

RNA-Seq-Based Transcriptome Analysis of Nitric Oxide Scavenging Response in *Neurospora crassa*

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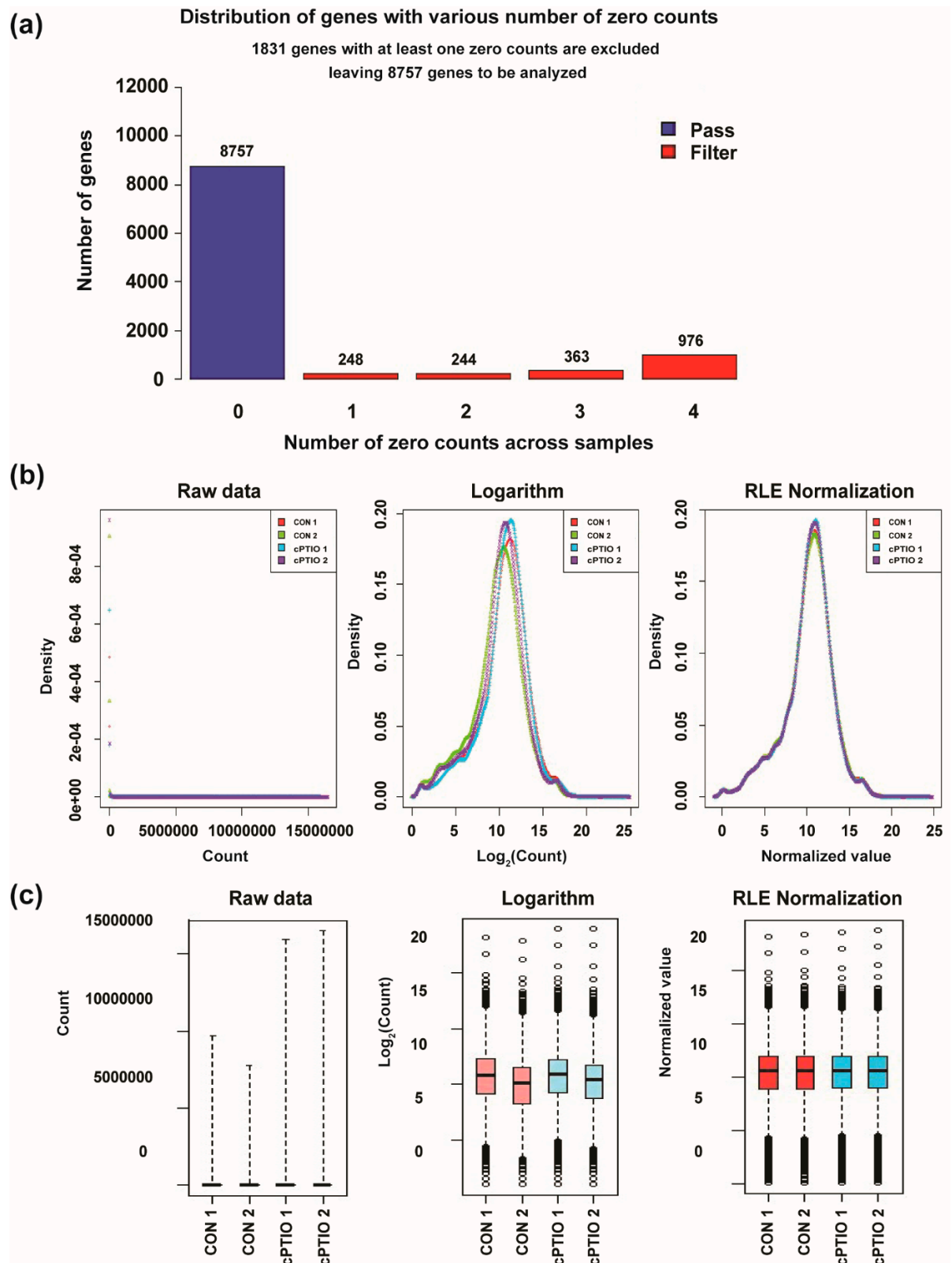
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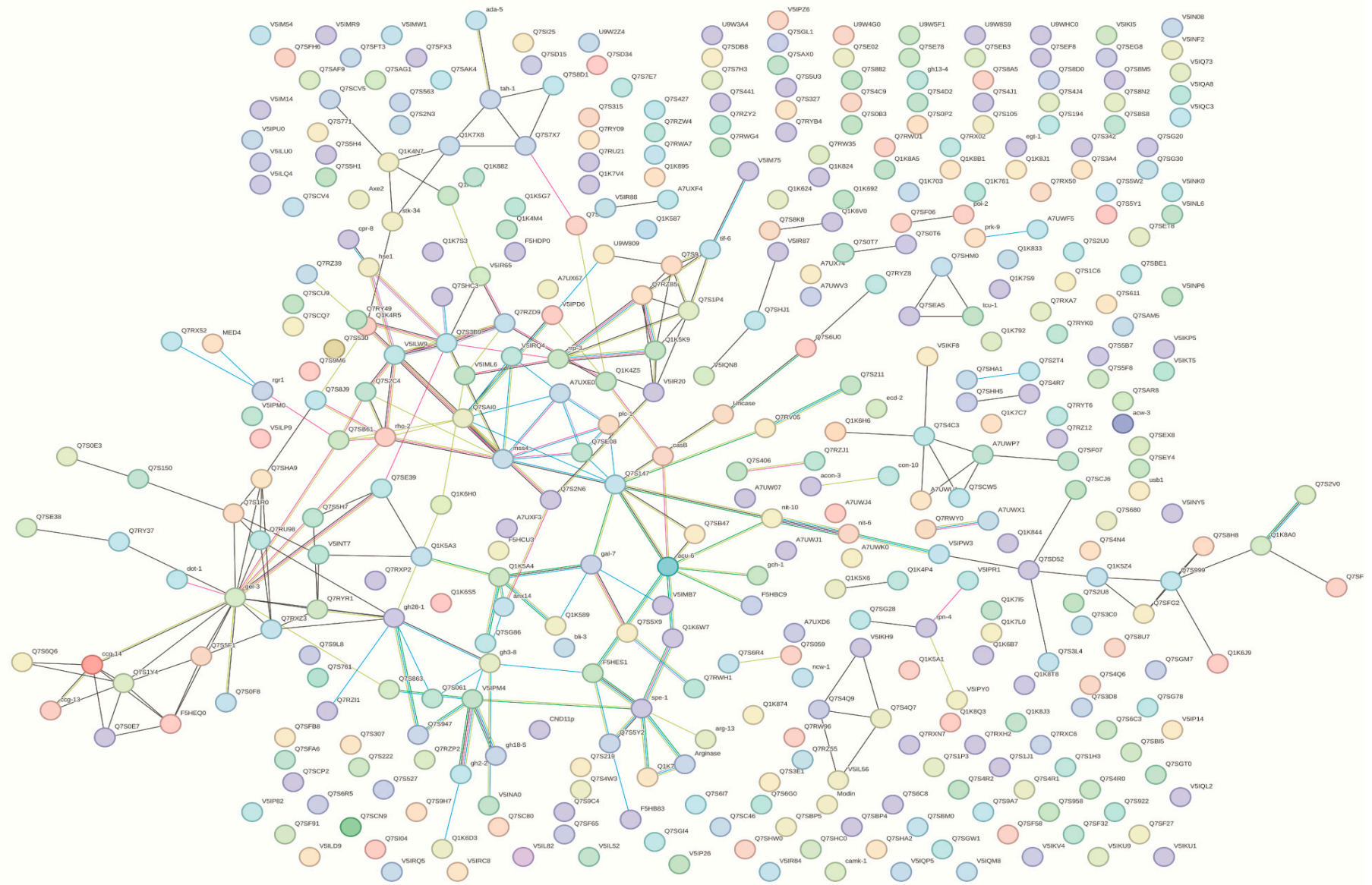
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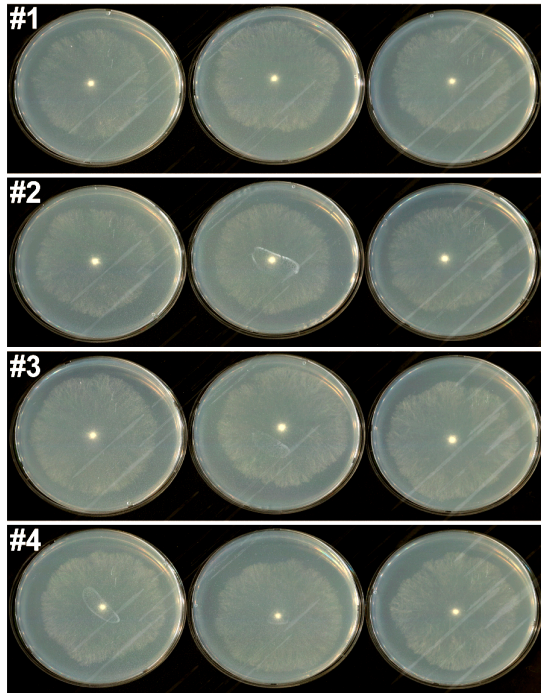
Supplementary Figure S1. Quality checking of RNA-seq data. (a) Number of zero counts across samples. A total of 1831 genes with at least one count value of 0 in 4 samples were filtered out and leaving 8,757 genes were counted for analysis. (b and c) Density plot (b) and

