Supplementary information

Table S1. 2–h and 6–h common C17 mycosubtilin response DEGs. Sheet 1. Genes related to membrane components. Sheet 2. Genes related to DNA replication. Sheet 3. Genes related to the cell cycle. Sheet 4. Genes related to hydrolase activity. Sheet 5. For genes related to oxidoreductase activity, the red font represents the gene enriched in the dioxygenase activity term. Sheet 6. Genes related to energy metabolism.

Table S2. 2–h specific C17 mycosubtilin response DEGs. Sheet 1. Genes related to hydrolase activity. Sheet 2. Genes related to energy metabolism.

Table S3. 6–h specific C17 mycosubtilin response DEGs. Sheet 1. Genes related to transmembrane transport. Sheet 2. Genes related to translation. Sheet 3. Genes related to energy metabolism.

Table S4. Primers used for qRT–PCR.

Figure S1. C17 mycosubtilin affects the spore viability and hyphal microscopic morphology of Vd 991. (A) Growth of Vd 991 in Czapek–Dox broth medium containing different concentrations of C17 mycosubtilin. (B) Vd 991 colony area statistics after growing in different concentrations of C17 mycosubtilin for 72 h. In this experiment, the blank control (CK) group was only supplemented with Czapek–Dox Broth medium but without C17 mycosubtilin and spores; the control group was only supplemented with Czapek–Dox Broth medium and spores but without C17 mycosubtilin. The minimum concentration that inhibited the growth of Vd 991 for 72 h was defined as the MIC. The IC50 was defined as the concentration at which the

colony growth area of Vd 991 was half of that of the control at 72 h. Statistical analyses were performed by t test. The data represent the mean ± SD, n ≥ 3. ns indicates no significant difference, and **** indicates extreme significance at P < 0.0001. (C,D) SEM images of Vd 991 hyphae. Vd 991 hyphae was treated with C17 mycosubtilin at IC50 (C) and MIC (D) concentrations at different times. a, untreated Vd 991 hyphae (control); b1-e1, Vd 991 hyphae treated for 6 h, 12 h, 18 h, and 24 h, respectively. The red-framed area is the area displayed in the next row (b2-e2, and b3-e3, respectively).

Figure S2. The effect of C17 mycosubtilin at the MIC concentration on the morphology and structure of different organelles of Vd 991. (A) TEM images of Vd 991 spores. a, d, untreated Vd 991 spores (control); b, e, Vd 991 spores treated with C17 mycosubtilin for 2 h at MIC concentrations; c, f, Vd 991 spores treated with C17 mycosubtilin for 6 h at MIC concentrations. M, mitochondria; N, nucleus; Ld, lipid droplets; CW, cell wall; dCW, damaged cell wall; PM, plasma membrane; dPM, damaged plasma membrane. (B) Mitochondrial aspect ratio chart. The aspect ratio of spore mitochondria treated with the MIC concentration of C17 mycosubtilin for 0 h, 2 h, and 6 h was calculated. At least 30 mitochondria in each group were counted in each experiment, and the experiment was repeated 3 times. Statistical analyses were performed using one-way ANOVA. No less than 30 mitochondria per group were counted, and the values are the mean ± SD. *** indicates extreme significance at p < 0.001.

Figure S3. The conidial necrosis of Vd 991 was analysed by flow cytometry after treatment with C17 mycosubtilin. (A) The spore population of Vd 991 after treatment

with C17 mycosubtilin at the MIC concentration at different times by annexin V-FITC/PI double staining. a, untreated Vd 991 spores (control); b–e, Vd 991 spores treated for 2 h, 6 h, 12 h, and 24 h, respectively; f, summary of spore population data. Each dot represents a cell, and the color represents the cell density. (B) Vd 991 spores treated with C17 mycosubtilin at the MIC concentration at different times by PI staining. a, Untreated Vd 991 spores (control); b–e, Vd 991 spores treated for 2 h, 6 h, 12 h, and 24 h, respectively; f, statistical diagram of spore staining rate at different treatment times. Statistical analysis in this figure is based on one-way ANOVA. Values are mean ± SD, where $n \geq 3$; ns means no significant difference, *** means significant difference at $p < 0.001$, and **** means significant difference at $p < 0.0001$.

