Supplementary File S1:

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CACCACCAACTGCCTTGCGCCCCTGGCCAAGATCATCCACGACAAGTTCGGCATTGTTGAGGGCTTGATG
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GACGCTCCGTCAACAACAACATCATTCCCTCTTCCACTGGTGCCGCCAAGGCCGTCGGAAAGGTCATTCC
TTCGCTGAACGGCAAGCTCACgtacgtgcttttcaagccatactatctggagaataggctaacgtatatc
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CCAACGCCGGAATCCAGCTGAGCCCCAACTTCGTCAAGCTGATCTCCTGGTACGACAACGAGTGGGGTTA
CTCCCGTCGTGTCTGCGACCTCATCAACTACGTCGCTGCTAAGGATGCCGCCGCTGGCATTTAA

Fig. S1 The coding region sequence of the glycerol-3-phosphate dehydrogenase gene (*SrGPD*) from *S. rugosoannulata* HC7. Uppercase letters indicate exons, while lowercase letters indicate introns.

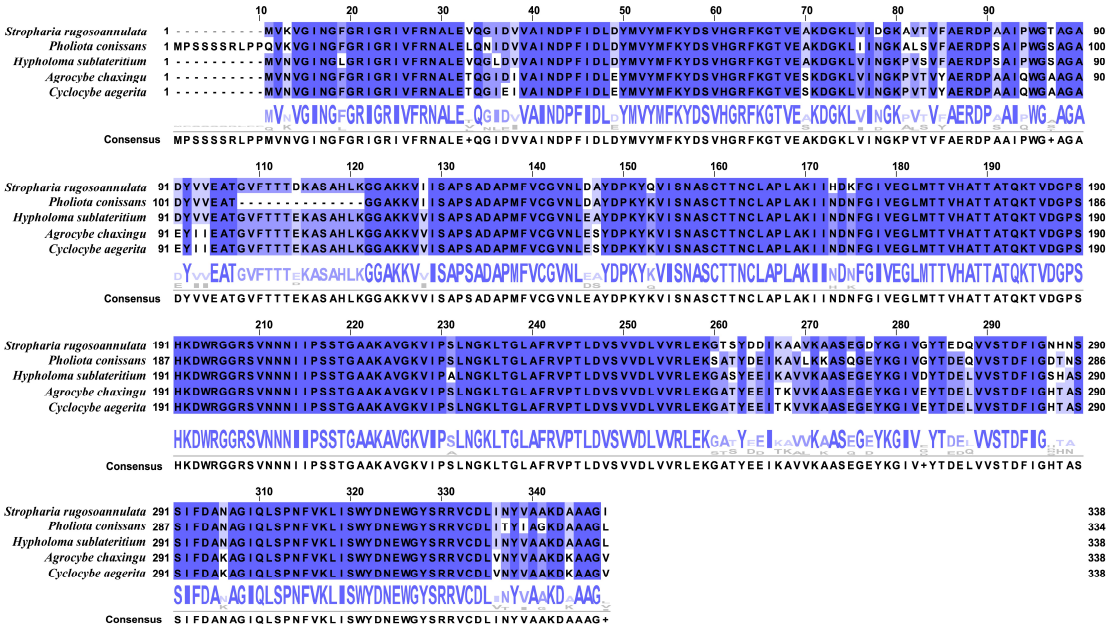


Fig. S2 Multiple sequence alignment of GPD protein sequences. The multiple sequence alignment results comparing the SrGPD protein sequence with GPD protein sequences from other species are presented.

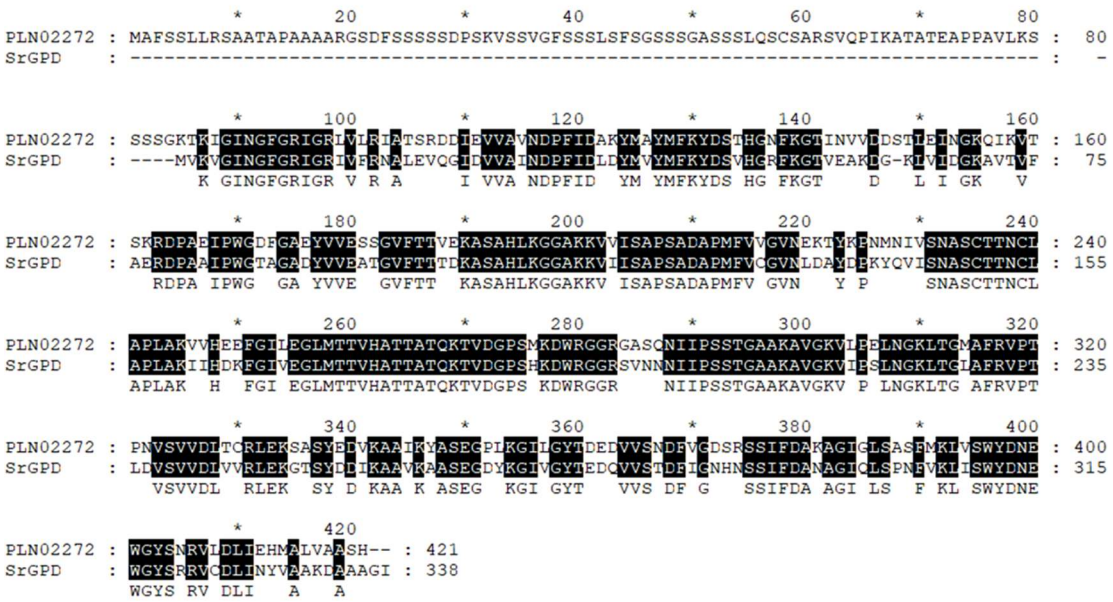


Fig. S3 Sequence alignment of *SrGPD* with the PLN02272 superfamily. Amino acids that are identical in the alignment results are highlighted with a black background.