box plots (c) of normalized data using Relative Log Expression (RLE) normalization method based on raw signal (count) and logarithm (based 2). A line in the middle of each box column represents the median of data, and the top and bottom of box column correspond to the 75th and 25th percentiles of data, respectively. Whiskers show the maximum and minimum values, excluding outliers (circles).

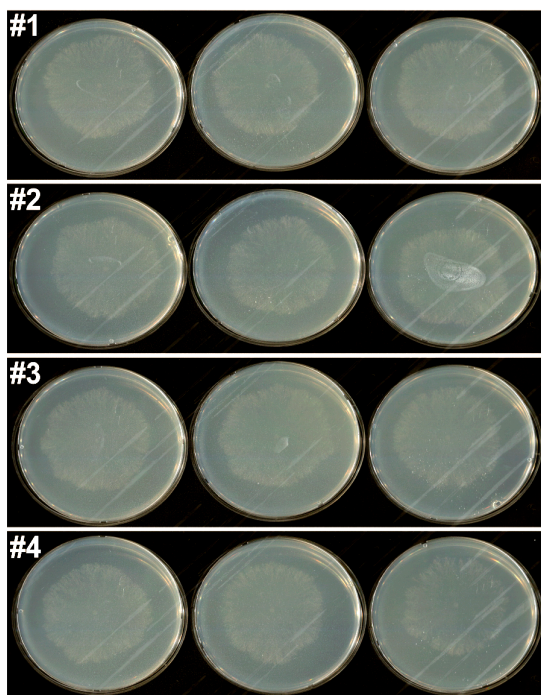


Supplementary Figure S2. The protein-protein interaction (PPI) network constructed using STRING version 11.5. The PPI network of all differentially expressed genes includes 235 edges (interaction) among 403 nodes (protein). Each circle and line represent protein and connectivity, respectively. Different color indicates; purple: experiments, blue: co-occurrence, yellow: text mining, light blue: databases, green: neighborhood, black: co-expression with 0.400 medium confidence score.

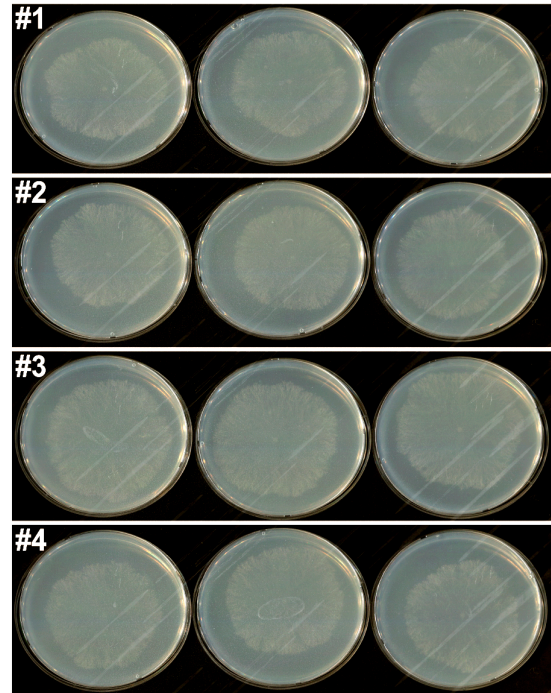
(a) Wild Type (FGSC4200)



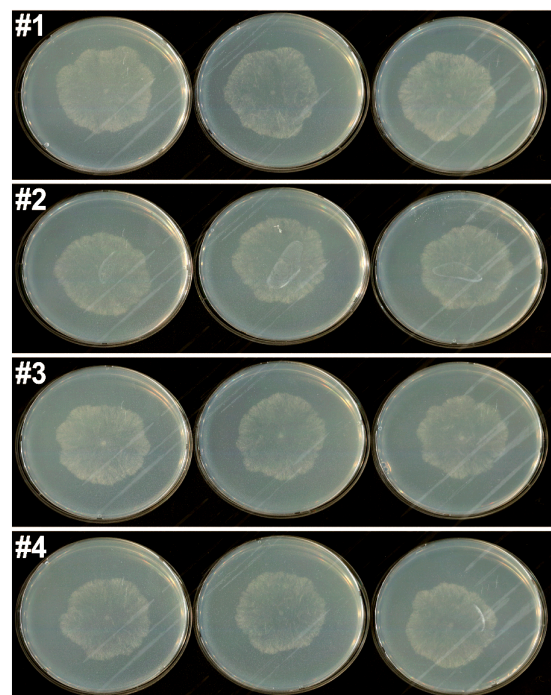
Δ gel-3 (FGSC12976)



Δ mss-4 (FGSC15509)