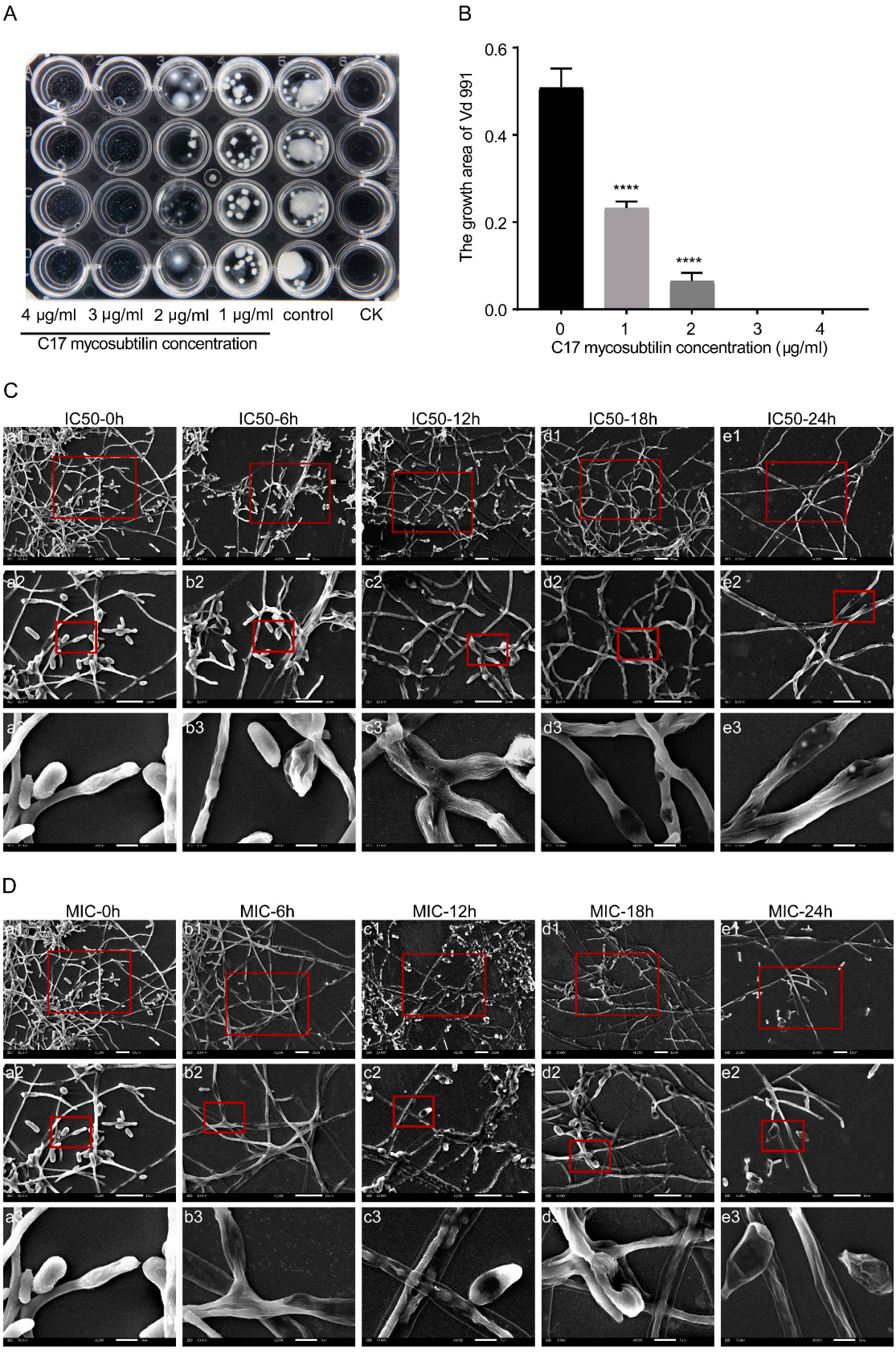
Figure S4. Assessment of RNAseq sequencing data quality. (A,B) Principal component analysis (PCA) of transcriptomic samples. Dots of the same color represent each biological repetition in the group, and the distance between dots represents the overall expression difference of the samples; PC1, PC2, and PC3 represent different principal component dimensions, and the numbers in parentheses represent the interpretation degree of principal components. The higher the degree of component interpretation, the greater the overall difference of the samples in the dimension of the principal component vector. (C) Heatmap of the correlation coefficient between samples. M0H6 and M0H2 indicated that the spores grew naturally in Czapek–Dox broth medium without C17 mycosubtilin treatment for 6 h and 2 h, respectively; M3H6 and M3H2 indicated that the spores grew in Czapek–Dox broth medium containing C17 mycosubtilin at the IC50 concentration for 6 h and 2 h, respectively.

