

Supplementary Material

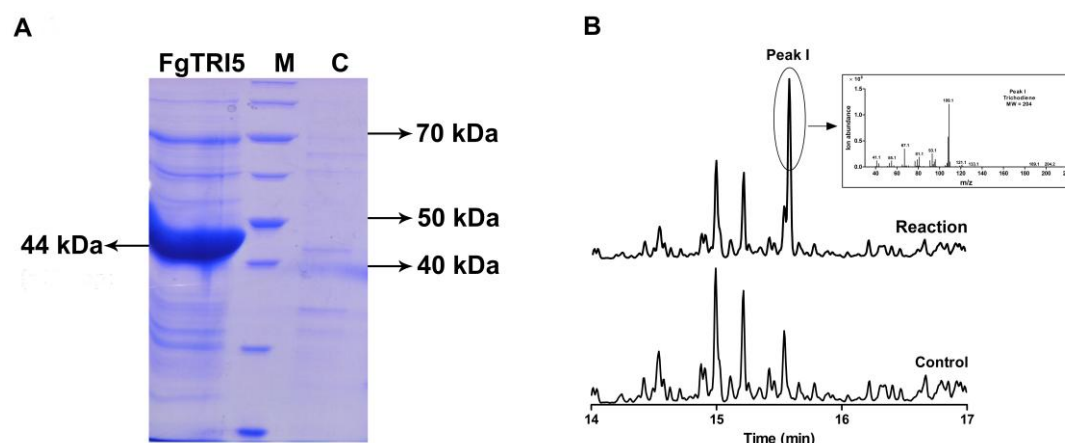
Heterologous Biosynthesis of the Fungal Sesquiterpene

Trichodermol in *Saccharomyces cerevisiae*

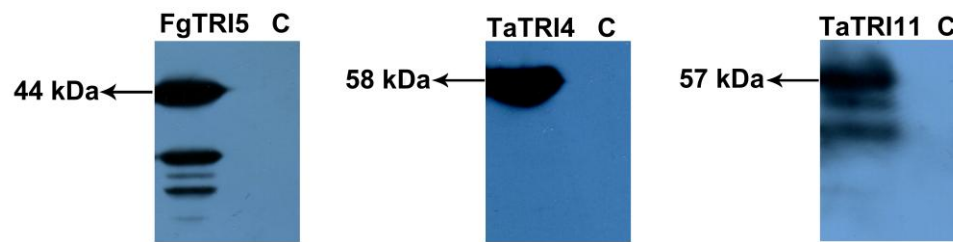
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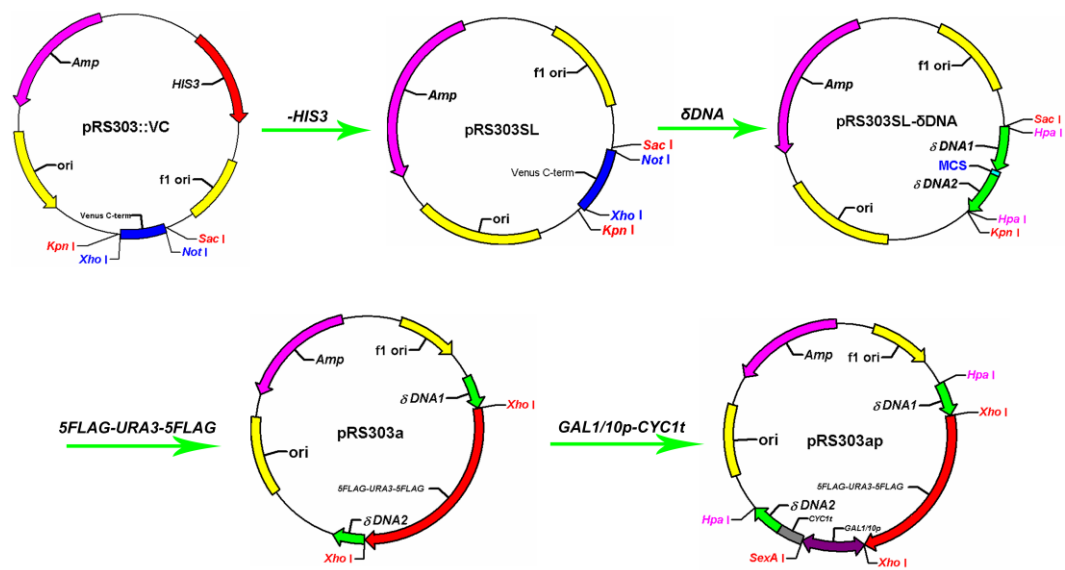
1.1 Supplementary Figures



