Supplementary Information

The autophagy gene ATG8 affects morphogenesis and oxidative stress tolerance in *Sporisorium scitamineum*

Bin Zhang, Guobing Cui, Changqing Chang, Yixu Wang, Haoyang Zhang, Baoshan Chen, Yi Zhen Deng[1], Zide Jiang[1]

1. To whom correspondence should be addressed. Email: Z Jiang (email: zdjiang@scau.edu.cn) or YZ Deng (dengyz@scau.edu.cn).

This PDF file includes

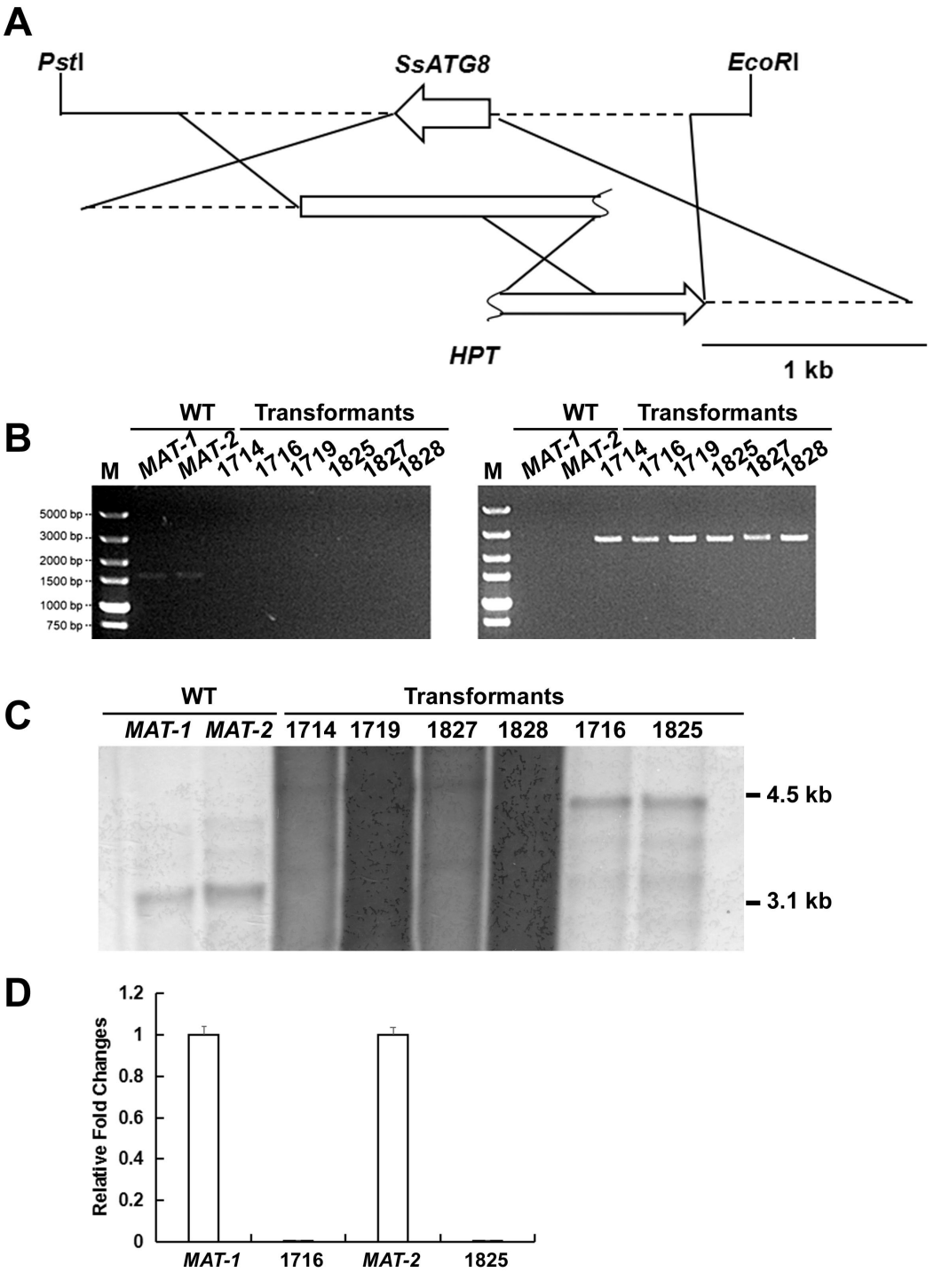
Figure and Legend

Figure S1 Generation and verification of *ssatg8*Δ mutants. (A) Schematic representation of two partial-overlapped fragments with *HPT* resistant marker flanked with 5’- and 3’- region (dashed line) of *SsATG8* ORF (open reading frame; arrowed box) drawn to scale. Homologous recombination between these two fragments and *S. scitamineum* genome results in replacement of *HPT* gene with *SsATG8* ORF. PCR amplified flanking DNA fragment denoted by the 1 kb scale bar, were used as the probe for DNA gel blot analysis shown in (C). (B) PCR verification of deletion of *SsATG8* gene in the transformants. Left: PCR amplification of *SsATG8* coding region with primers as listed in Table 1; Right: PCR amplification with the primers located outside the homologous region of 5’- *SsATG8* and inside the *HPT* gene, as listed in Table 1. (C) Southern blot analysis for confirmation the deletion mutants. Genomic DNA from the wild-type *MAT-1* and *MAT-2*, as well as the indicated transformants in these two mating type background, was digested with the restriction enzyme *Pst*I and *EcoR*I, followed by probing with the denoted probe as in (A). The wild-type 3.1 kb *SsATG8* locus was lost while a 4.4 kb band detected in the transformants, which is diagnostic of correct gene replacement event. (D) *SsATG8* gene transcription was undetected in the transformants 1716 and 1825, confirming the deletion of *SsATG8* gene. Relative gene expression level was calculated with -ΔΔCt method (Livak and Schmittgen, 2001) with *ACTIN* as internal control.

Reference

Livak, K. J., Schmittgen, T. D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 25, 402-

Figure S1


A
PstI
SsATG8
EcoRI
HPT
1 kb
B
WT
Transformants
M
MAT-1
MAT-2
1714
1716
1719
1825
1827
1828
5000 bp
3000 bp
2000 bp
1500 bp
1000 bp
750 bp
C
WT
Transformants
MAT-1
MAT-2
1714
1719
1827
1828
1716
1825
4.5 kb
3.1 kb
D
Relative Fold Changes
1.2
1
0.8
0.6
0.4
0.2
0
MAT-1
1716
MAT-2
1825