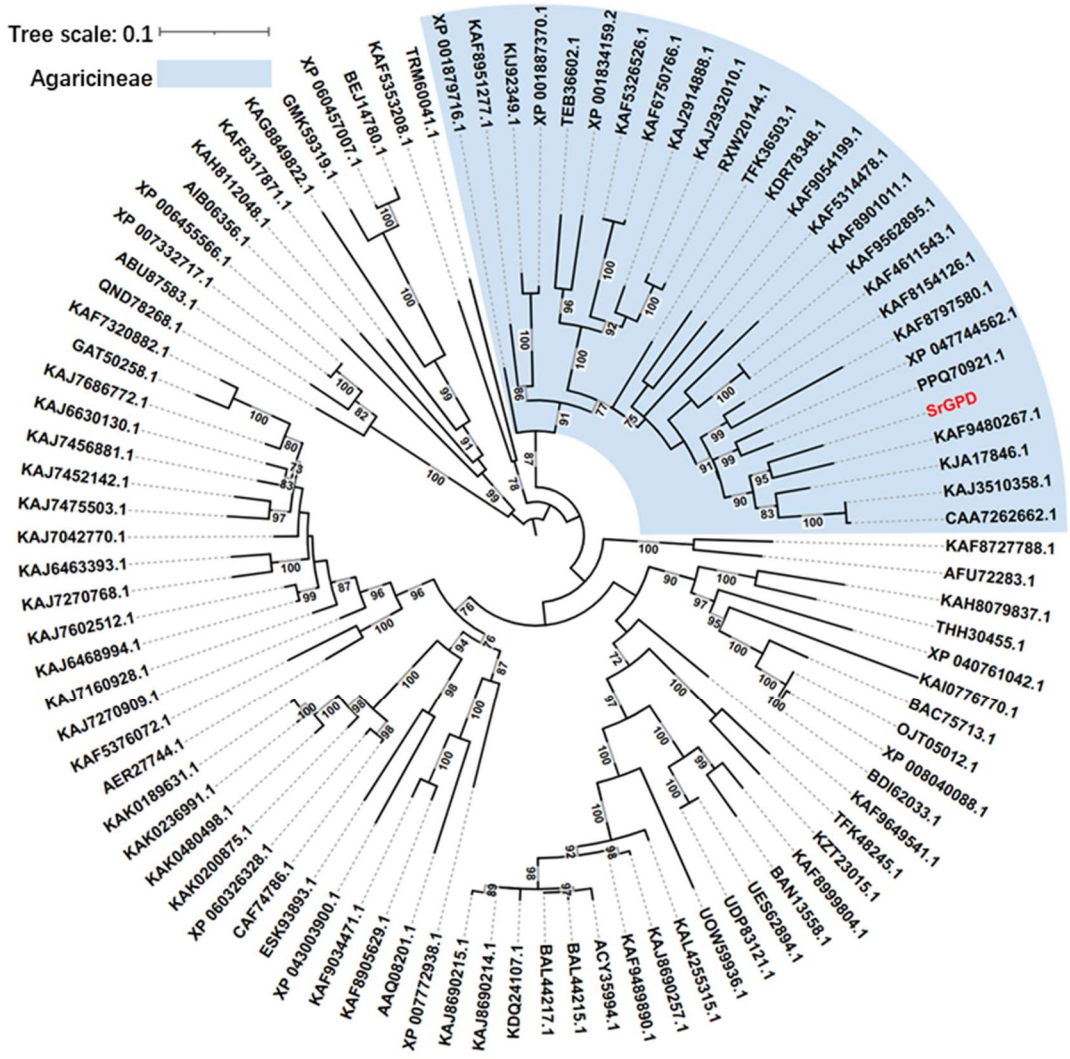


Fig. S4 Phylogenetic tree of GPD protein sequences from *S. rugosoannulata* and other selected fungi. The sequences used for constructing the phylogenetic tree included the SrGPD protein sequence and 94 GPD protein sequences derived from 82 fungal species (The correspondence between the sequence accession numbers and their respective species is detailed in Table S4). The tree is midpoint rooted and is drawn to scale with branch lengths computed using the Maximum Likelihood method with 10,000 ultrafast bootstrap replicates and measured in the number of expected substitutions per site. the ultrafast Bootstrap values which more than 70% are shown above or below the branches. The species corresponding to accession number which covered by the blue background all belong to Agaricineae.

accgtccattccacgacggcgacacagaaaaccgtggacggcccatctcacaaggactggcgtgggggtc
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ccttaatggaaagttaacgtaagtagaatgttttaattggtaacgaagaaaataaacttaacagcattta
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tataggttataccgatgagcaagttgtatcaaccgatttcattggaagcgactattcttccatcttcgat
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ctggcgggactatataagttgggccgccgtcggactttcgctcctcgcgcatttttgcattcaacctcta
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Fig. S5 The putative promoter sequence of the *GPD* in *S. rugosoannulata* HC7.