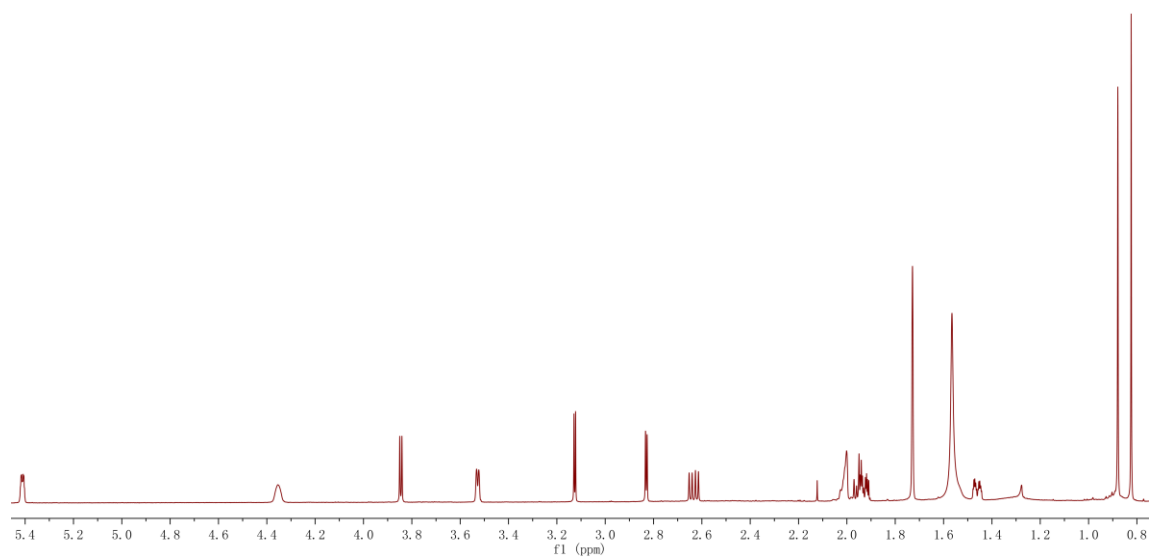
Supplementary Figure 1. SDS-PAGE result of purified recombinant trichodiene synthase FgTRI5 from *E. coli* BL21 (DE3) and GC-MS analyses of *in vitro* enzymatic assay products. **(A)** SDS-PAGE of purified and concentrated FgTRI5 (ca 44 kDa). M: PageRuler™ Broad Range Unstained Protein Ladder (Thermo Fisher Scientific; Waltham, MA, USA); C: The negative control. **(B)** GC-MS analyses of the organic phases (*n*-pentanes) of the reaction mixture and the control in enzymatic assay (top: the total ion chromatogram of trichodiene with mass spectrum inserted; bottom: the control). Peak I represented the newly observed product, showing the same molecular mass and fragments as trichodiene.



