

Figure legends

Figure S1. (A) Multiple alignments of 5'- and 3'-untranslated regions (UTRs) of the five identified dsRNA segments with respect to the coding strand (ORF sense strand). Consensus sequences and conservation rates are shown below the nucleotide sequences. (B) RNA sequencing of the Af293 strain was performed, and mapped to the genome of AfPmV1-M. The reads were mapped except for 1 to 2 bases at the very tip, which are difficult to map, indicating that the genome sequencing was correct. We also confirmed the results by viral RNA sequencing using fragmented and primer ligated dsRNA sequencing (FLDS; Urayama et al., 2016; 2018).

Figure S2. Phylogenetic analysis of the putative RNA-dependent RNA polymerase (RdRp) sequences of AfuPmV-1M and other selected mycoviruses belonging to Chrysoviridae, Totiviridae, Partitiviridae, Reoviridae, and Polymycoviridae. The phylogenetic tree was generated using the neighbor-joining (NJ) method. Datasets were subjected to 1000 bootstrap replicates. The scale bars indicate the number of replacements per site. The numbers at the branch points indicate the bootstrap percentage. The full names and sequence information are given in Table S1.

Figure S3. (A) Comparison of dsRNA bands between the AfuPmV-1M-infected strain (lane V) and the virus-free strain (lane F) by agarose gel electrophoresis. Lane M, molecular weight marker. (B) Comparison of dsRNA amounts between the AfuPmV-1M-infected strain and the virus-free strain by RNA sequencing. For Virus-free strain all the expression were undetectable levels.

Figure S4. Correlation of differential gene expression between virus-infected (Virus) and virus-free (Free) strains analyzed by RNA-seq. A and B: Scatter plots of transcript levels (RPKMs) at the swelling stage (A: 4 h) and the hyphal stage (B: day 6). C: Transition of gene expression patterns in the virus-infected (circles) and virus-free (triangles) strains during the swelling stage (4 h, white) and hyphal stage (day 6, black). The pattern was examined using principal component analysis (PCA).

Figure S5. Functional catalog analysis of down-regulated (A) and up-regulated (B) genes at 4 h in the swelling stage and down-regulated (C) and up-regulated (D) genes on 6 day in conidia-forming stage. Common genes down-regulated (E) and up-regulated (F) at 4 h and day 6. The analysis was performed using the FungiFun web application (Priebe et al.,

2011). Genes which had more than two-fold reductions between the virus-infected and virus-free strains were analyzed. Detailed data underlying Figure S5 are attached as an MS Excel file (Table S2).

Figure S6. HPLC analysis of gliotoxin and fumagillin production by *A. fumigatus* on day 6 of the mycelial stage. Gliotoxin in the supernatant and fumagillin in mycelia were detected by absorbance at wavelengths of 254 and 330 nm, respectively.

Figure S7. Viral gene expression in each transformants during sporulation.

Figure S8. Comparisons of the whole-virus infected KU strain and the native virus-free KU. A-B. Radial growth of colonies. 2 strain of whole-virus infected KU strain (#2 and #3) were tested. C-D. Growth of each strain under stress conditions. Whole-virus infected KU strain #3 was used for these analysis.

Table S1. Information on the virus isolates used for sequence alignment and phylogenetic analysis of their RdRps.

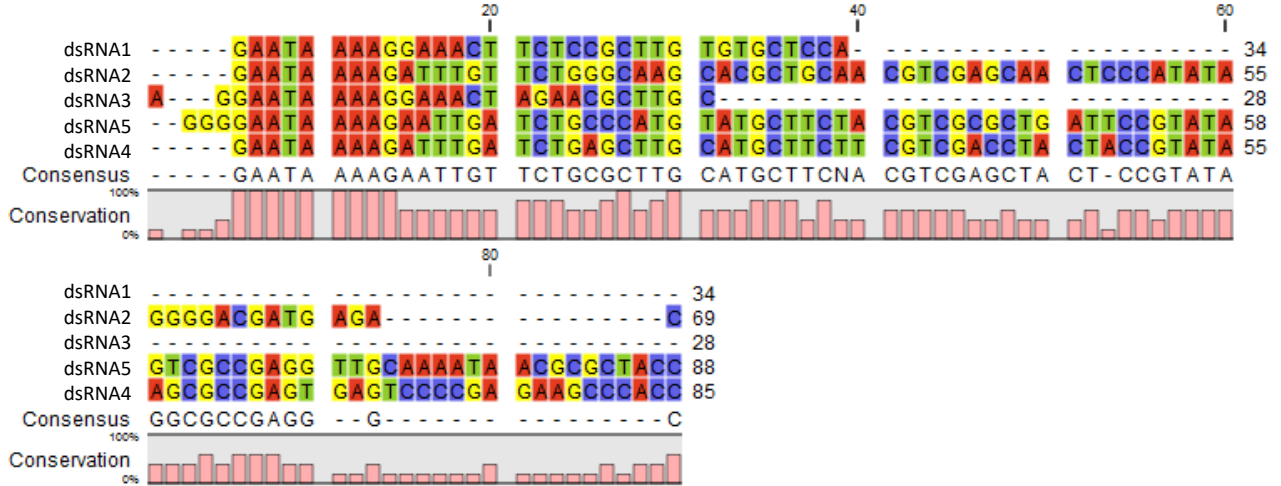
Table S2. RNA-seq analysis data to compare gene expression between virus-infected and virus-free strains at two distinct time points: just before the start of swelling (4 h after the start of incubation, a stage referred to here as the 4-h swelling stage) and at the hyphal stage (day 6). (A) 4h down. Genes that are down-regulated in virus-infected strains compared to virus-free strains at 4h. (B) 4h Up. (C) 6days down. (D) 6days up. (E) Common genes down-regulated at 4h and day 6. (F) Common genes up-regulated at 4 h and day 6. Subsequently, GO data is also attached.