Figure S5. Effect of C17 mycosubtilin on gene expression in Vd 991 spores presented with gene expression heatmap. (A) DNA replication-related genes (2–h and 6–h common DEGs). (B) Genes related to the mitotic cell cycle process (2–h and 6–h common DEGs). (C) Downregulated genes related to the cell wall and cell membrane (2–h and 6–h common DEGs). (D) Upregulated genes related to the cell wall and cell membrane (2–h and 6–h common DEGs). (E) Genes related to the translation process (6–h specific DEGs). (F) Downregulated genes related to transmembrane transport activity (6–h specific DEGs). (G) Upregulated genes related to transmembrane transport activity (6–h specific DEGs). M0H6 and M0H2 indicated that the spores grew naturally in Czapek–Dox broth medium without C17 mycosubtilin treatment for 6 h and 2 h, respectively; M3H6 and M3H2 indicated that the spores grew in Czapek–Dox broth medium containing C17 mycosubtilin at the IC50 concentration for 6 h and 2 h, respectively.

Figure S6. Effect of C17 mycosubtilin on gene expression in Vd 991 spores presented with gene expression heatmap. (A) Upregulated genes related to hydrolase activity (2–h and 6–h common DEGs). (B) Downregulated genes related to hydrolase activity (2–h and 6–h common DEGs). (C) Downregulated genes related to oxidoreductase activity (2–h and 6–h common DEGs). (D) Upregulated genes related to oxidoreductase activity (2–h and 6–h common DEGs). (E). Upregulated genes related to hydrolase activity (2–h specific DEGs). (F). Downregulated genes related to hydrolase activity (2–h specific DEGs). M0H6 and M0H2 indicated that the spores grew naturally in Czapek–Dox broth medium without C17 mycosubtilin treatment for

6 h and 2 h, respectively; M3H6 and M3H2 indicated that the spores grew in Czapek–Dox broth medium containing C17 mycosubtilin at the IC50 concentration for 6 h and 2 h, respectively.

Figure S1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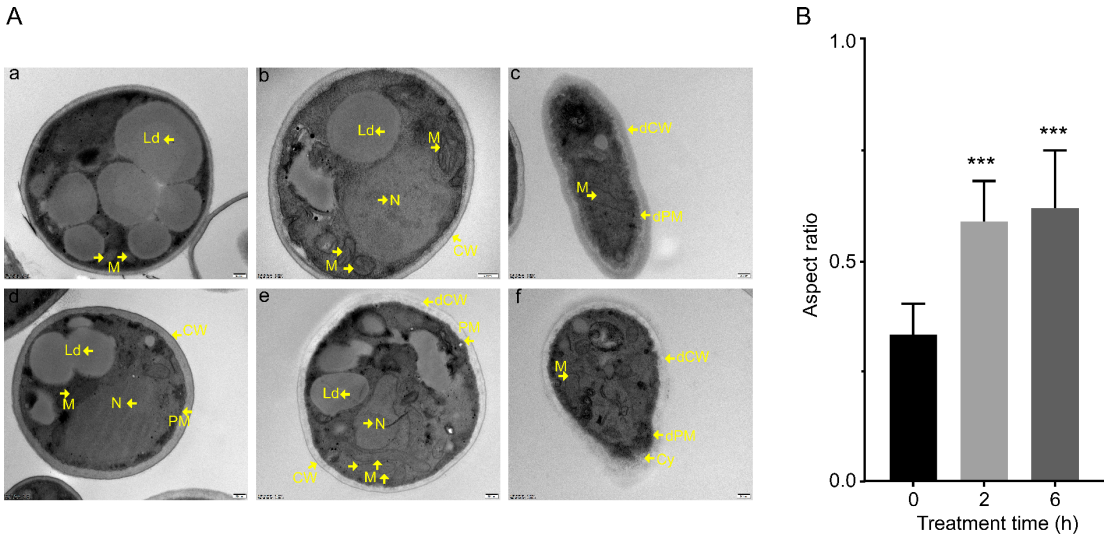
4 μg/ml
3 μg/ml
2 μg/ml
1 μg/ml
control
CK
C17 mycosubtilin concentration
B
The growth area of Vd 991
0.6
0.4
0.2
0.0
****
****
0
1
2
3
4
C17 mycosubtilin concentration (μg/ml)
C
IC50-0h
IC50-6h
IC50-12h
IC50-18h
IC50-24h
D
MIC-0h
MIC-6h
MIC-12h
MIC-18h
MIC-24h

