Figure S1. Ali et al.

Ustilago hordei life cycle
Figure S2. Ali et al.

A

EtBr autorad

EtBr autorad

EtBr autorad

Uh364 yeast Uh362

kb

1,100+ 1,100+ 1,100+

1,100+ 945 915

1,100+ 915 815

915 785 745

785 680 610

680 555 295

667

Uh364 Chr18 = 667 kb

probe: UHOR_10021 gene 16

probe: UHOR_10022 gene 17

probe: UHOR_08123 gene 23

B

EtBr autorad

EtBr autorad

EtBr autorad

Uh364 Uh362

~ 150 kb

probe: 1.5 kb UHOR_10022 3’-flank
Figure S3A, continued
**Figure S3C**

*UHOR_10021 - *Uhavr1* locus region in virulent isolates*

983

| **Uh364brk** | CAAGTAGTGTATCTAATTTCA------------- |
| **Uh822** | CAAGTAGTGATCTATTCCAGTTGAGTCATTTTCAGTCAGGCTTCTTCGGGTCATTGTCGAGGTTTCCGGTTAGATGGTGATGGTAG |
| **Uh811** | CAAGTAGTGATCTATTCCAGTTGAGTCATTTTCAGTCAGGCTTCTTCGGGTCATTGTCGAGGTTTCCGGTTAGATGGTGATGGTAG |
| **Uh818** | CAAGTAGTGATCTATTCCAGTTGAGTCATTTTCAGTCAGGCTTCTTCGGGTCATTGTCGAGGTTTCCGGTTAGATGGTGATGGTAG |
| **Uh815** | CAAGTAGTGATCTATTCCAGTTGAGTCATTTTCAGTCAGGCTTCTTCGGGTCATTGTCGAGGTTTCCGGTTAGATGGTGATGGTAG |
| **Uh805** | CAAGTAGTGATCTATTCCAGTTGAGTCATTTTCAGTCAGGCTTCTTCGGGTCATTGTCGAGGTTTCCGGTTAGATGGTGATGGTAG |
| **Uh362** | CAAGTAGTGATCTATTCCAGTTGAGTCATTTTCAGTCAGGCTTCTTCGGGTCATTGTCGAGGTTTCCGGTTAGATGGTGATGGTAG |
| **Uh820** | CAAGTAGTGATCTATTCCAGTTGAGTCATTTTCAGTCAGGCTTCTTCGGGTCATTGTCGAGGTTTCCGGTTAGATGGTGATGGTAG |

**Comparison of *Uhavr1* locus sequences among seven virulent isolates, focusing on the region after the ‘breakpoint’ where Uh362 (grey) diverges from sequences in avirulent isolate Uh364 (brk, highlighted in yellow). The 10-bp repeat unit is in red. The base position is as in panel B.**
Figure S3D
Figure S4. Ali et al.

A. Diagram showing the genes C18A2, C18A3, and C18A4 with their respective deletions (ΔC18A2, ΔC18A3, and ΔC18A4) and the probes used ( Probe 3F).

B. Gel showing the wild type (wt) and ΔC18A2 samples with the probes used.

C. Gel showing the wild type (wt) and ΔC18A3 samples with the probes used.

D. Gel showing the wild type (wt) and ΔC18A4 samples with the probes used.
Figure S5. Ali et al.
Figure S6. Ali et al.

A

\text{gene 16} \quad \text{gene 17} \\
\text{10022-5F} \quad \text{10022-3F} \\
\Delta \text{UhAvr1}

B

\text{wild type Uh364} \\
\text{BgIII} \quad 1.8 \text{ kb} \\
\text{UhAvr1} \quad \text{Probe 3F} \\
\text{Uh364} \quad 1 \quad 2 \quad 3 \quad 4 \quad 5

\text{\Delta UhAvr1} \\
\text{BgIII} \quad 3.6 \text{ kb} \\
\text{Cbx} \\
\text{Uh364} \quad 1 \quad 2

C

\text{BgIII} \quad 7.5 \text{ kb} \\
\text{UhAvr1} \quad \text{GFP} \quad \text{Zeo}\text{r} \\
\text{Uh1289} \quad 1 \quad 2

\text{7.5} \quad \text{3.6} \quad 1.8
Figure S7. Ali et al.