Table S3. Summary of results comparing phenotypic changes in *A. fumigatus* strains infected with the AfuPmV-1M virus (native virus) or transformed with plasmids by ectopic expression of each AfuPmV-1M ORF (ORF1–5). Downward and upward arrows indicate decreases and increases, respectively, in comparison with the respective control (virus-free strain or strain transformed with an empty vector). Effects are indicated only when statistically significant ($p < 0.05$).

Table S4. Primers used in the present study.

Figure S1A

5'-terminus



3'-terminus

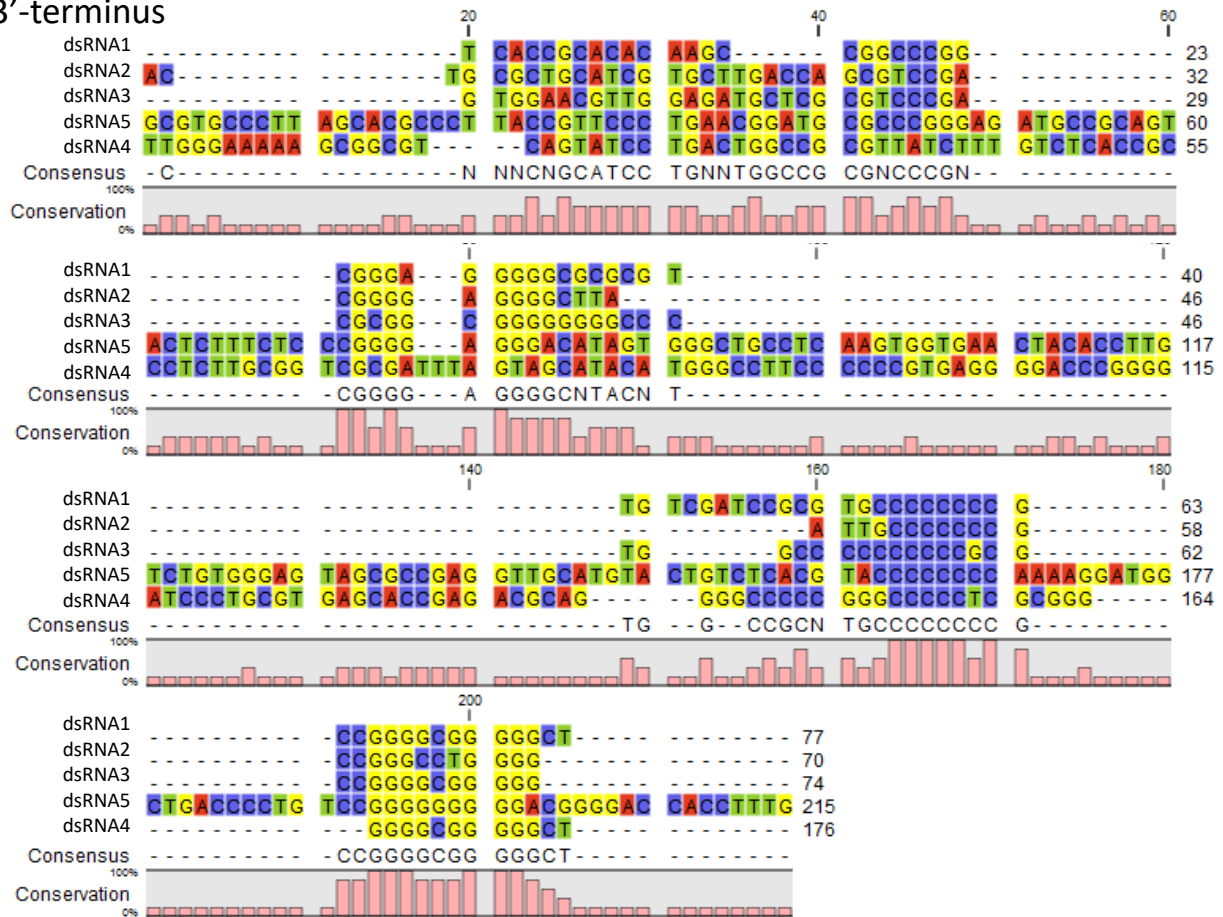


Figure S1B

| Name | Consensus length | Total read count | Average coverage | Reference length | Coverage length ratio (%) |
|-------------------|-------------------------|-------------------------|-------------------------|-------------------------|----------------------------------|
| AfuPmV1M1 mapping | 2402 | 22865 | 999 | 2403 | 100.0 |
| AfuPmV1M2 mapping | 2230 | 67045 | 3058 | 2230 | 100.0 |
| AfuPmV1M3 mapping | 1970 | 33402 | 1755 | 1971 | 99.9 |
| AfuPmV1M5 mapping | 1156 | 9435 | 814 | 1156 | 100.0 |
| AfuPmV1M4 mapping | 1133 | 29937 | 2727 | 1140 | 99.4 |