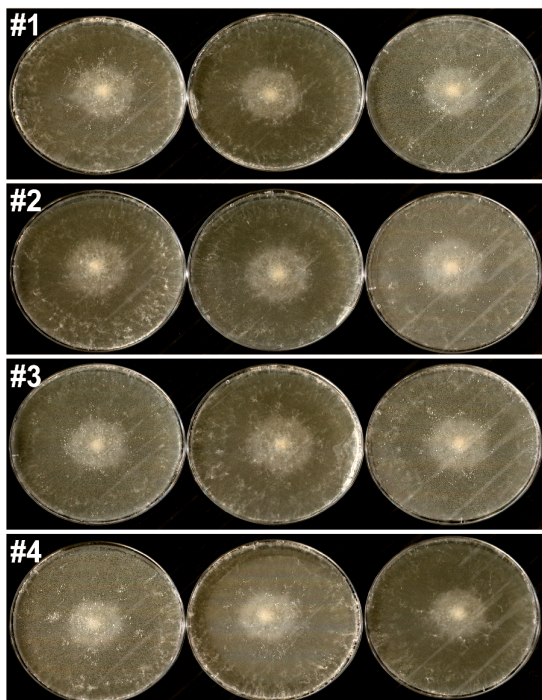


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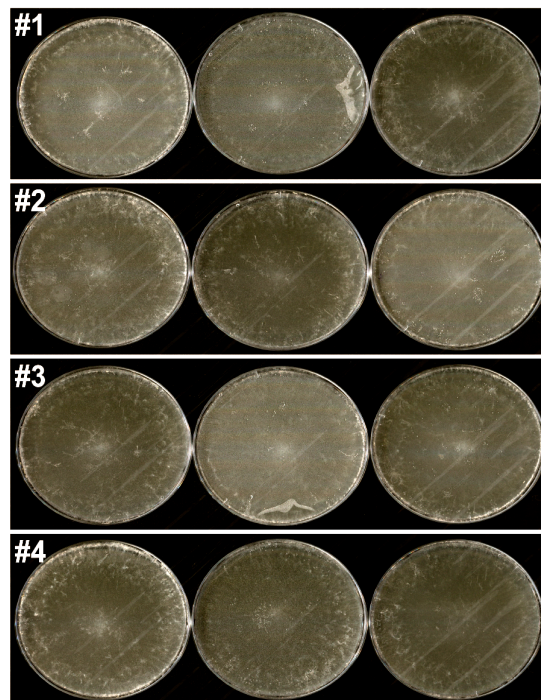


(b)

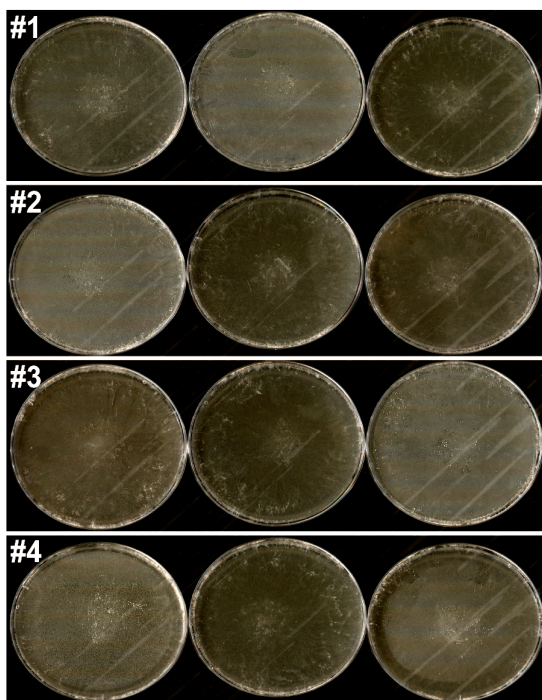
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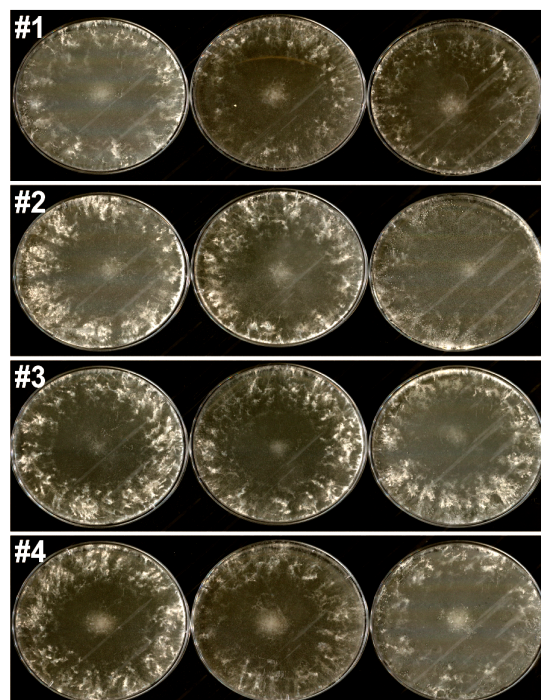
$\Delta mss-4$ (FGSC15509)



$\Delta gel-3$ (FGSC12976)

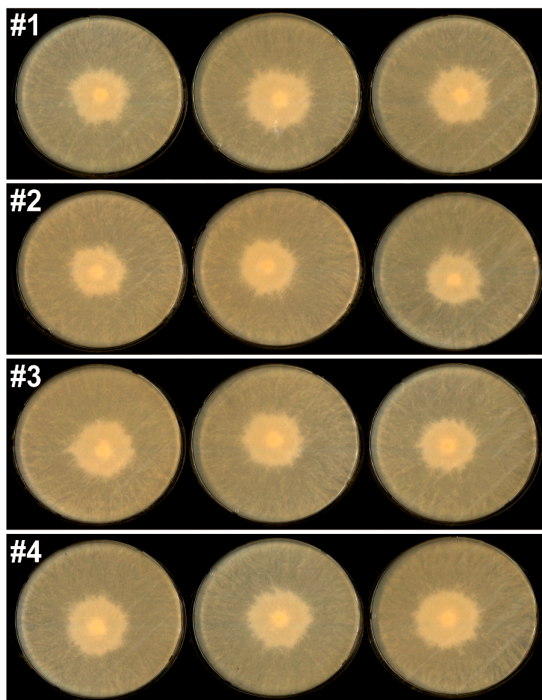


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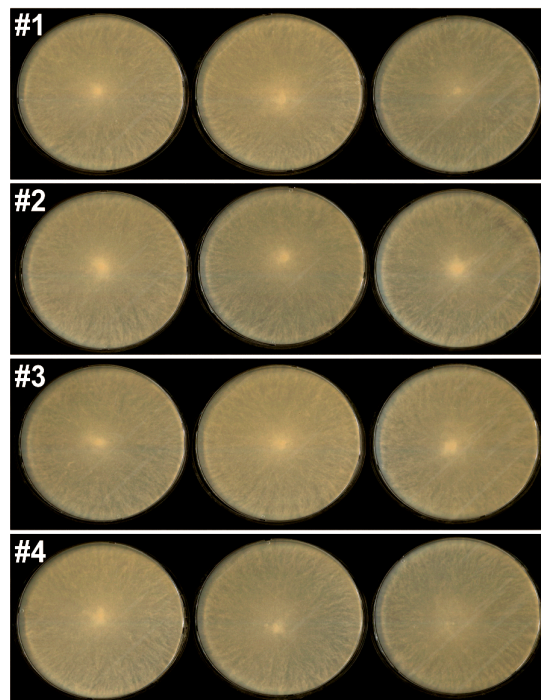