A

Avr1 (Uh364)

BAC3-A2 (117 kb)

C18A2

(Δ38.5 kb)

B

Infected Plants (%) vs. Odessa, Hannchen, Odessa, Hannchen, Odessa, Hannchen, Odessa, Hannchen

wild type  ΔC18A2 complemented with BAC1-6  ΔC18A2
Identification of specific domains in UhAVR1p and comparison to other Ustilaginaceae effectors. Full-length UhAVR1p is 190 aa and has a calculated Mw of 21 kDa and an estimated pI of 8.17 (Protein Calculator v3.3). SignalP 4.1 predicts a 19 aa signal peptide (SP, in red) resulting in a processed protein of 18.9 kDa and an estimated pI=7.75. If 20 aa are cleaved off, then the protein is predicted as myristoylated (prediction by http://mendel.imp.ac.at/myristate/SUPLpredictor.htm). K39 (in blue) has a high probability of being a sumoylation site / SUMO protein attachment site (score 0.85 in SUMOplot Analysis Program, http://www.abgent.com/tool, and 0.967 in http://sumosp.biocuckoo.org/index.php).

A. Secondary structure prediction using SWISS-MODEL http://swissmodel.expasy.org/workspace[1]; C, coil; E, extended beta; H, helix. B. A CLUSTAL 2.1 multiple sequence alignment of UhAVR1p and three effector homologs from U. maydis and Sporisorium reilianum (um05295, um10554 and Sr10052). The RxLR motifs (highlighted) which have been implicated in membrane PI3P binding and effector uptake in other fungal and oomycete effectors, line up with the PDFR motif in UhAVR1p (orange in A).

Figure S9. Ali et al.

A

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*C* grown with zeomycin selection

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Infected plants (%)

- gene 16
- gene 16-SP
- UhAvr1
- UhAvr1-SP
Figure S10. Ali et al.

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**wild type**

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**ΔC18A2**

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B

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Infected Plants (%)
Table S1. *U. hordei* genes located on BAC3-A2 (117 kb) and their homologs in *U. maydis*

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1. Number corresponds to predicted genes in the figures
2. MIPS *U. hordei* strain Uh364 (*MAT-I*) Database gene ID number available at [http://mips.helmholtzmuenchen.de/genre/proj/MUHDB/](http://mips.helmholtzmuenchen.de/genre/proj/MUHDB/); color indicates homology / likely family members, and corresponds to Figure 3
4. Arbitrary number given to the corresponding *U. maydis* gene in the Figures
5. Reported expect value based on BLASTp [109]
percent identical amino acids over the length of the matching protein sequences
percent similar amino acids over the length of the matching protein sequences
SIMAP results of the best hit; SIMAP is a program that measures protein similarity based on identities of amino acids in homologous fragments multiplied by the length of the homologous region and divided by the protein length [63]
annotated function of *U. hordei* gene; predicted SSPs indicated with an asterisk

Table S2. Strains used in this work

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<td>wild type; Um521 [110]</td>
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**World-wide field isolates**

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Uh, *U. hordei*; Um, *U. maydis*. All mutants were generated in the Uh364 background. R, resistant to the indicated antibiotic: hyg, hygromycin B; zeo, zeomycin / zeocin; cbx, carboxin; integrative complementing plasmids are in between square brackets. Δ, deletion mutant, indicating specific gene or region.


Table S3. Pathogenicity data of *U. hordei* controls, deletion mutants and complementing transformants.

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1 For descriptions of strains and deletion mutants, see Table S2. Complementing plasmid constructs are given between square brackets, expressing the corresponding gene chimer (either linked to GFP or the HA epitope tag) from either the otef or U. maydis HSP70 promoter. –SP indicates the effector gene is lacking the predicted signal peptide sequence.

2 Pathogenicity tests are variable; ratings for the same cross should be compared with respect to infection on universal susceptible ‘Odessa’
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#, primer inventory number. F, forward; R, reverse. 3F and 5F indicates primers were used for the amplification of 3’- and 5’-ends of deleted regions. The I-SceI recognition sequence is in bold type and underlined, while only bold type represents the attB1 and attB2 sequences on the primers used for the deletion constructs. The tetranucleotide CACC in bold type indicates the sequence used for directional cloning in the pENTR/D™ Gateway plasmid (Invitrogen).
Table S5. Annotated genes in the region of the *U. maydis* 19A cluster.

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1 Number corresponds to predicted *U. maydis* genes in Figure 4
2 MIPS *U. maydis* Database gene ID number available at [http://mips.helmholtz-muenchen.de/genre/proj/ustilago/](http://mips.helmholtz-muenchen.de/genre/proj/ustilago/); color indicates homology / likely family members, and corresponds to Figure 4
3 annotated function of *U. maydis* gene; the 24 predicted SSPs are indicated with an asterisk [26]