Figure S2

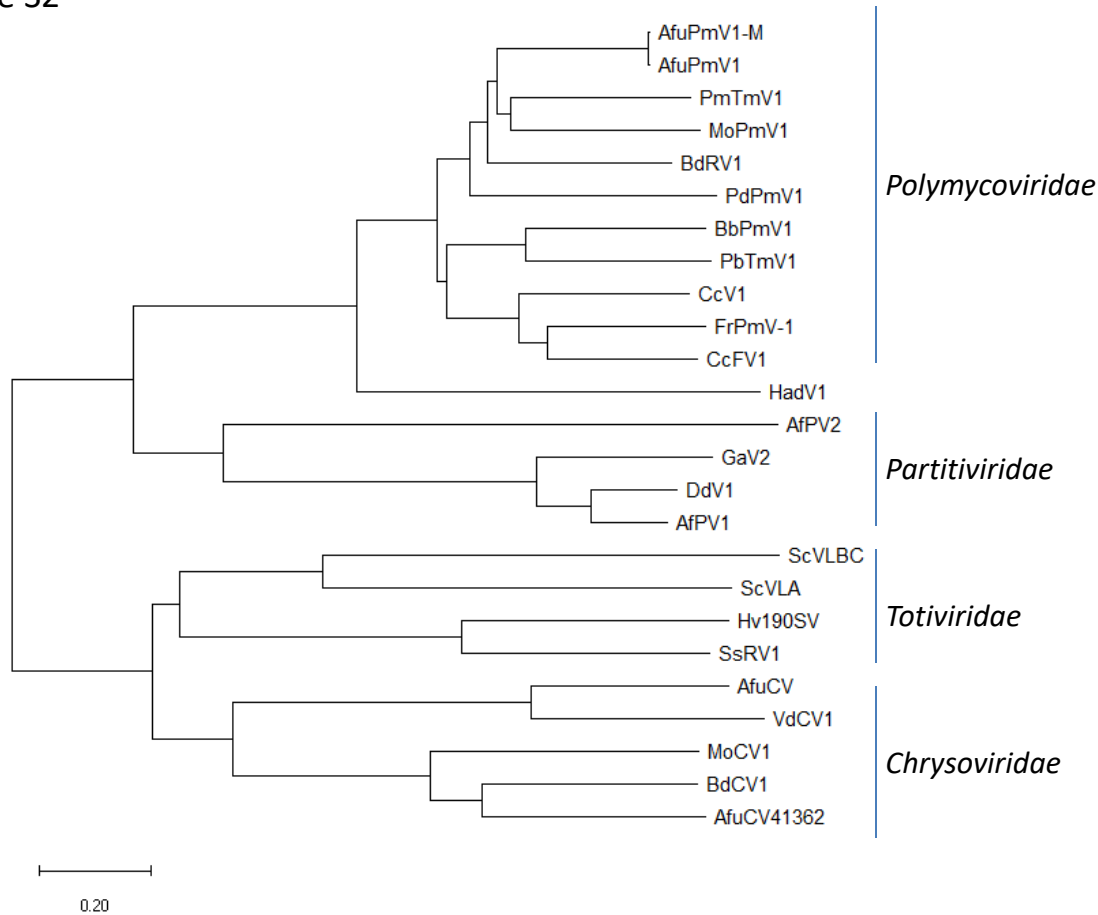


Table S1 Information on the virus isolates used for sequence alignment and phylogenetic analysis of their RdRps.