Figure S2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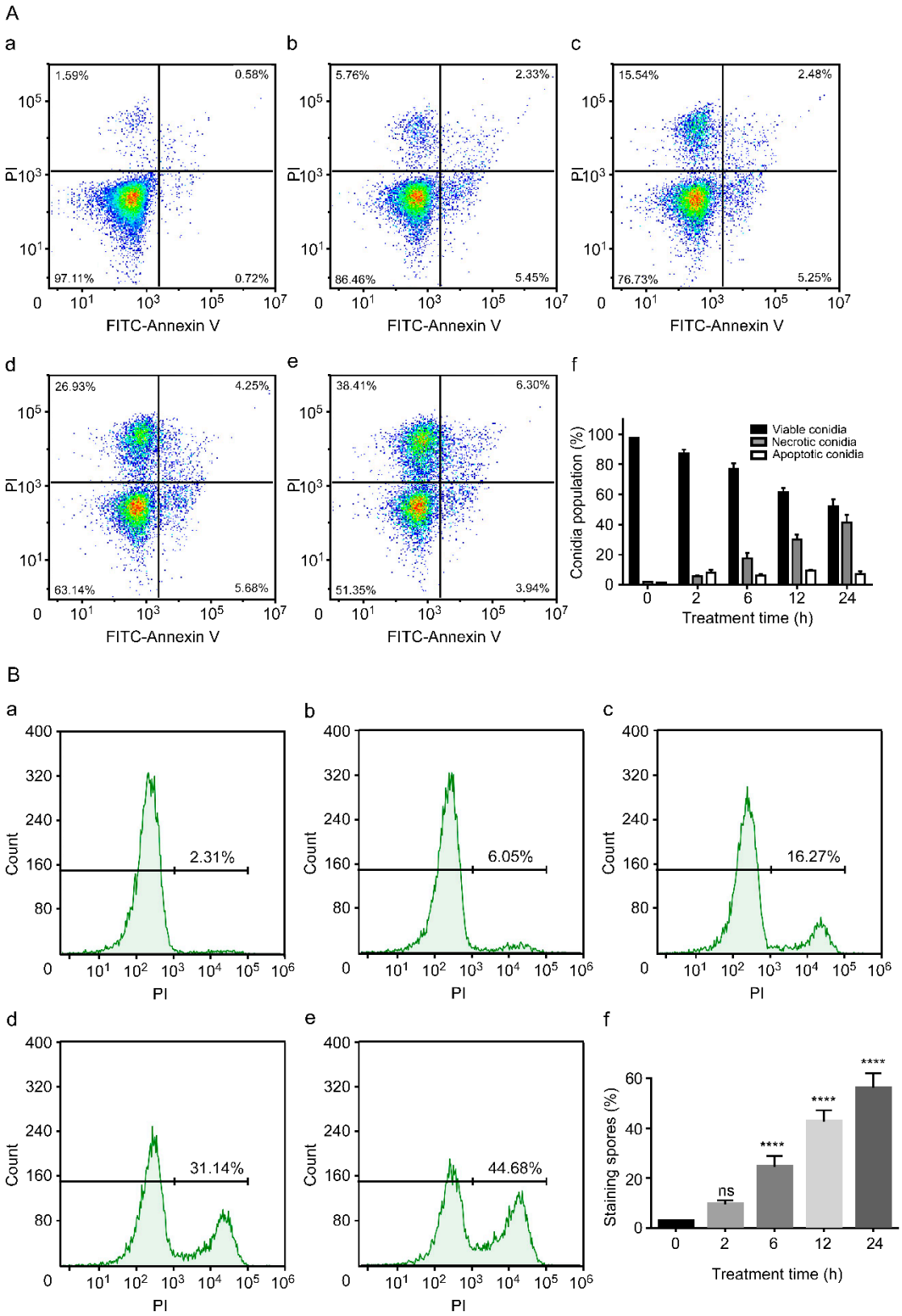
a
Ld
M
b
Ld
M
N
CW
M
c
dCW
M
dPM
d
CW
Ld
M
N
PM
e
dCW
PM
Ld
N
CW
M
f
M
dCW
dPM
Cy
B
1.0
0.5
0.0
Aspect ratio
***
***
0
2
6
Treatment time (h)