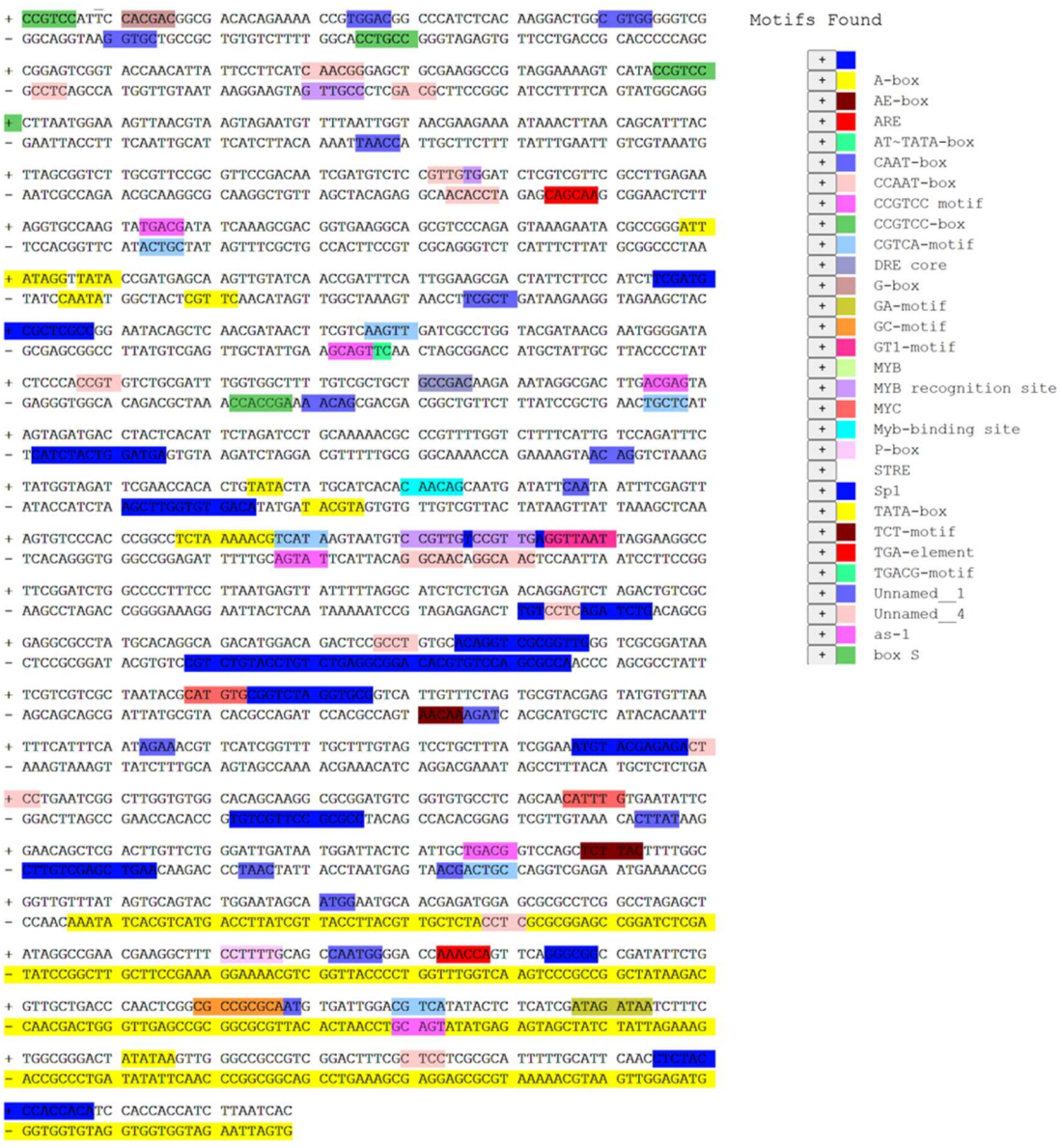


Fig. S6 Prediction of the *GPD* promoter elements in *S. rugosoannulata*. The PlantCARE online tool was employed to identify motifs within the putative *GPD* promoter region of *S. rugosoannulata*. Different base background colors indicate the distinct motifs that have been predicted.

gtgttctctgagtttcaggggtaccgagtcaaaattaatgaaagatatagcagaatttatgtgtgtaatt
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tggagtatttttcaaccatccaagatcataaatttaaaatacgtttgaagtcgaacaagaattcgttatc
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gaatggtgaaaaaaaacgaaatagagaaggtggtactcgggatgcttgccgagacgttgacgtttctccg
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tcacccgcagcttcgcgcggctcgataccactcgatagcaatatccaatttaccgtcaccgataccgagc
acgttcgttatccagtcgataaggatcccgtctccgatgactcaggttcgacggtggtaacggtttgcta
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atgggtatattgttcagagtgcgcgctactgggaatggcgagcgtgtcgtgcgcgtcagcgtgcgctctg
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ttcatattaatcacatactt

Fig. S7 The putative terminator sequence of the *GPD* in *S. rugosoannulata* HC7.