(c)

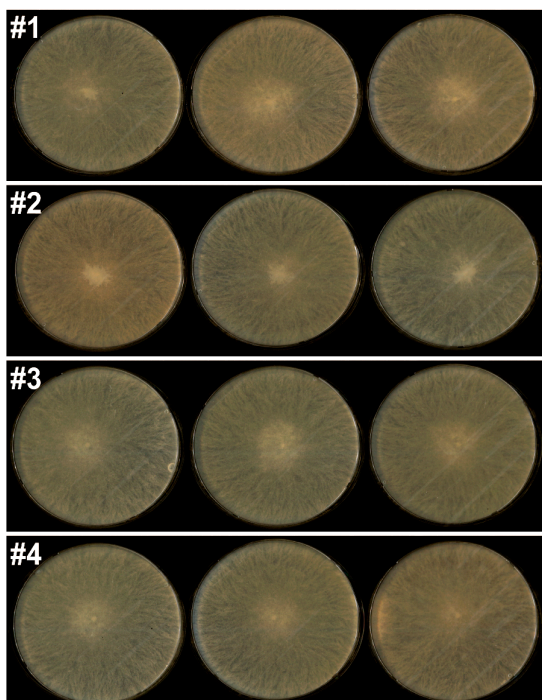
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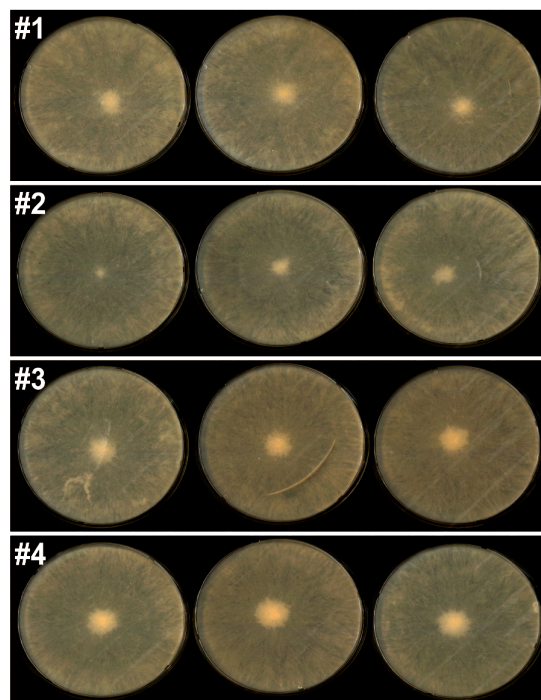
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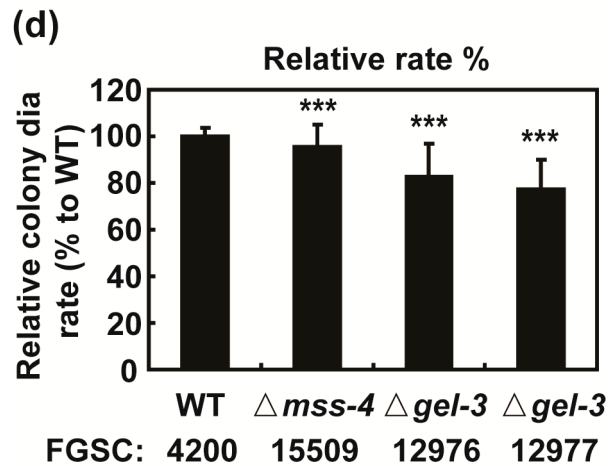


$\Delta gel-3$ (FGSC12976)



$\Delta gel-3$ (FGSC12977)





Supplementary Figure S3. Analysis of basal hyphal growth in *N. crassa*. Images of all strains (Wild Type/FGSC4200, $\Delta mss-4$ /FGSC15509, $\Delta gel-3$ /FGSC12976 and $\Delta gel-3$ /FGSC12977) grown on plates containing VM agar medium for 24 hours (a) and 48 hours (b, c). (b) and (c) are top and bottom view of plate, respectively. Colony diameters of growing cultures were measured after 24 h and relative growth rates were calculated (d). Twelve replicate plates (three replicates per experiment and four independent experiments) per strain were analyzed.

(a) $\Delta gel-3$ $\Delta gel-3$ $\Delta mss-4$ Wild Type
 (FGSC12977) (FGSC12976) (FGSC15509) (FGSC4200)
 1 day

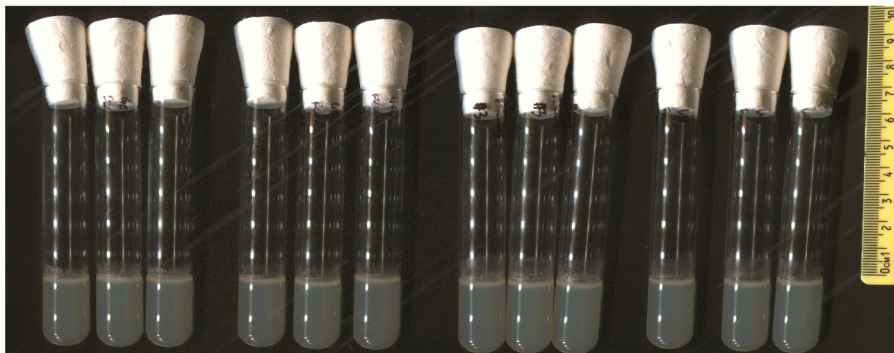
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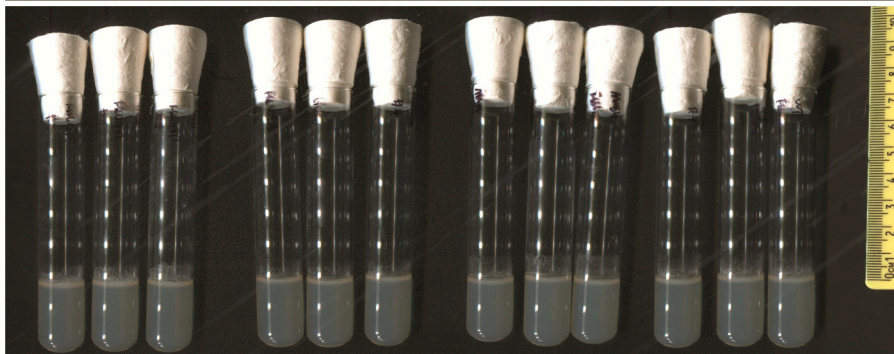
#2



#3



#4



(b) $\Delta gel-3$ $\Delta gel-3$ $\Delta mss-4$ Wild Type
 (FGSC12977) (FGSC12976) (FGSC15509) (FGSC4200)
 2 days

#1



#2



#3



#4



(c) $\Delta gel-3$ $\Delta gel-3$ $\Delta mss-4$ Wild Type
 (FGSC12977) (FGSC12976) (FGSC15509) (FGSC4200)
 3 days