Figure S3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a
1.59%
0.58%
97.11%
0.72%
b
5.76%
2.33%
86.46%
5.45%
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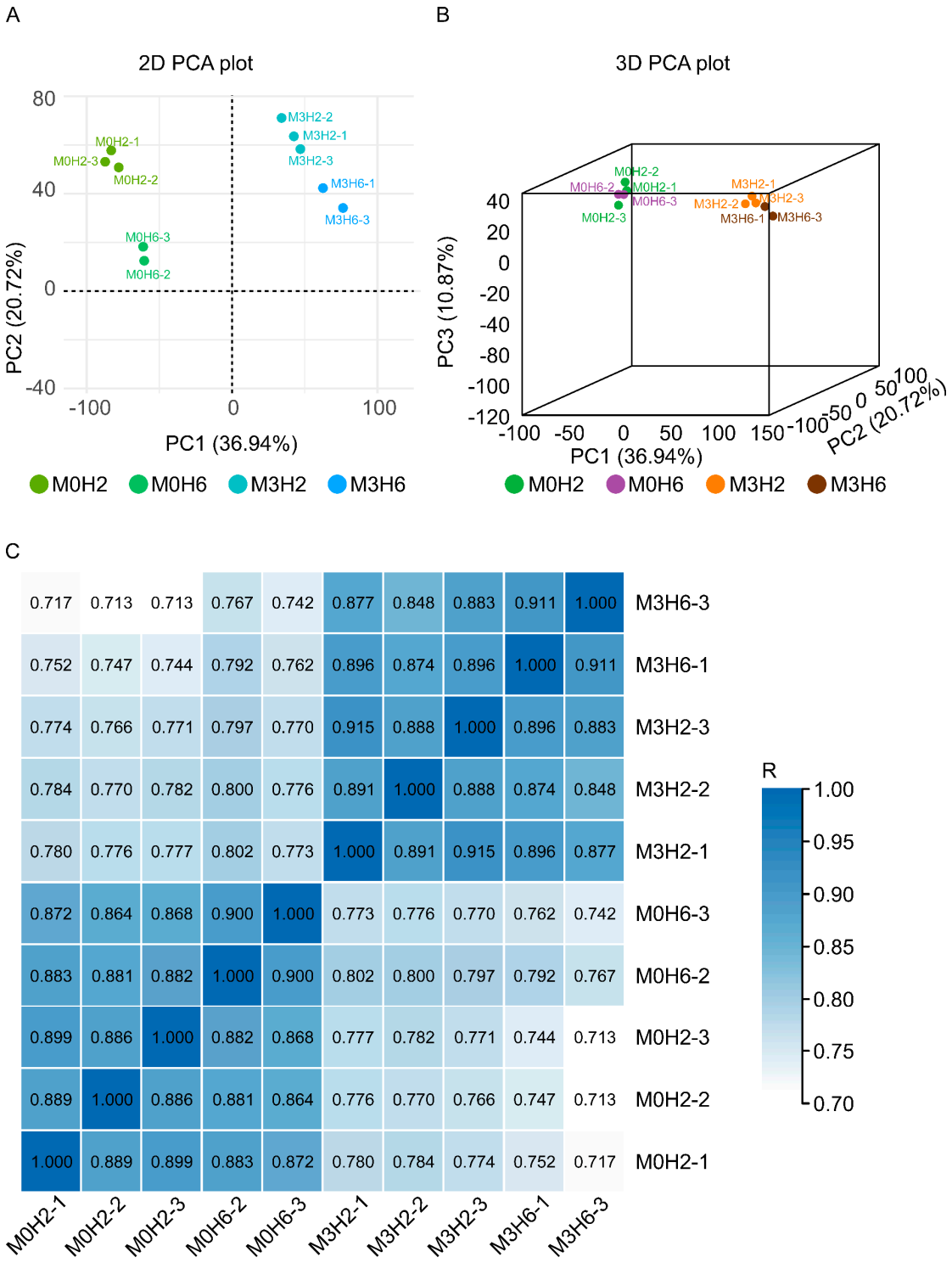
15.54%
2.48%
76.73%
5.25%
d
26.93%
4.25%
63.14%
5.68%
e
38.41%
6.30%
51.35%
3.94%
PI
FITC-Annexin V
f
Viable conidia
Necrotic conidia
Apoptotic conidia
Conidia population (%)
Treatment time (h)
B
a
2.31%
b
6.05%
c
16.27%
d
31.14%
e
44.68%
Count
PI
f
Staining spores (%)
ns
****
Treatment time (h)