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CAAAACGAGAACCCCCCAGCACAGGgtatggattgccatgttcagattcaaatattttcttattatccaa
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TGCGCTTGCTGCTCTACTTCCTGCCCATCGTACTGGTGGAATCAGGATGGGTACCTTGGTCCTGCGACAT
TAATGCAGGCGTACCGATGGATCGCTGACTCTCGGgtatgtactttcagcttgacggggataaagcggaa
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GAGCTTGTACCGTTGCCATACCATCTTTAACTgtctgtatttccgctgctactgctgctttgattgtgtg
tttgctcactggctcatcttgcagGTTCGCGCACCTGCCCCAAGGGTCTTAACCCTGCCGCAGCCATCGC
TAAGATCAAACTAGAAATGGCCATGGATTAA

Fig. S8 The coding region sequence of the succinate dehydrogenase [ubiquinone] iron-sulfur subunit (*SrSDI*) from *S. rugosoannulata* HC7. Uppercase letters indicate exons, while lowercase letters indicate introns.

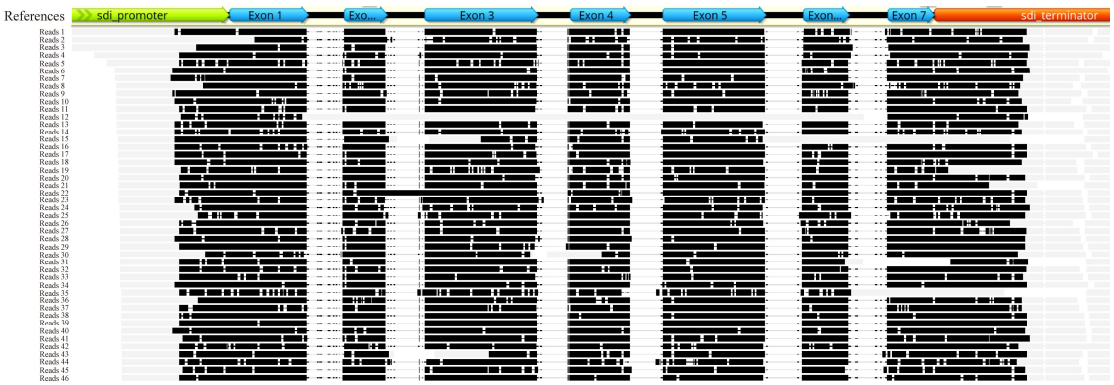


Fig. S9 Alignment of transcripts obtained via full-length transcriptome sequencing against the reference sequence of the *SrSDI*. The transcript sequence (indicated by black boxes) is largely consistent with the exon regions (marked in blue) of the reference

sequence.