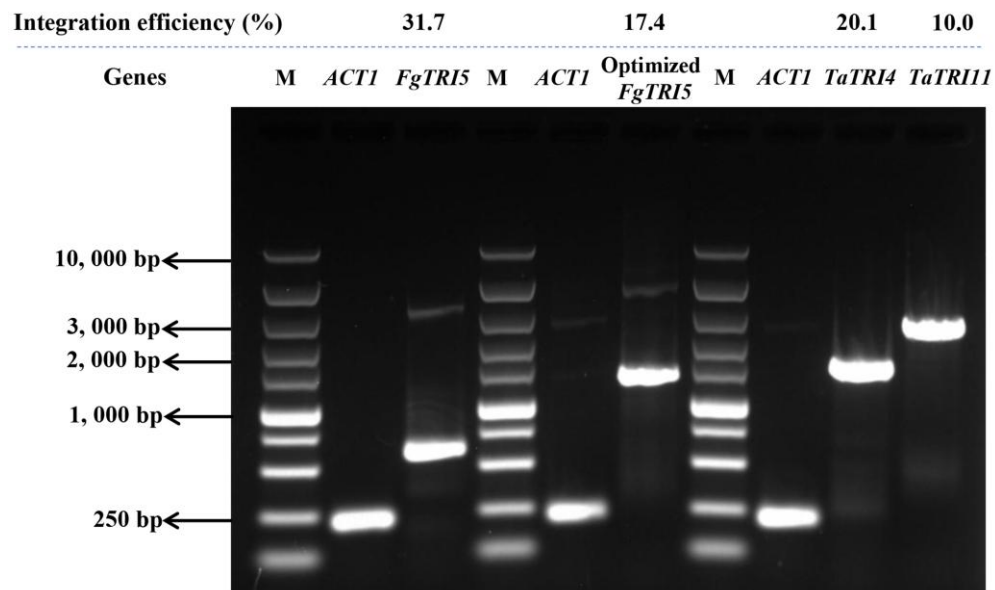
Supplementary Figure 2. Western blot results for the expression of *FgTRI5* in TD1 (left; *ca* 44 kDa), *TaTRI4* in TD5 (middle; 58 kDa), and *TaTRI11* (right; 57 kDa) in TD5. C: The negative control.



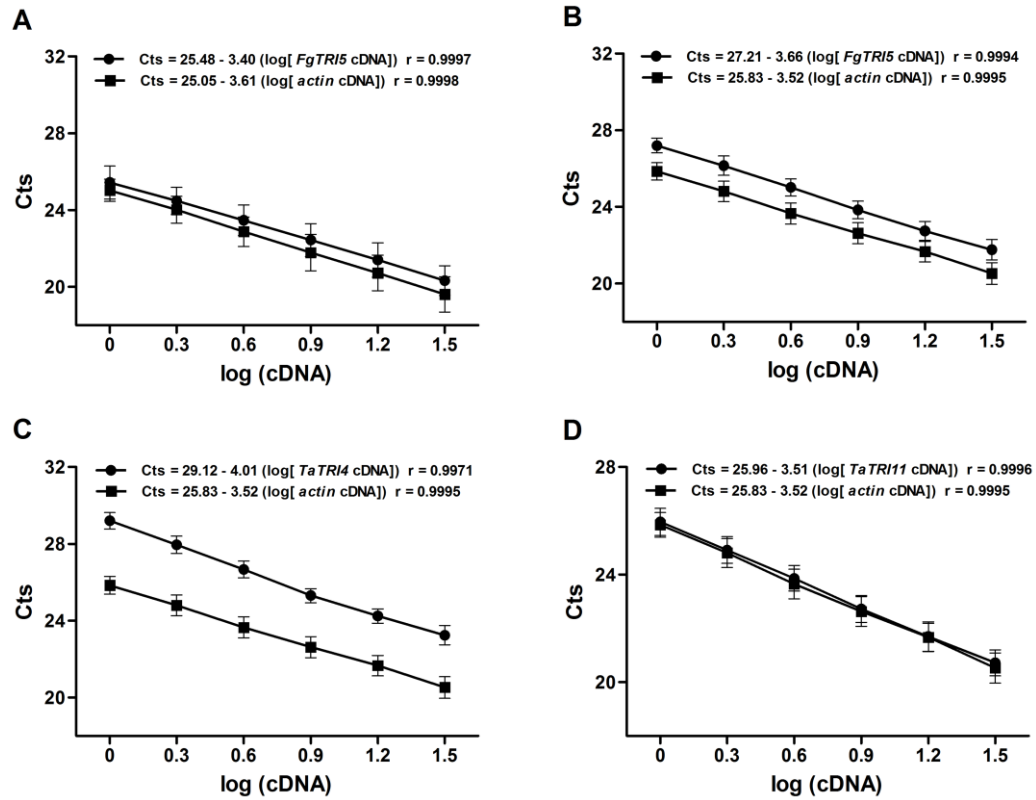
Supplementary Figure 3. Construction of the integration plasmid pRS303ap.