Figure S4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PC2 (20.72%)
PC1 (36.94%)
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M0H2-3
M0H2-2
M0H6-3
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M3H2-2
M3H2-1
M3H2-3
M3H6-1
M3H6-3
M0H2
M0H6
M3H2
M3H6
B
3D PCA plot
PC3 (10.87%)
PC1 (36.94%)
PC2 (20.72%)
M0H2
M0H6
M3H2
M3H6
C
R
1.00
0.95
0.90
0.85
0.80
0.75
0.70
M0H2-1
M0H2-2
M0H2-3
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M3H6-3

Figure S5

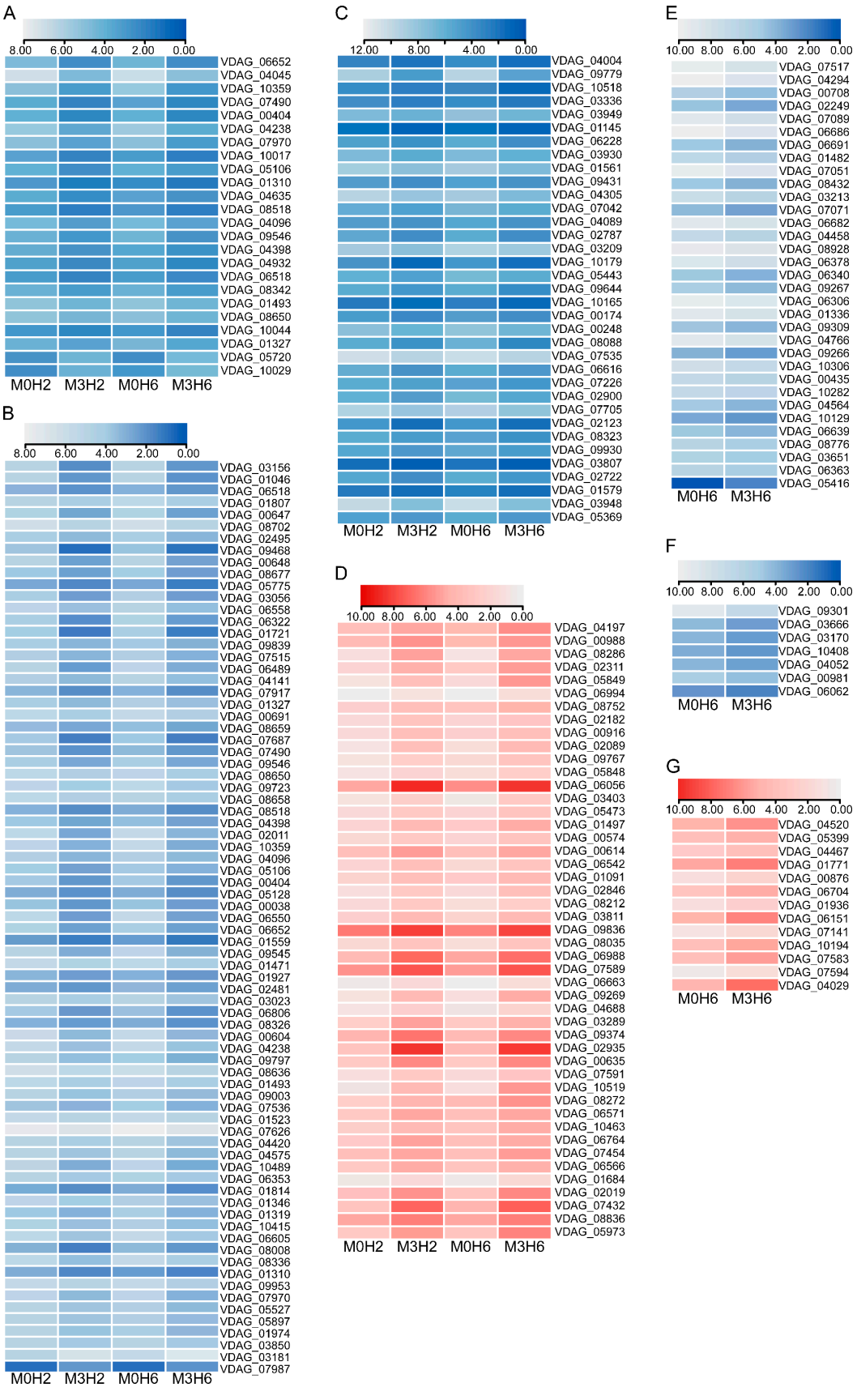
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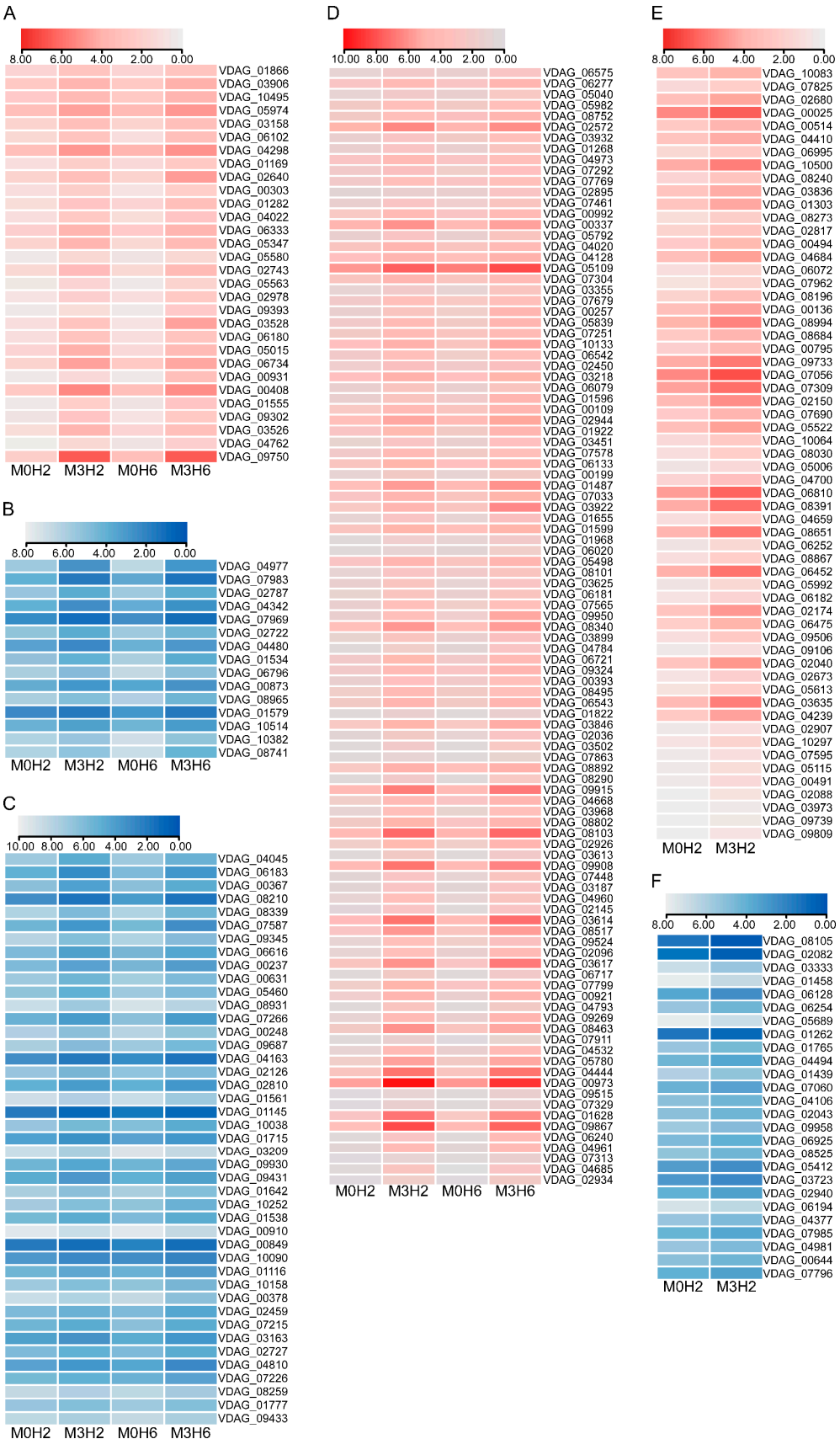
Figure S6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