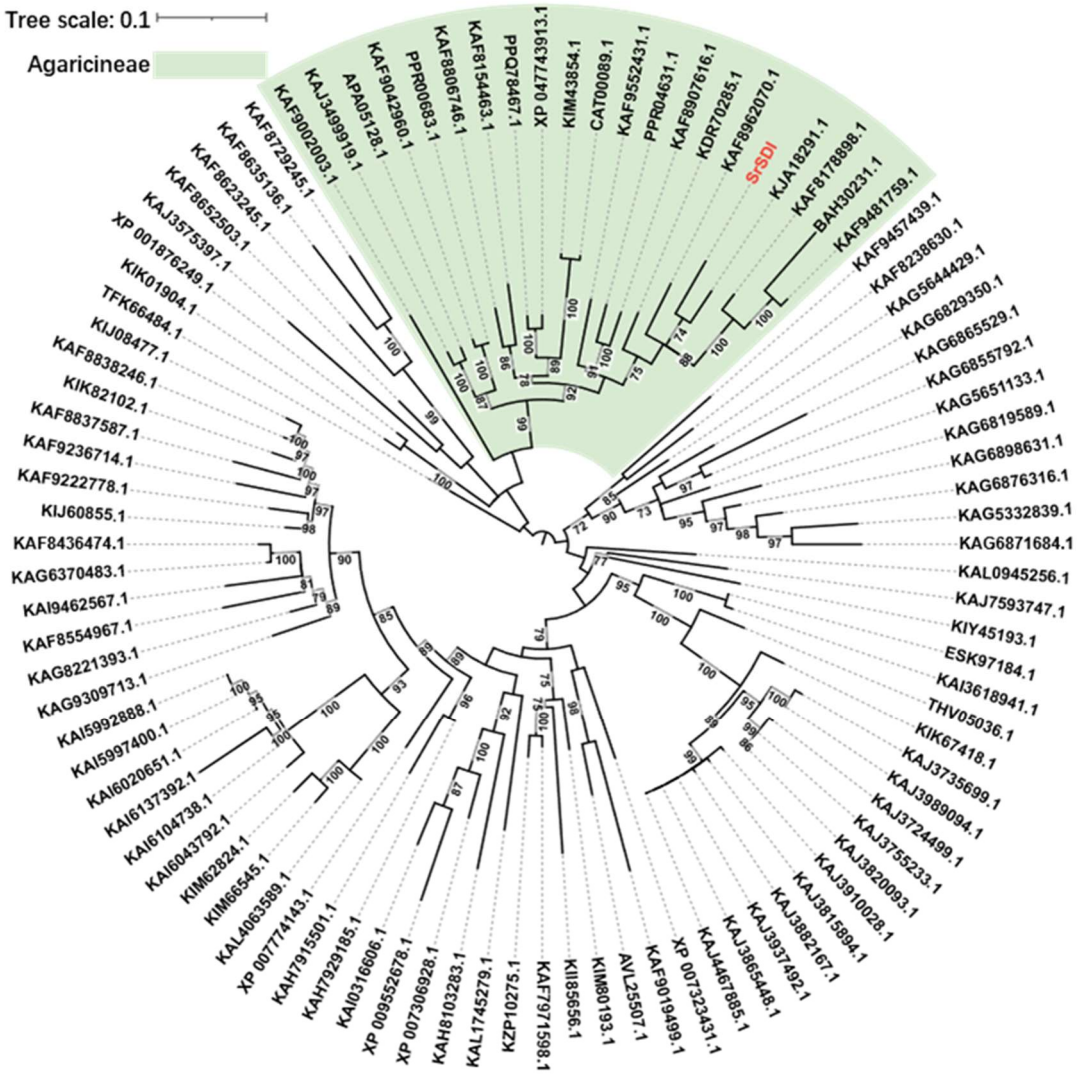


Fig. S10 Phylogenetic tree of SDI protein sequences from *S. rugosoannulata* and other selected fungi. The sequences used for constructing the phylogenetic tree included the SrSDI protein sequence and 95 SDI protein sequences derived from 87 fungal species (The correspondence between the sequence accession numbers and their respective species is detailed in Table S5). The tree is midpoint rooted and is drawn to scale with branch lengths computed using the Maximum Likelihood method with 10,000 ultrafast bootstrap replicates and measured in the number of expected substitutions per site. the ultrafast Bootstrap values which more than 70% are shown above or below the branches. The species corresponding to accession number which covered by the blue background all belong to Agaricineae.



Fig. S11 Sequence alignment of *SrSDI* with the PLN00129 superfamily. Amino acids that are identical in the alignment results are highlighted with a black background.

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ctcagtgtgtttcccctata

Fig. S12 The putative promoter sequence of the *SDI* in S. rugosoannulata HC7.

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ttttatttttctggagcgtgtacttctaagttgcactttcgatcgcctccgcagtcttcgcaacggttct
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Fig. S13 The putative terminator sequence of the *SDI* in *S. rugosoannulata* HC7.

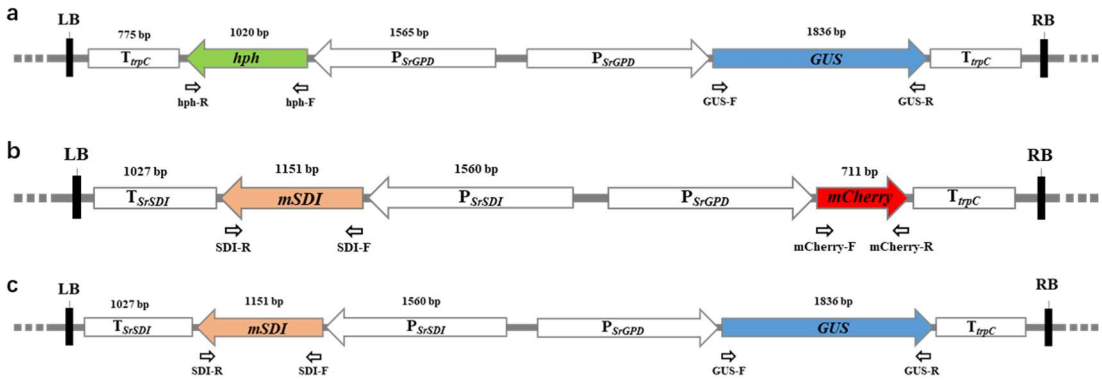


Fig. S14 Schematic diagrams of the recombinant plasmids used for protoplast transformation in *S. rugosoannulata* HC7. a The recombinant plasmid pCM-GHT-GGT. **b** The recombinant plasmid pCM-mSDI-GMT. **c** The recombinant plasmid pCM-mSDI-GGT.

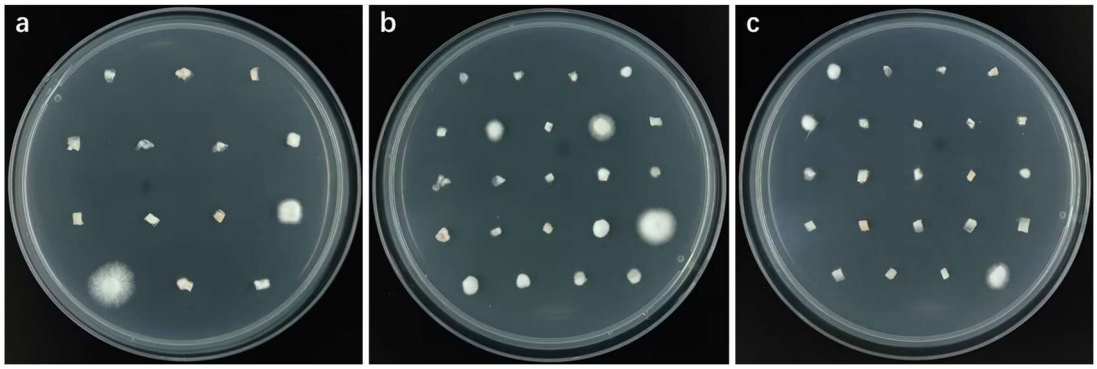


Fig. S15 The re-screening plates for S. rugosoannulata transformants obtained through Agrobacterium-mediated transformation with plasmid pCM-GHT-GGT (a), pCM-mSDI-GMT (b), or pCM-mSDI-GGT (c).