| Virus name | Abbreviation | GenBank accession no. | Family |
|--|---------------------|------------------------------|------------------------|
| Aspergillus fumigatus chrysovirus | AfuCV | CAX48749 | Chrysoviridae |
| AfuCV41362 | AfuCV41362 | LC350094 | Chrysoviridae |
| Botryosphaeria dothidea chrysovirus 1 | BdCV1 | AGZ84312.1 | Chrysoviridae |
| Magnaporthe oryzae chrysovirus 1 | MoCV1 | AB560761 | Chrysoviridae |
| Verticillium dahliae chrysovirus 1 | VdCV1 | ADG21213.1 | Chrysoviridae |
| Aspergillus fumigatus partitivirus 1 | AfPV1 | CAY25801 | Partitiviridae |
| Aspergillus fumigatus partitivirus 2 | AfPV2 | YP_009553311 | Partitiviridae |
| Discula destrucriva virus 1 | DdV1 | AAG59816 | Partitiviridae |
| Grapevine associated Partitivirus-2 | GaV2 | ADU86896 | Partitiviridae |
| Aspergillus spelaeus tetramycovirus 1 | AsTmV1 | AYP71805 | Polymycovirus |
| Phaeoacremonium minimum tetramycovirus 1 | PmTmV1 | QDB74985 | Polymycovirus |
| Aspergillus fumigatus polymycovirus-1 | AfuPmV1 | HG975302 | Polymycovirus |
| Aspergillus fumigatus polymycovirus-1M | AfuPmV1-M | LC517041 | Polymycovirus |
| Cladosporium cladosporioides virus 1 | CcV1 | YP_009052470 | Polymycovirus |
| Penicillium brevicompactum tetramycovirus 1 | PbTmV1 | AYP71801 | Polymycovirus |
| Colletotrichum camelliae filamentous virus 1 | CcFV1 | ASV63092 | Polymycovirus |
| Beauveria bassiana polymycovirus 1 | BbPmV1 | CUS18595 | Polymycovirus |
| Fusarium redolens polymycovirus 1 | FrPmV1 | QDH44656 | Polymycovirus |
| Botryosphaeria dothidea virus 1 | BdRV1 | AKE49495 | Polymycovirus |
| Magnaporthe oryzae polymycovirus 1 | MoPmV1 | QAU09249 | Polymycovirus |
| Penicillium digitatum polymycoviruses 1 | PdPmV1 | YP_009551548 | Polymycovirus |
| Helminthosporium victoriae virus 190S | Hv190SV | NP_619670 | Totiviridae |
| Saccharomyces cerevisiae virus L-A | ScVLA | NP_620495 | Totiviridae |
| Saccharomyces cerevisiae virus L-BC | ScVLBC | NP_042581 | Totiviridae |
| Sphaeropsis sapinea RNA virus 1 | SsRV1 | NP_047558 | Totiviridae |
| Hadaka virus 1 | HadV1 | BBU94038 | Unclassified Riboviria |

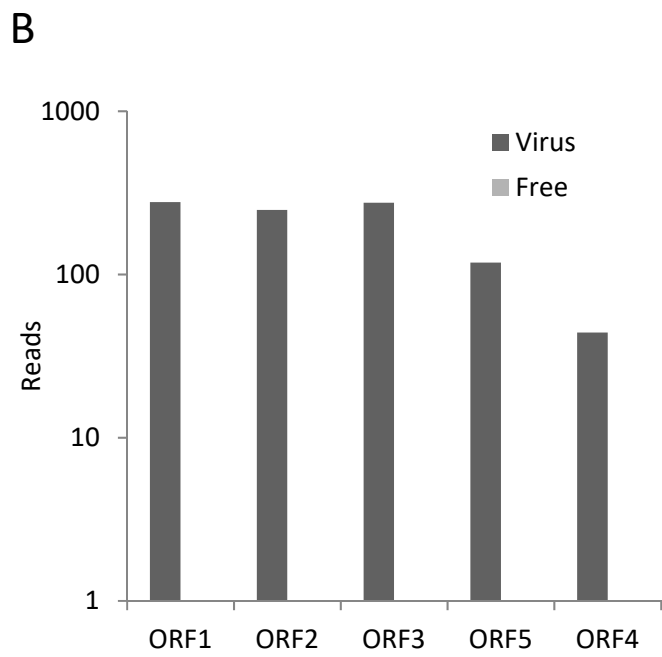
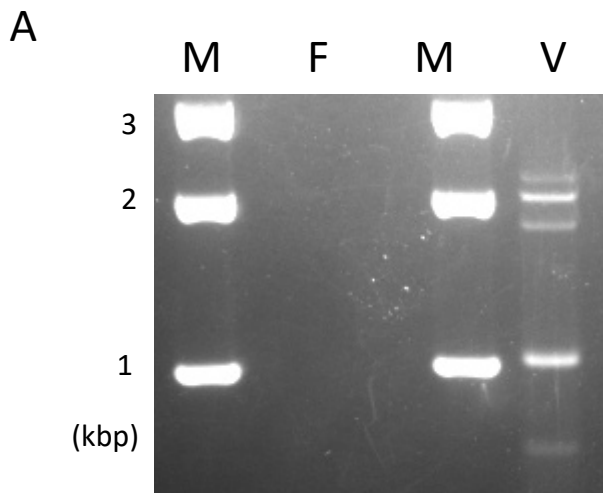
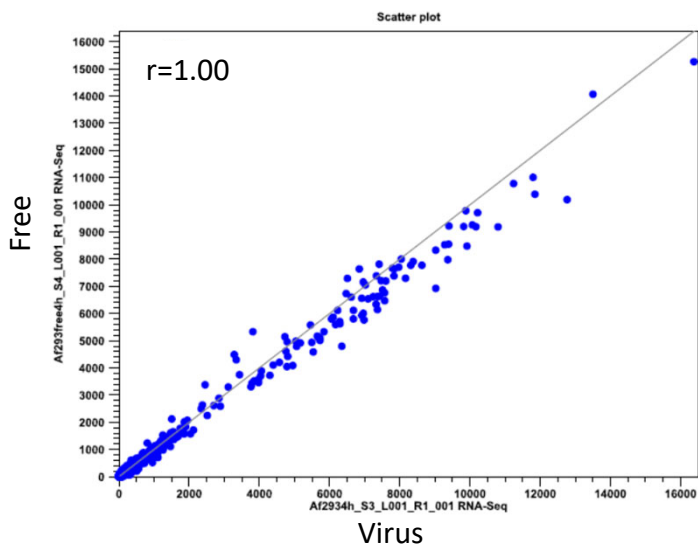
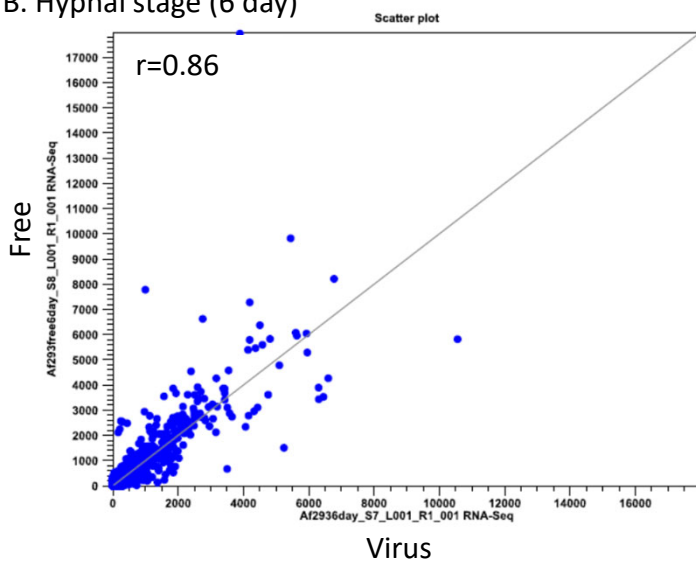