#1



#2



#3



#4



(d) Δ_{gel-3} Δ_{gel-3} Δ_{mss-4} Wild Type
 (FGSC12977) (FGSC12976) (FGSC15509) (FGSC4200)
 5 days

#1



#2

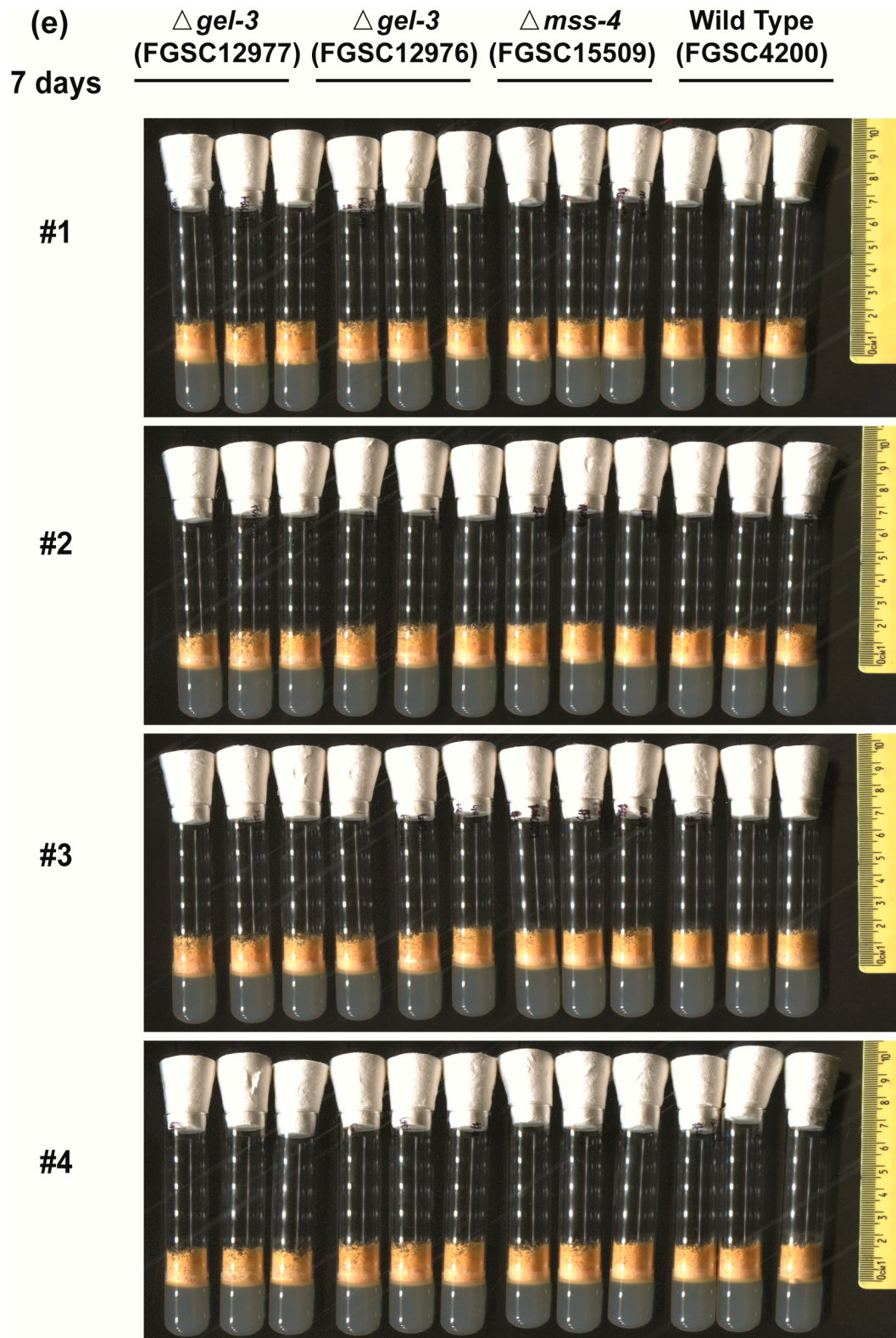


#3



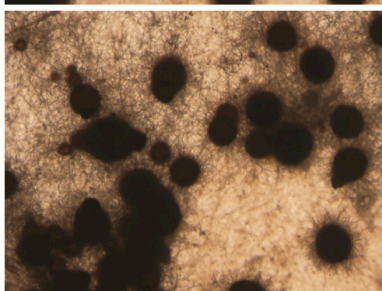
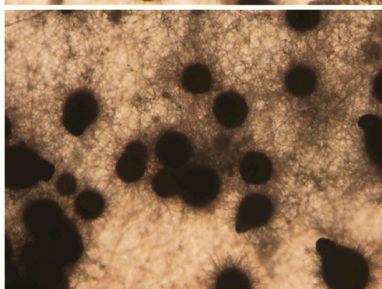
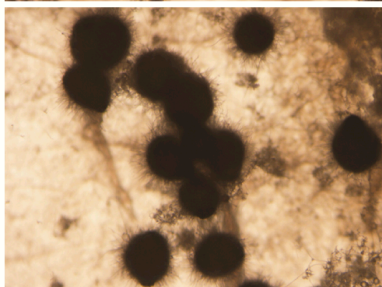
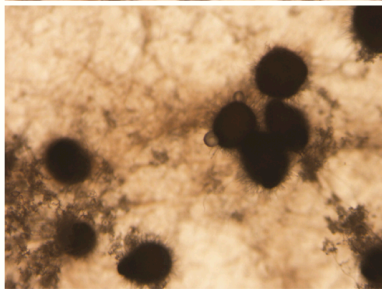
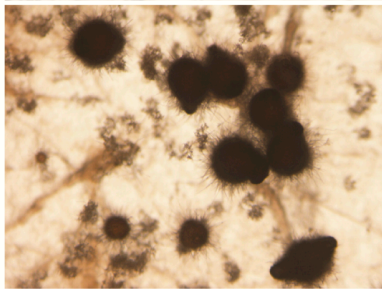
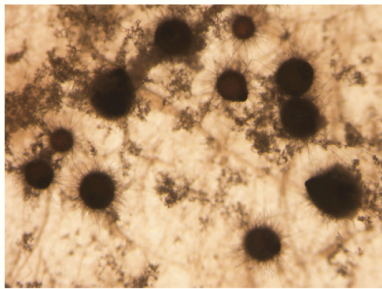
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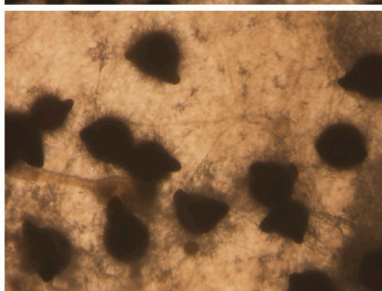
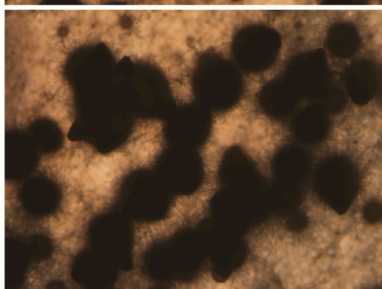
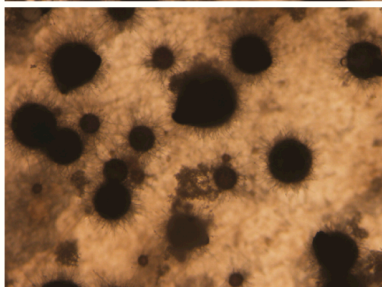
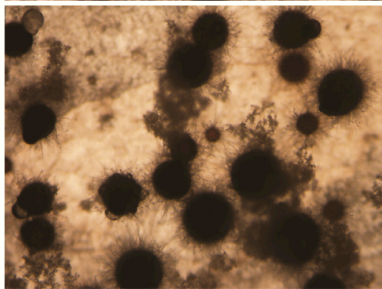
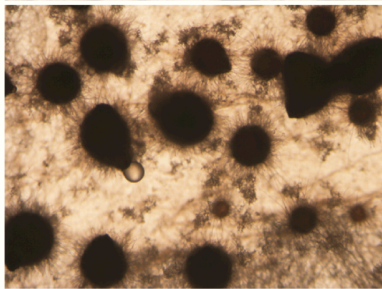
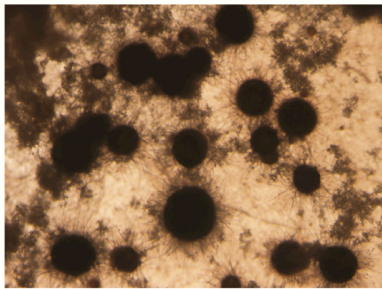