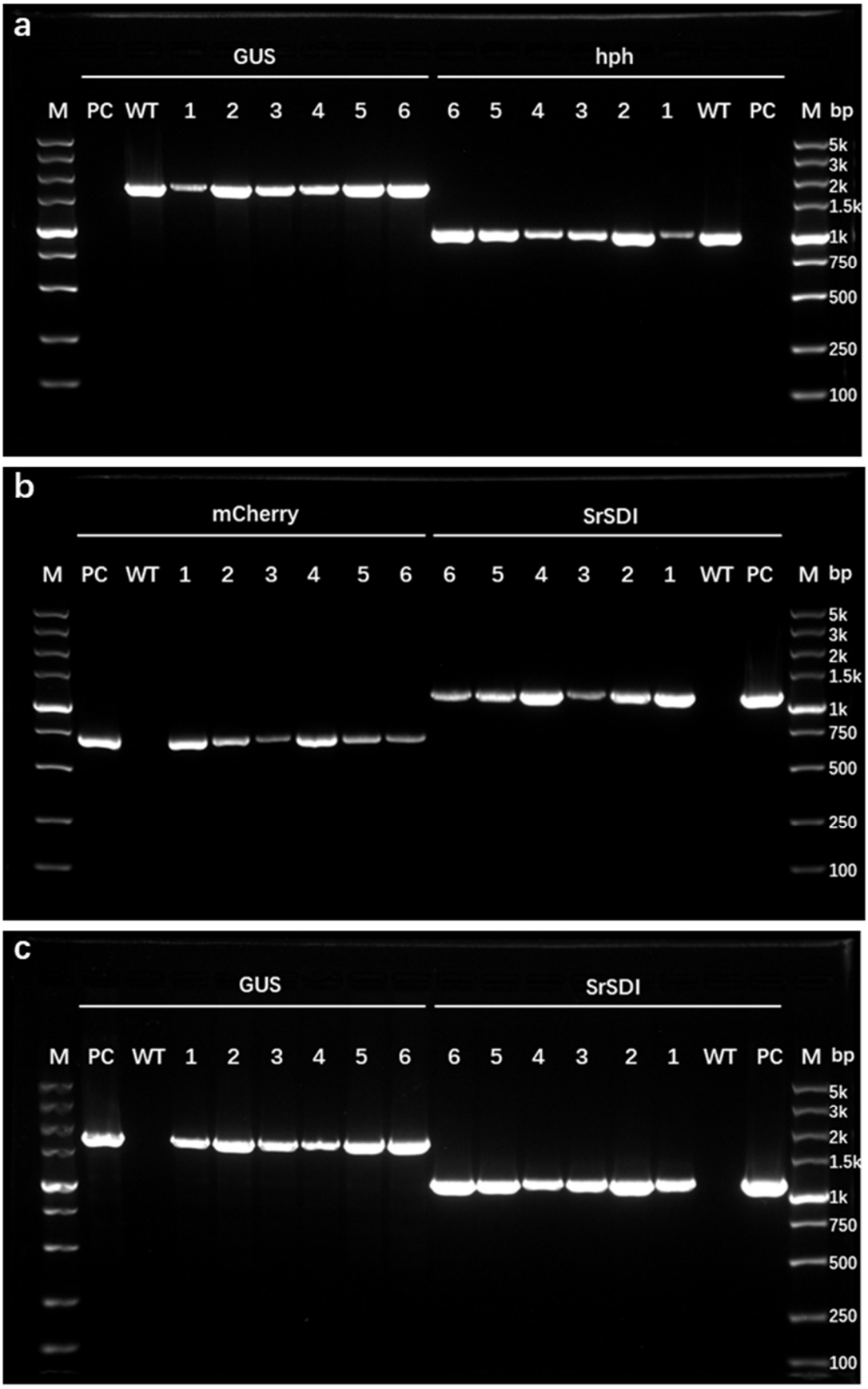


Fig. S16 PCR-based identification of the insertion fragments in *S. rugosoannulata*

transformants generated using plasmids pCM-GHT-GGT (a), pCM-mSDI-GMT (b), and pCM-mSDI-GGT (c). The primers employed are those depicted in Fig. S14. PC, plasmid positive control. WT, wild type. 1-6, transformants after re-screening. M, DNA molecular marker.