Figure S3

A. Swelling stage (4 h)



B. Hyphal stage (6 day)



C. Principal component analysis (PCA)

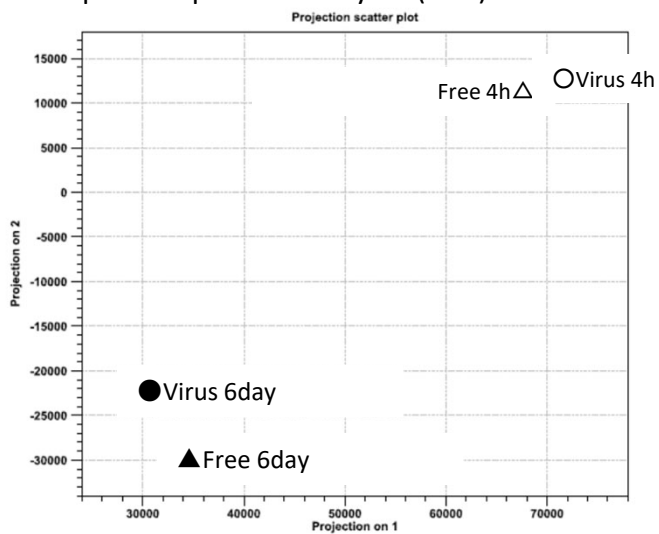
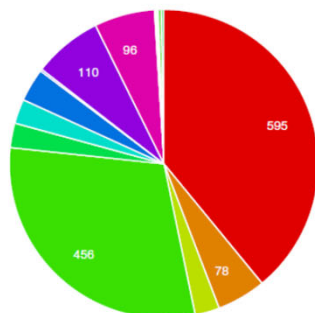


Figure S4.

Figure S5

A. 4h down

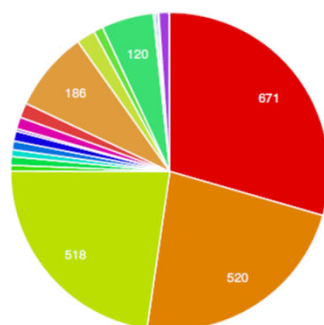
All (15) significantly enriched categories
Method: GO



- cellular_component
- zinc ion binding
- sequence-specific DNA binding
- RNA polymerase II transcription factor activity
- biological_process
- transcription from RNA polymerase II promoter
- regulation of transcription from RNA polymerase II promoter
- regulation of transcription, DNA-templated
- signal peptide processing
- oxidation-reduction process
- oxidoreductase activity
- molybdopterin synthase activity
- mating-type factor pheromone receptor activity
- N-acetylglucosamine-6-phosphate deacetylase activity
- damaged DNA binding
- cellular aromatic compound metabolic process

B. 4h up

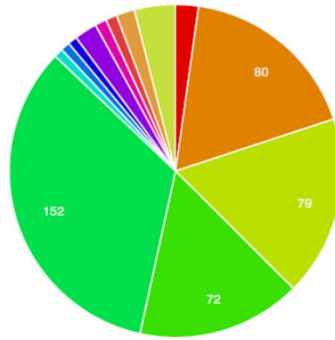
20 most significantly enriched categories
Method: GO



- cellular_component
- biological_process
- molecular_function
- carbohydrate transport
- transmembrane transporter activity
- substrate-specific transmembrane transporter activity
- transporter activity
- heme binding
- microtubule binding
- hydrolase activity, hydrolyzing O-glycosyl compounds
- hydrolase activity, acting on glycosyl bonds
- metabolic process
- carbohydrate metabolic process
- monooxygenase activity
- oxidation-reduction process
- microtubule-based movement
- microtubule motor activity
- glutathione biosynthetic process
- iron ion binding
- gamma-glutamylcyclotransferase activity

