology* **111**, 1245-1262 (2019).
6. H. Son *et al.*, A phenome-based functional analysis of transcription factors in the cereal head blight fungus, *Fusarium graminearum*. *PLoS Pathog* **7**, e1002310 (2011).