Supplementary Figure S4. Analysis of aerial hyphal growth and conidia production in *N. crassa*. Images of all strains (Wild Type/FGSC4200, $\Delta mss-4$ /FGSC15509, $\Delta gel-3$ /FGSC12976 and $\Delta gel-3$ /FGSC12977) grown in tubes containing VM agar medium for 1 day (a), 2 days (b), 3 days (c), 5 days (d), and 7 days (e). Twelve replicate tubes (three replicates per experiment and four independent experiments) per strain were analyzed.

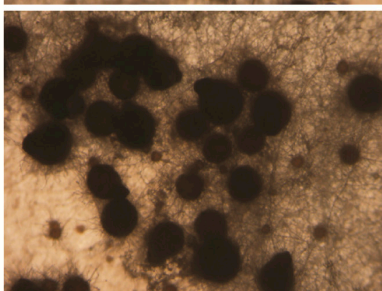
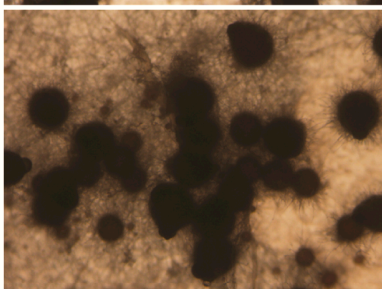
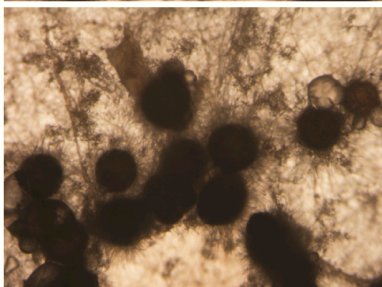
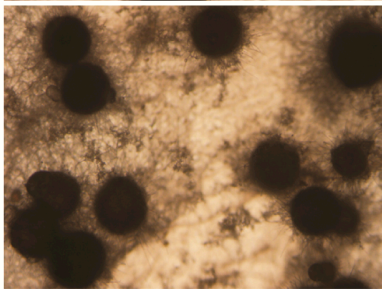
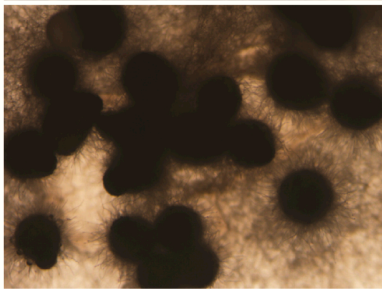
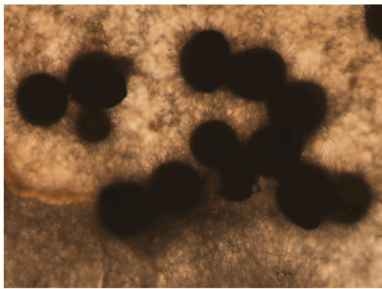
(a) wild type
#1