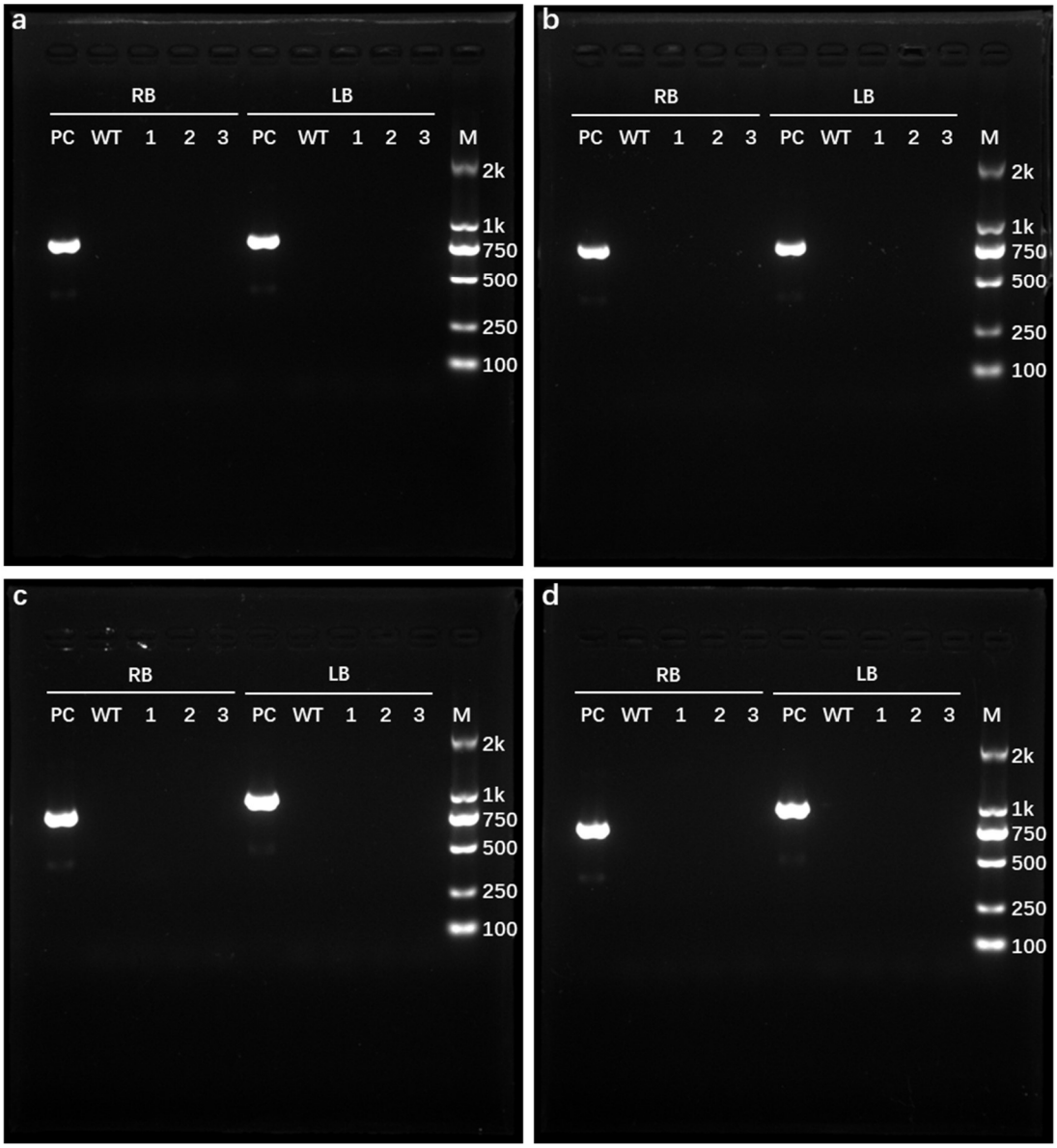


Fig. S17 Verification of exogenous DNA contamination in *S. rugosoannulata* transformants generated using the recombinant vectors pCM-GHT-GMT (a), pCM-GHT-GGT (b), pCM-mSDI-GMT (c), and pCM-mSDI-GGT (d). The right border sequence (RB) of the plasmid insertion fragment was validated using the primer pair LPU-R/RPU-R. The left border sequence (LB) was validated using the primer pairs LPU-F/LPU-R (for plasmids pCM-GHT-GMT and pCM-GHT-GGT) or LPU-F/LPUS-R (for plasmids pCM-mSDI-GMT and pCM-mSDI-GGT). PC, plasmid positive control. WT, wild type, used as a negative control to exclude non-specific binding of genomic DNA to the primers. 1-3: re-screened *S. rugosoannulata* transformants. M, DNA molecular marker.