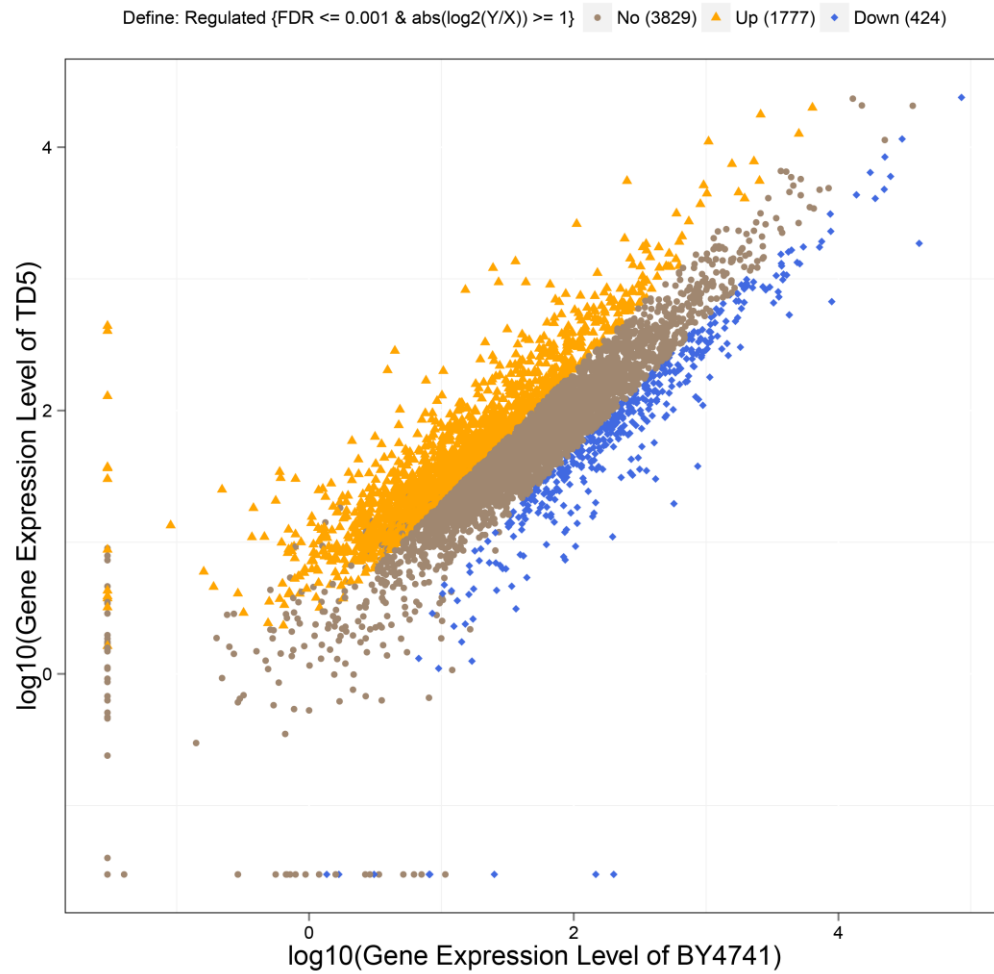
Supplementary Figure 4. ¹H-NMR spectrum of authentic trichodermol prepared by hydrolysis of trichodermate A using deuterated chloroform (CDCl₃) as the solvent.