#2



#3

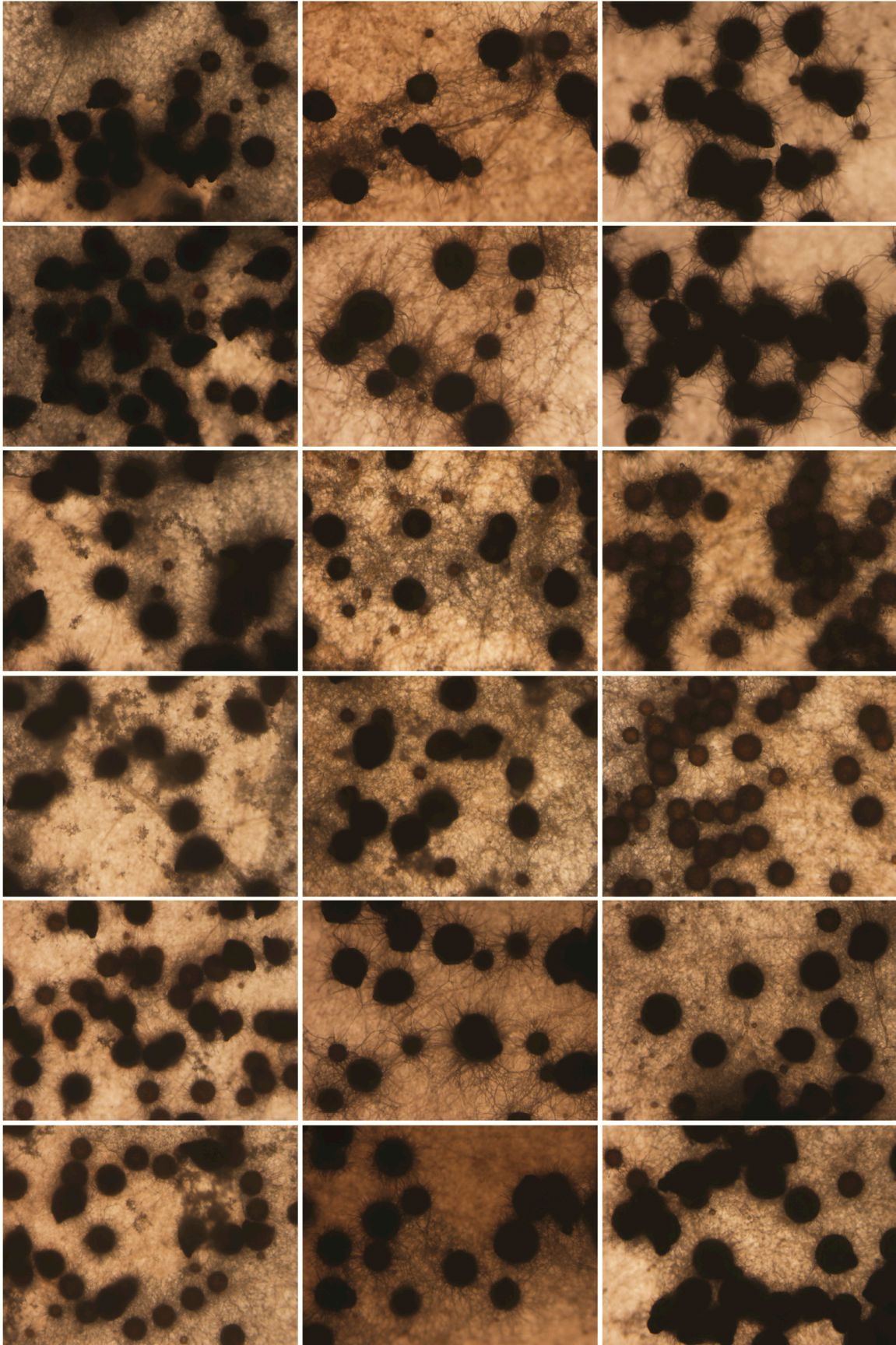


(b) *mss-4* knockout mutant

#1

#2

#3

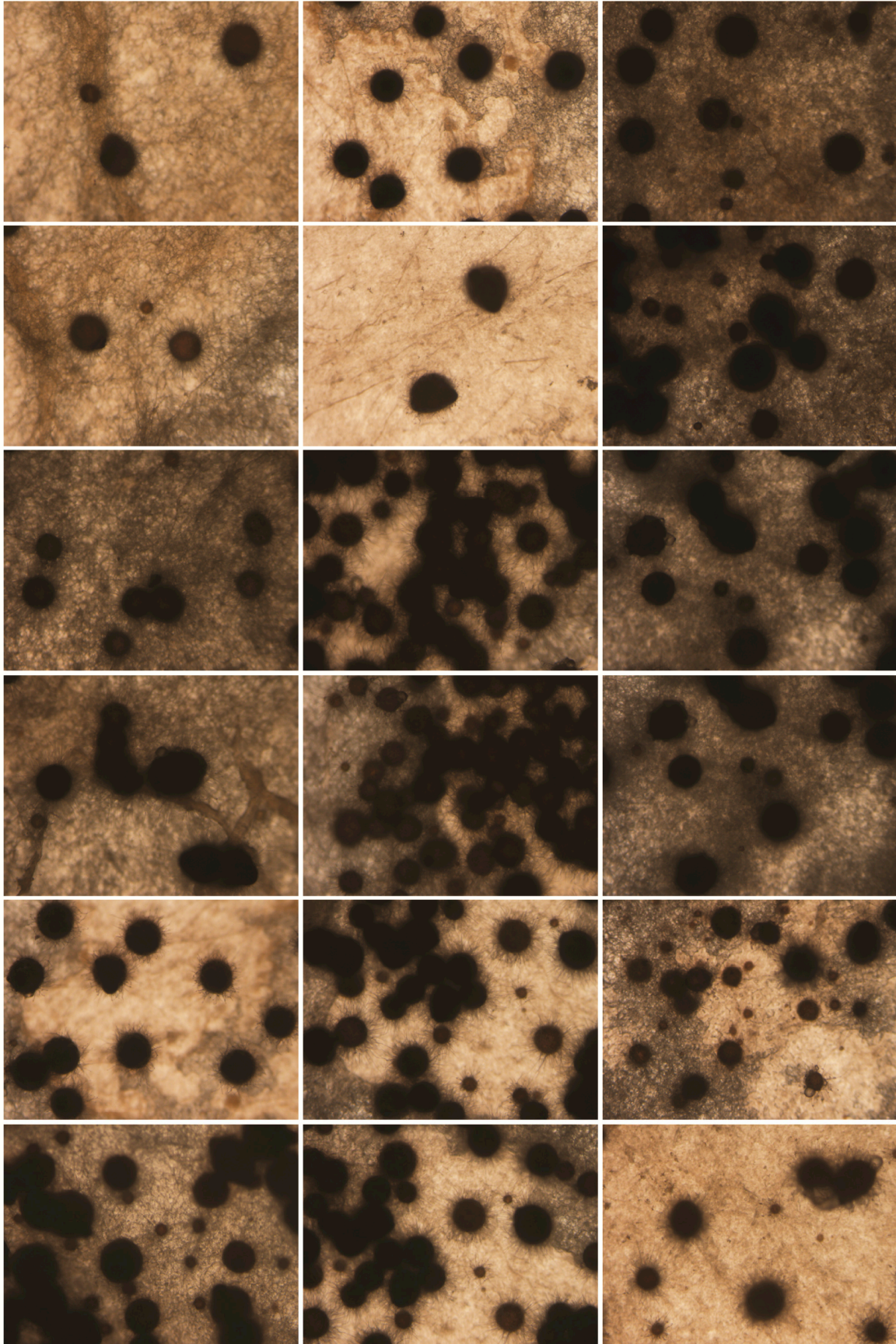


(c) *gel-3* knockout mutant (FGSC12976)