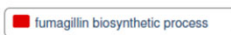
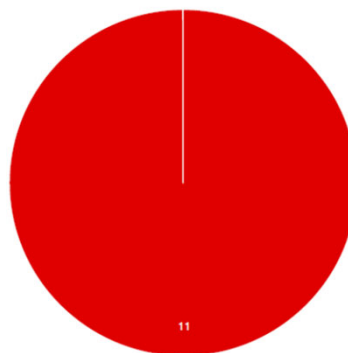
C. 6days down

All (13) significantly enriched categories
Method: GO



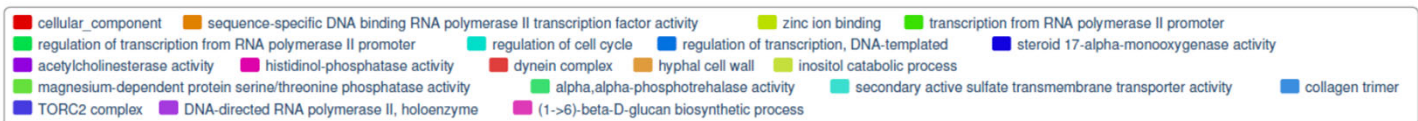
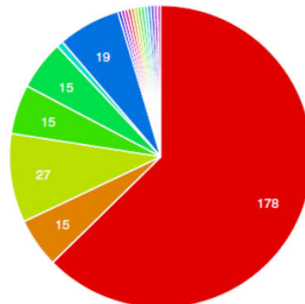
D. 6days up

All (1) significantly enriched categories
Method: GO



E. Common genes down-regulated at 4h and day 6.

20 most significantly enriched categories
Method: GO



F. Common genes up-regulated at 4 h and day 6.

20 most significantly enriched categories
Method: GO

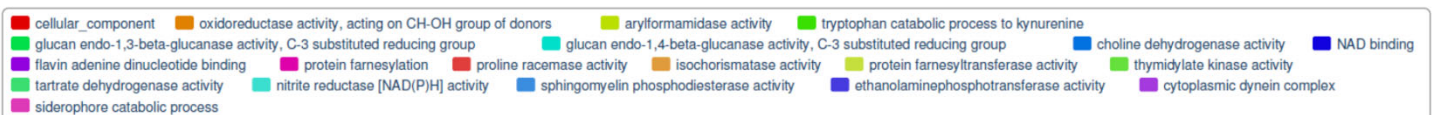
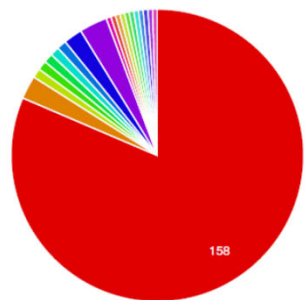
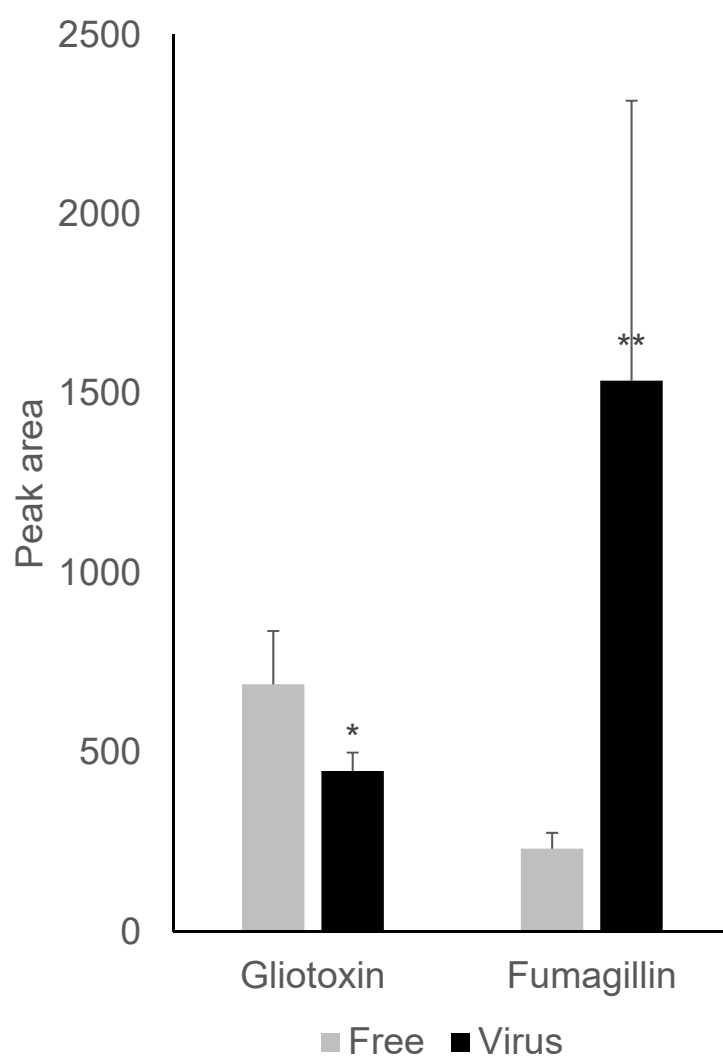