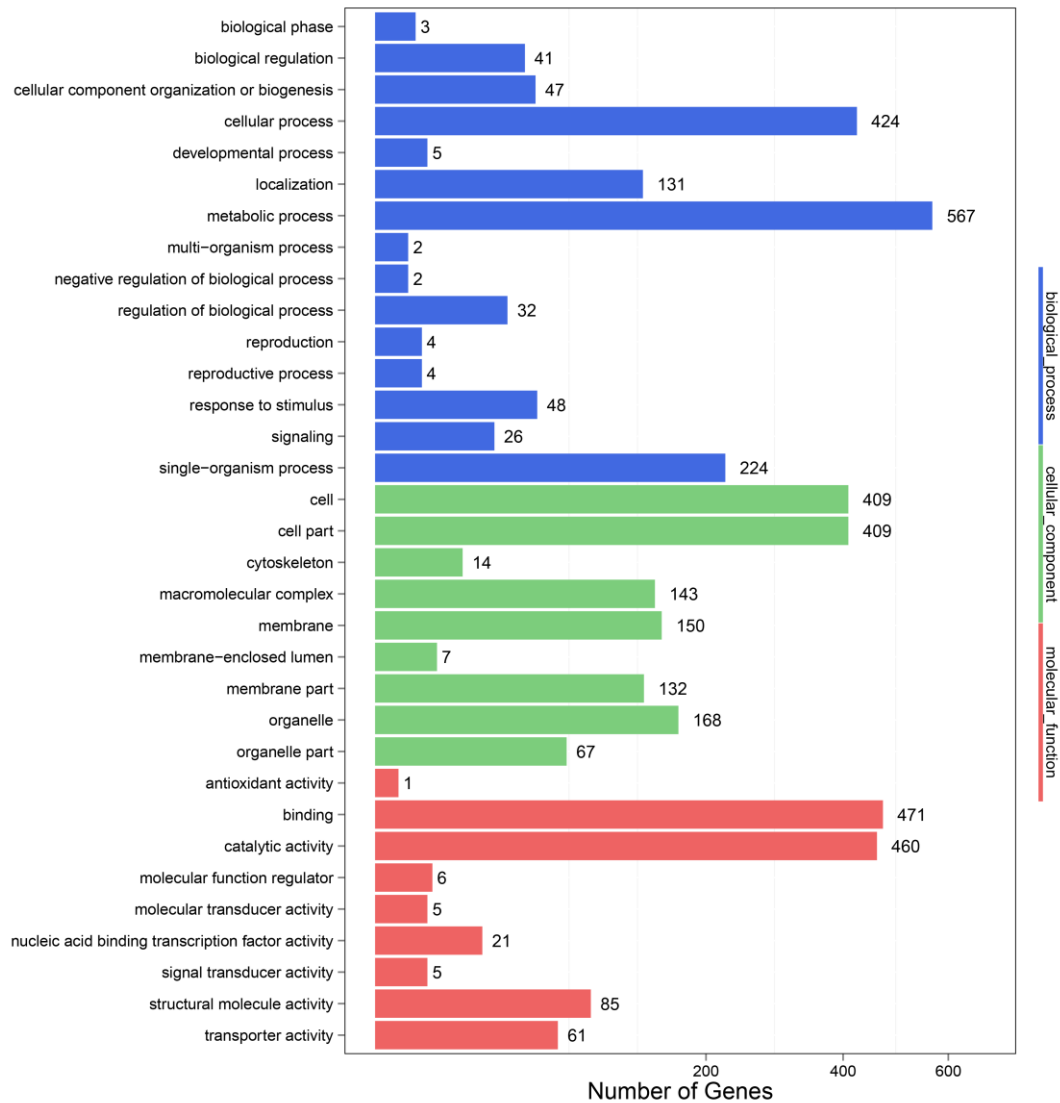
Supplementary Figure 5. Integration efficiencies for *FgTRI5* in TD3, codon-optimized *FgTRI5* in TD4, and *TaTRI4* and *TaTRI11* in TD5, based on Integrated Density Value (IDV) analysis. M: Super DNA Marker (CWBIO, Beijing, China).