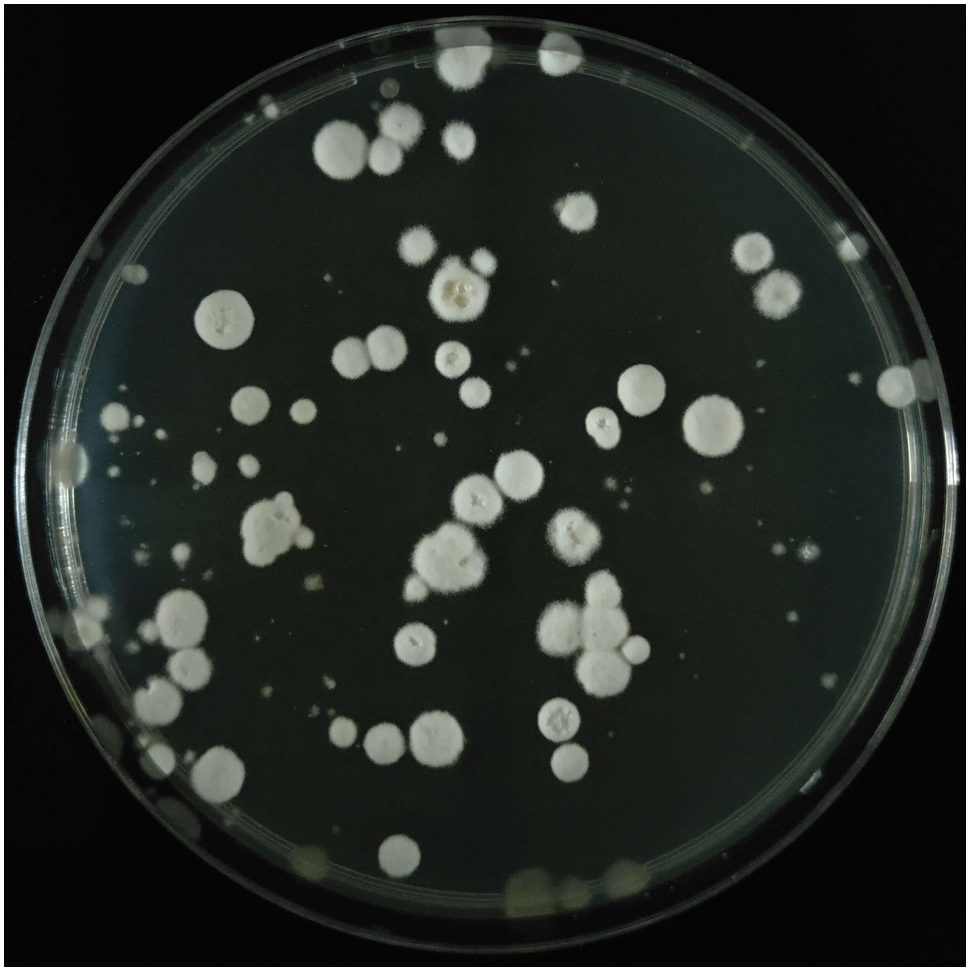


Fig. S18 Regeneration of *S. rugosoannulata* protoplasts. The figure shows the regeneration status of 10^4 protoplasts cultured on the regeneration medium for 10 days at 25°C.

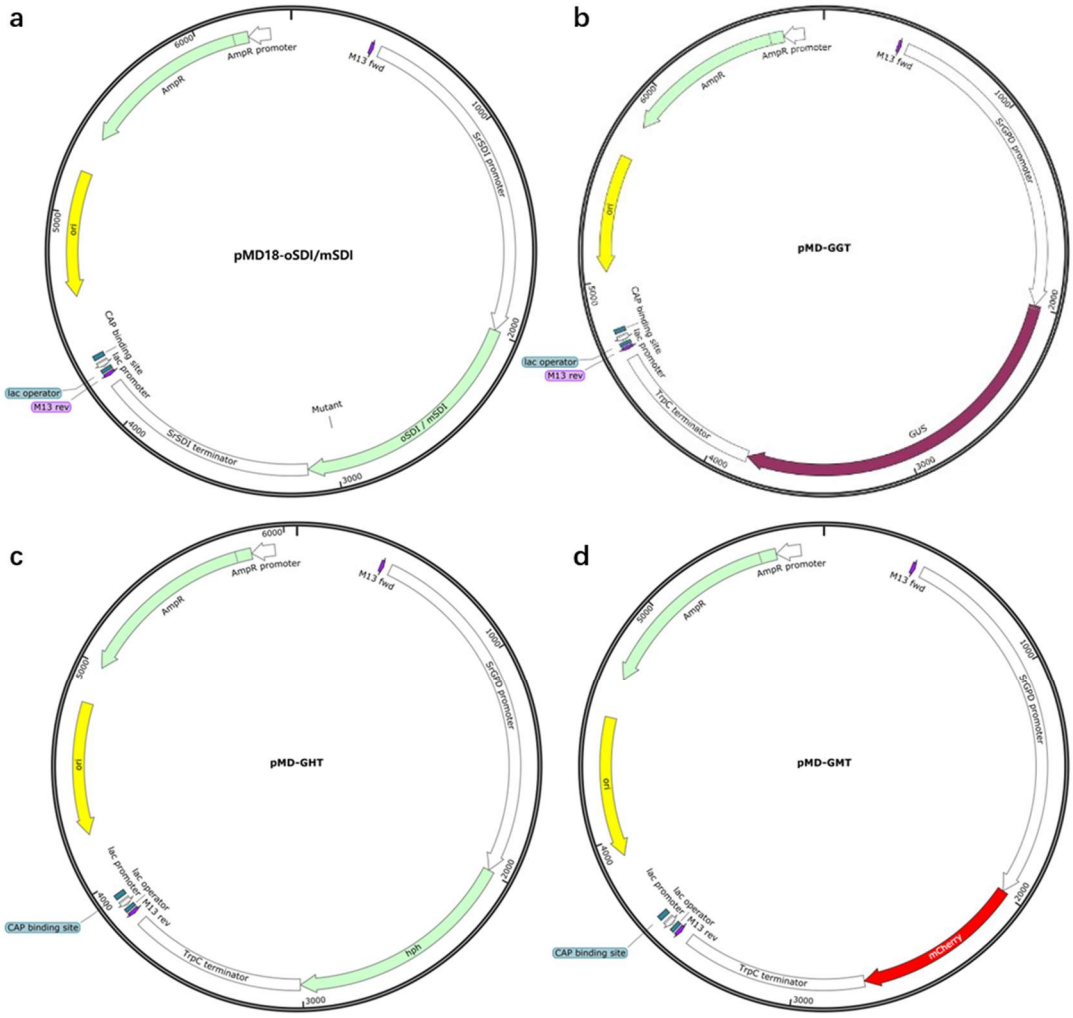


Fig. S19 Schematic diagrams of recombinant plasmids used for constructing binary vectors. a Plasmid maps of pMD-oSDI or pMD-mSDI. **b** Plasmid map of pMD-GGT. **c** Plasmid map of pMD-GHT. **d** Plasmid map of pMD-GMT.