Figure S6



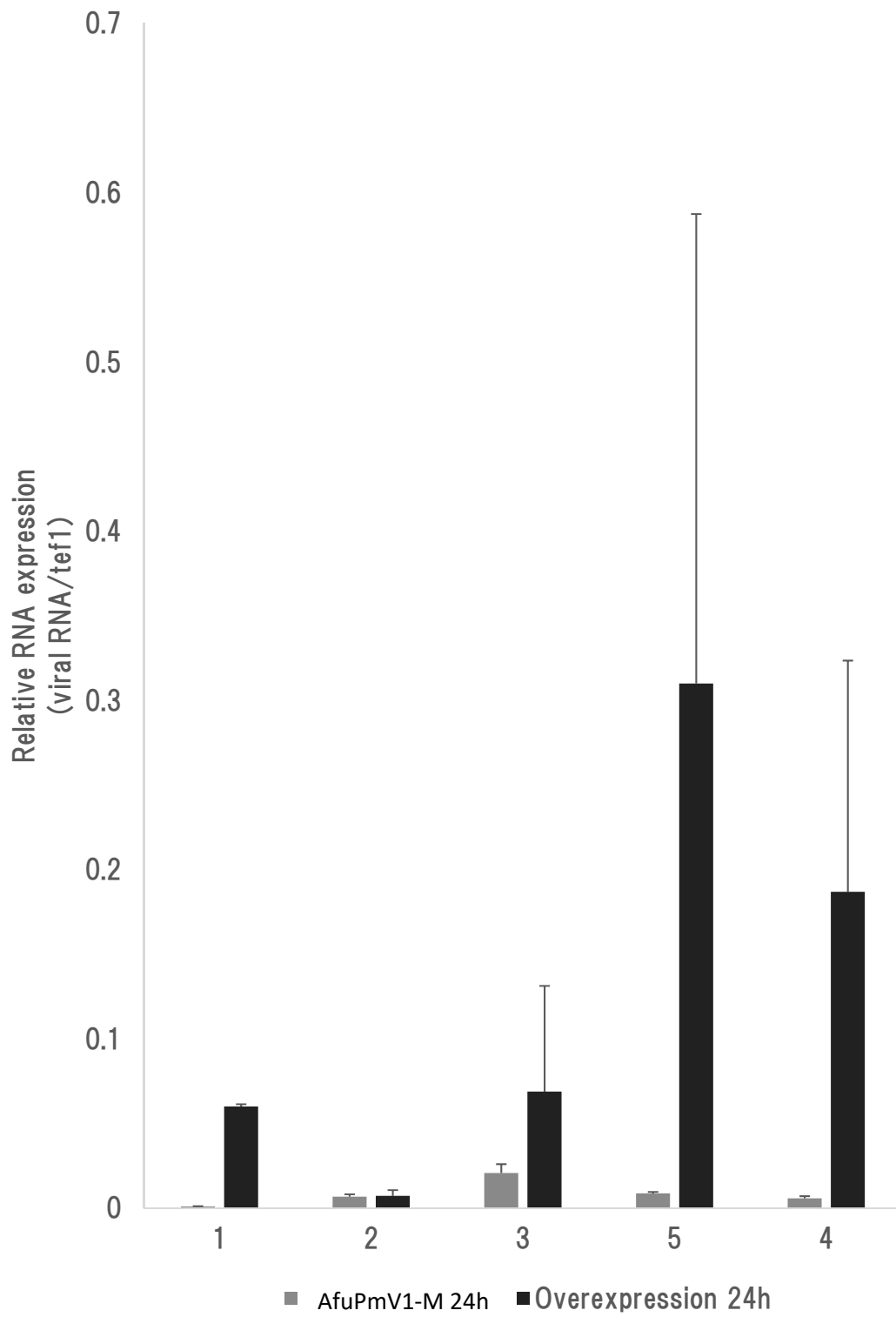


Figure S7

Table S3

| Parameters | | Virus | ORF | | | | | |
|-------------------------------|--------------------------------|-------------------------------|-----|---|---|---|---|---|
| | | | 1 | 2 | 3 | 5 | 4 | |
| Fungal phenotype | Influence at sporulation stage | Number of conidia | ↓ | | ↓ | | | |
| | | Tolerance to oxidative stress | ↓ | | ↓ | ↓ | | |
| | | Tolerance to osmotic stress | ↓ | | | ↓ | ↓ | |
| | | Adherence to lung cells | ↓ | ↓ | ↓ | | | |
| | | Sensitivity to phagocytosis | ↓ | | | ↓ | | |
| | Influence at germination stage | Swelling of conidia (6 h) | ↓ | ↓ | | ↓ | | |
| | | Germination of conidia (8 h) | ↓ | | | | | ↓ |
| | Influence to mycelial growth | Radial growth (on agar plate) | ↓ | | ↓ | ↓ | | |
| | | Dry weight (in fluid medium) | ↓ | | | | | |
| | | Damage to lung cells (LDH) | | | | ↑ | | ↑ |
| Influence to fungal virulence | Mouse lung CFU | ↓ | | ↓ | | ↓ | | |