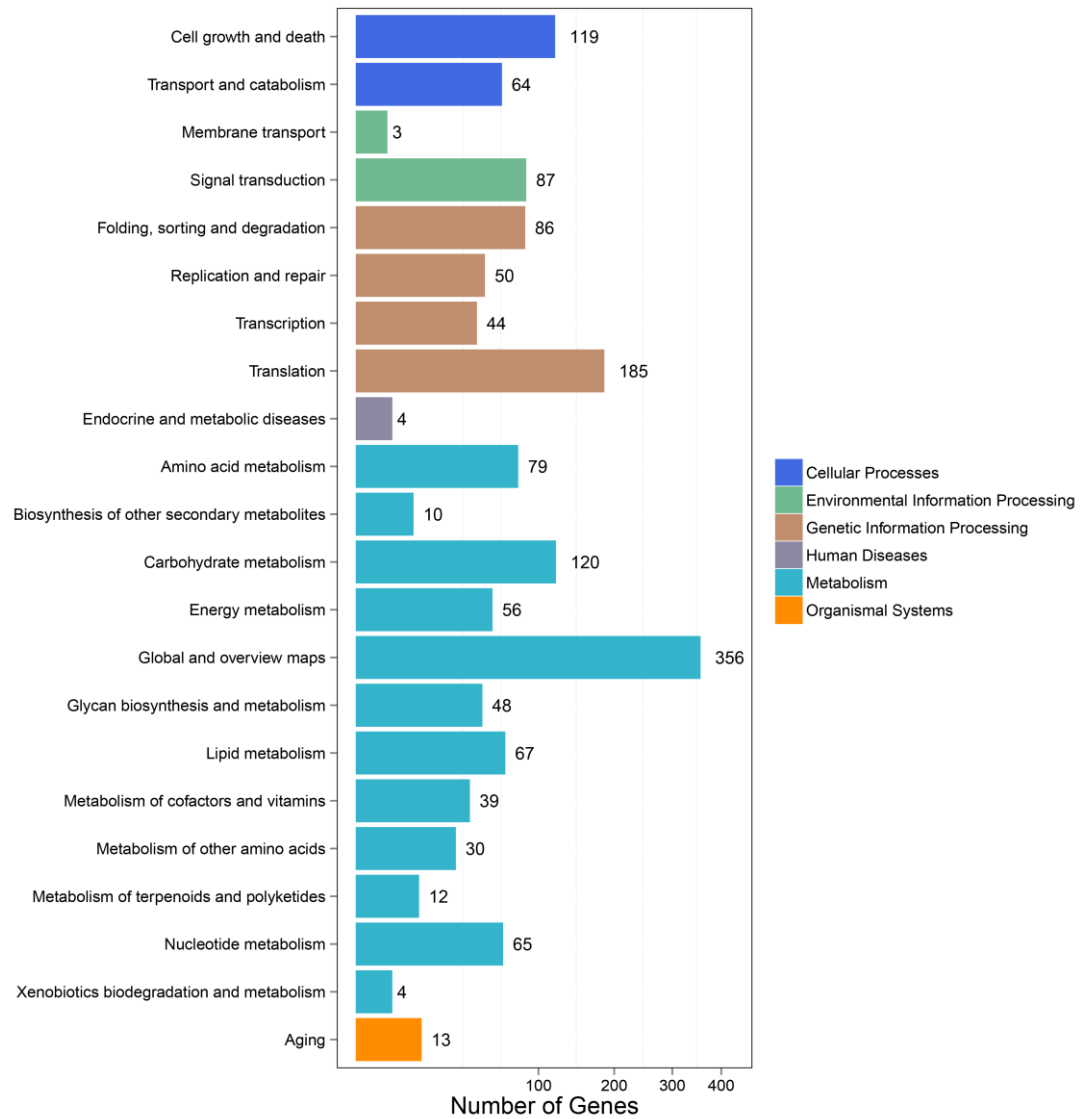
Supplementary Figure 6. Calibration curves from the genomic DNAs of transformants TD3–TD5 used to calculate the copies of *FgTRI5* (A), the codon-optimized *FgTRI5* (B), and *TaTRI4* (C) and *TaTRI11* (D) integrated into the genomes of TD3, TD4, and TD5, respectively.



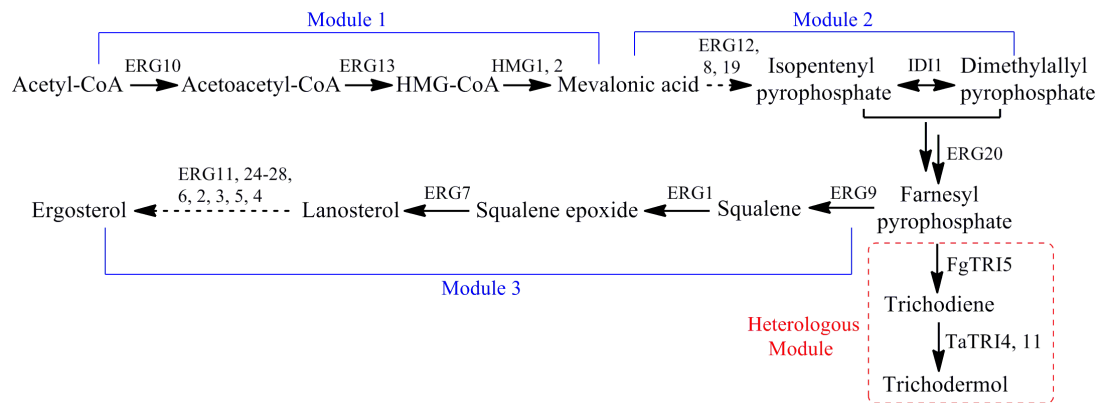
Supplementary Figure 7. Scatter plot for differential gene expressions (DEGs) based on the results from RNA-Seq analysis of TD5 in comparison with BY4741. Blue, orange, and brown points represented the downregulated, upregulated, and unaffected genes, respectively.