#1

#2

#3

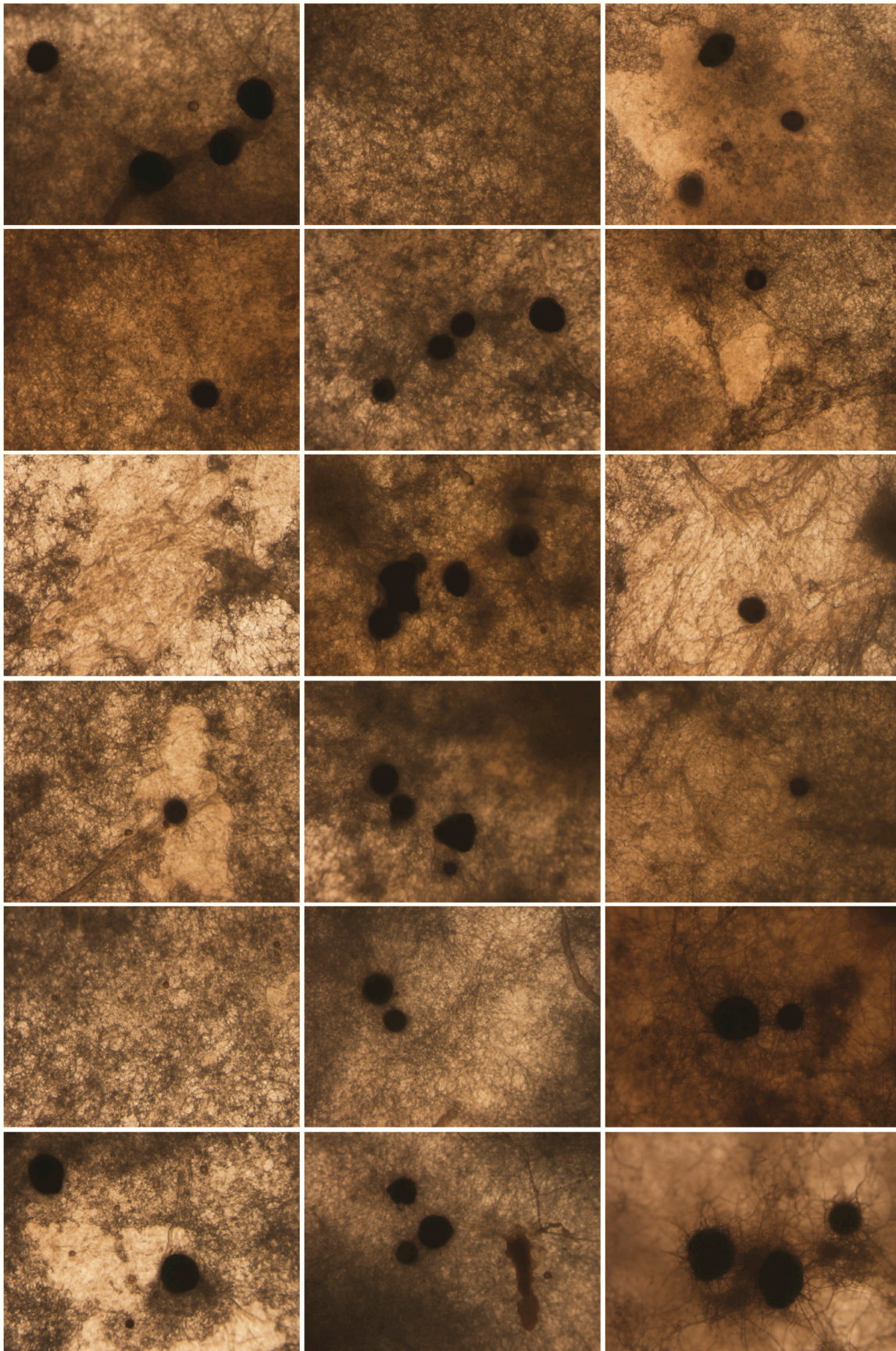


(d) *gel-3* knockout mutant (FGSC12977)

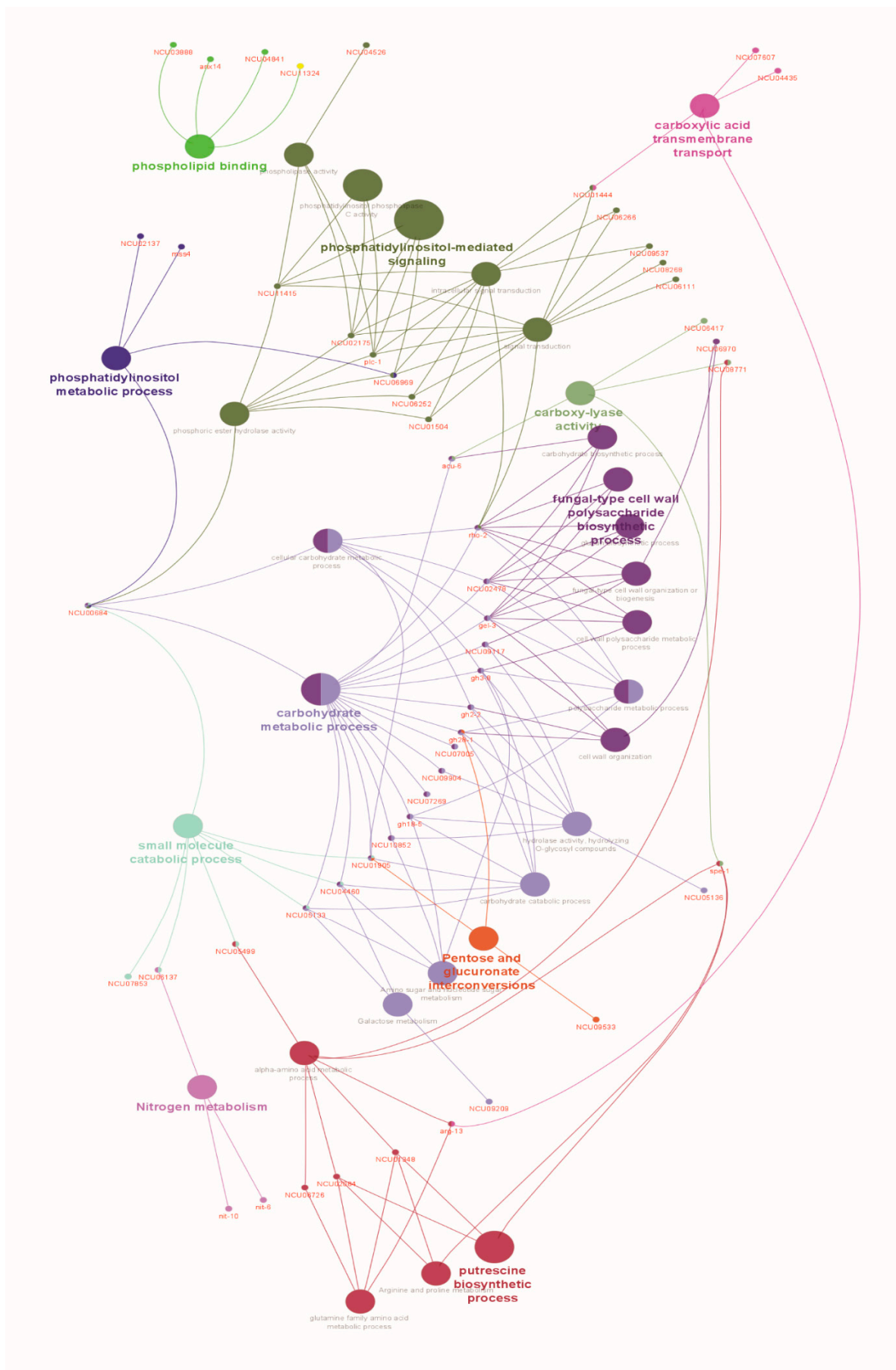
#1

#2

#3



Supplementary Figure S5. Analysis of perithecia formation in *N. crassa*. Perithecia formation was analyzed in wild type/FGSC4200 (a), $\Delta mss-4$ /FGSC15509 (b), $\Delta gel-3$ /FGSC12976 (c) and $\Delta gel-3$ /FGSC12977 (d) after fertilized with wild type of opposite mating type. Images were taken 7 days after fertilization. Eighteen replicate images from three independent experiments (three replicate plates per experiment) per strain were analyzed.



Supplementary Figure S6. GO terms and KEGG pathway visualized using the ClueGO/CluePedia plugin from Cytoscape. Significant GO and KEGG terms are presented together with specific gene interactions. Nodes of the same color represent a functional group. The gene names involved in each group are shown in red. The maximum node (minimum p -value) in the same functional group is shown in bold and was also considered the most abundant term. $p \leq 0.05$, Benjamini-Hochberg correction, and kappa score ≥ 0.4 were used as criteria for ClueGO analysis.