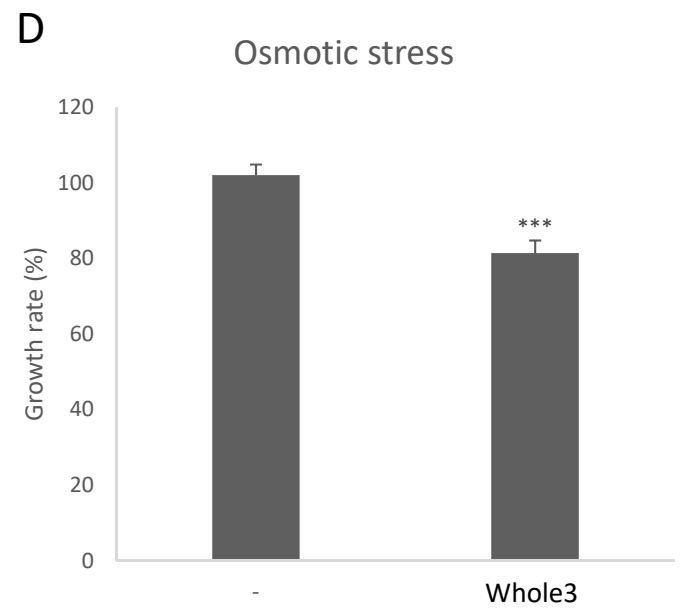
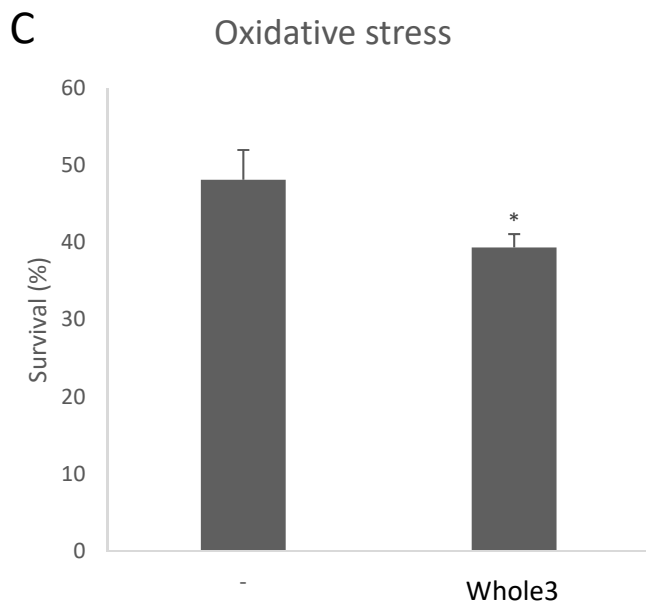
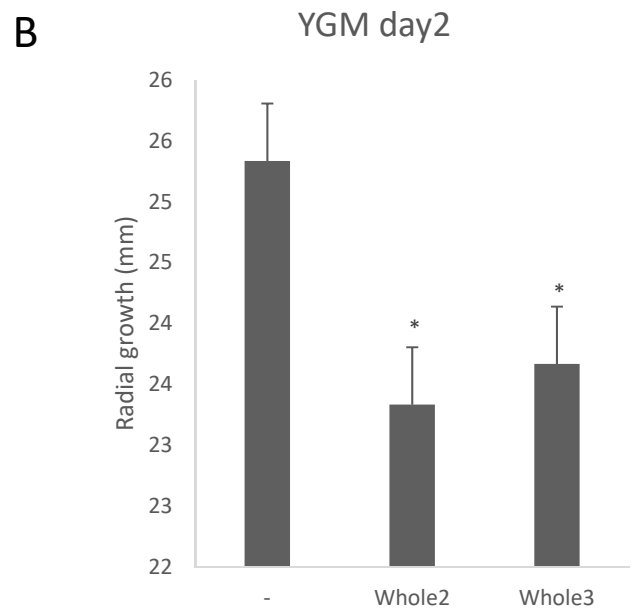
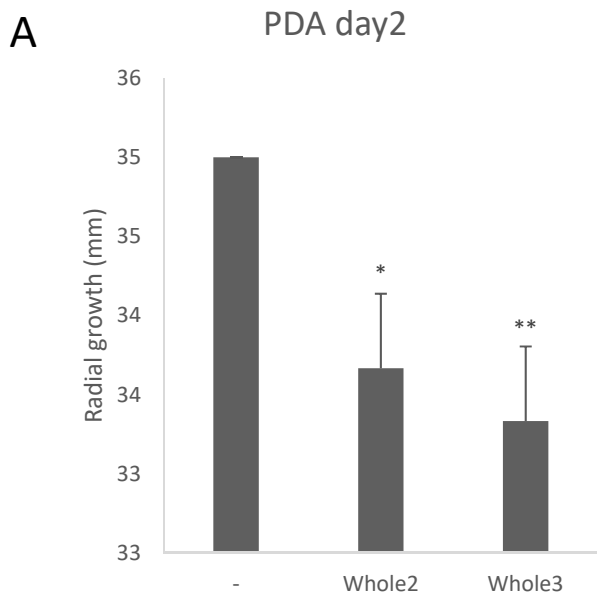


Figure S8