Supplementary Figure 8. Gene Ontology Consortium (GO) function classification of DEGs.



Supplementary Figure 9. Kyoto Encyclopedia of Genes and Genomes (KEGG) function classification of DEGs.



Supplementary Figure 10. Three modules involved in the biosynthetic pathway of ergosterol in *S. cerevisiae*. CoA: coenzyme A; HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A.

1.2 Supplementary Tables

Supplementary Table 1 Oligonucleotides used in this study

Primers	Sequence (5'-3') ^a
FgTRI5-30a-F	<u>GCGGCCGC</u> ATGGAAAAC TTTCCCACC
FgTRI5-30a-R	<u>GCGGCCGC</u> CTCCACTAGCTCAATTGAAC
FgTRI5-pESC-F	<u>GGATCC</u> ATGGAAAAC TTTCCCACCG
FgTRI5-pESC-R	<u>GGTACC</u> TTACTCCACTAGCTCAATTGAACT
FgTRI5-303ap-F	AAACCCTCCGGA <u>ACCWGGT</u> ATGGAAAAC TTTCCCACC G
FgTRI5-303ap-R	GAGCGGATA <u>ACCWGGT</u> TTAATGGTGATGGTGATGATGC TCCACTAGCTCAATTGAACT
pRS303-L-F	CTGAGAGTGCACCATAATTCCGGATCATATGCGGTGT G
pRS303-L-R	GACGAAAGGGCCTCGTGA
pRS303-S-F	TATCACGAGGCCCTTTTCGTCTCGCGCGTTTCGGTGATG
pRS303-S-R	GAATTATGGTGCACTCTCAGTACAA
δDNA1-F	<u>GAGCTC</u> GTTAACATGAAGCAGGTGTTGTTGTCTGT
δDNA1-R	<u>GGATCC</u> CCGCGGGCGGCCGCTCGAGGAGGAGAACT TCTAGTATATTC
δDNA2-F	GCGGCCGCCCCGCGGGGATCCGAGGATATAGGAATCCT CA
δDNA2-R	<u>GGTACC</u> GTTAACGTGTTGGAATAGAAATCAACTATC
FLAG-URA3-F	TAGAAGTTCTCCTC <u>CTCGAG</u> ATGGACTACAAGGACGA CGAT
FLAG-URA3-R	CCGCGGGCGGCCGC <u>CTCGAG</u> GAATTCGAGCTCGTTTA AACTA
GAL1-10p-F	GAATTCCTCGAGG <u>CGGCCG</u> CTTTCAAAAATTCTTACTT TTT
GAL1-10p-R	GGGTTTTTTCTCCTTGACG
CYC1t-F	ACGTCAAGGAGAAAAAACCC <u>TCCGGAACCWGGT</u> ATC CGCTCTAACCGAAAAGG
CYC1t-R	GGATCCCCGCGGG <u>CGGCCG</u> CCTTCGAGCGTCCCAAAA C
TaTRI40-F	AGAAAAAACCCCGGATCCATGTTGGATATCAACGCT
TaTRI40-R	CTTCTGTTCCATGTCGACCAATTTCTTAATAACTTTAA C
TaTRI110-F	TTTTGAAAATTCGAATTCATGGCTAATGCAATTTCTG
TaTRI110-R	TTAGTGAGGGTTGAATTCCTTAGAAGCAAATCTATCAT GC
rDNA1-F	ATGAGAGTAGCAAACGTAAGTC
rDNA1-R	CTCACTATTTTTTACTGCGG
Ta411-F	TATTTTCCGCTTCCGCTTCCGCAGTAAAAAATAGTGAG

Ta411-R	GAGCGACCTCATGCTATACCT
HIS3-F	CTTCGAGCGTCCCAAAAC
	GTACAGACGCGTGTACGCATGTAACATTATACTGAAA
	ACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGAGCT
	TGGTGAGCGCTAGGA
HIS3-R	ATGATCCGTCGAGTTCAAGAG
rDNA2-F	TTGCTTTTTCTTTTTTTTTCTCTTGAACCTCGACGGATCA
	TGAACCTGGGTACCCGGGG
rDNA2-R	TTTCCTCTAATCAGGTTCCACC
ERG1-F	TCGTTTGTCGGTATGTCT
ERG1-R	GCTGGCAAGTAGGAGTTT
ERG7-F	GGTGGGTTCATACTAGAGGTG
ERG7-R	ACAAGGCTGTTCGCAATA
ERG9-F	GTTTGACCCGTTTGATTG
ERG9-R	GGAGCGTATTGTGACCAG
ERG10-F	TTGGTTCATTCCAGGGTT
ERG10-R	TCATAGCGGATGCACAGA
ERG13-F	GCTGGTTCGGATGCTTTG
ERG13-R	TTCGGCGTCAACTTCTGG
ERG20-F	TCTTTCTACTTGCCTGTCG
ERG20-R	ATGCCTTGTTGATTACCC
HMG1-F	AAGTGGACGGTGATTTGA
HMG1-R	AGGTTTCCTGTTGTGGGT
IDI1-F	TGACGAAAGCGGAGAAAC
IDI1-R	AGACGGAGAATGCACGAT
UPC2-F	ACGATCAAGAAGGAGCAG
UPC2-R	ATGGCGGTAATAGTAAGC
PDR5-F	CTTACAGCGGCTACTCAG
PDR5-R	TTCGCATAACTTTCCCTA
PDR11-F	TATCTGACGGTGGAGCAAAC
PDR11-R	AAATTCTAGGGCGGTGGC
PDR12-F	GCTGGTGCTTTCTTCCAGT
PDR12-R	GGACCCATAAACCACCGA
PDR1-F	GGTCATTATCCGTGTCTG
PDR1-R	ACTGAAGTGGGTATTTGC
PDR3-F	GAAGACCCGCACATCACT
PDR3-R	TATGGCAACCAAACCTCGA
YOR1-F	AGAGCCTTTCCTATTTCC
YOR1-R	TCATTTTCGGGCTTTCTAT
SNQ2-F	GTCGCCAAACCAGACTTA
SNQ2-R	TTGTCCACCCTTCCTCAA
STB5-F	GAACAACATCGTGGGTCA
STB5-R	TTCGTCCTGAGAAACCATAC
RAM1-F	TCCCTTTGCGATAACATA

RAM1-R	CCACTTCTCCGACTTCTA
STE14-F	CAGACATCAGACGCAATC
STE14-R	GACAGGGCAATAATAAAGAG
ACT1-F	CGACGCTCCTCGTGCTGT
ACT1-R	TGGGGCAACTCTCAATTCGT
TRI5-RT-F	CATGGCGGATCTATCTATTC
TRI5-RT-R	TGGGAAAGTGCTCGTTGA
TRI5O-RT-F	TACGCATACAATAAGGCT
TRI5O-RT-R	ATTGACAAATCAGCCATA
TaTRI4-RT-F	TTTTCTTGTTTGCTGGTA
TaTRI4-RT-R	GTATGTTCCCTTGTATTGC
TaTRI11-RT-F	GTCTTACATCCCAGGTCC
TaTRI11-RT-R	GCATGATCGATTTCGTTA
TRI5- δ DNA2-F	ATACAGAGGACGCCAAGAAG
TRI5- δ DNA2-R	TGTTAGAAGATGACGCAAATGA
TRI5O- δ DNA2-F	CAAAGGAATGTATGGCTGAT
TRI5O- δ DNA2-R	TATACGGTGTTAGAAGATGACG
rDNA1-Ta11-F	GTCCGCATTGGGATGTTAC
rDNA1-Ta11-R	TGGAAGCTGGTGCTGCAT
Ta4-rDNA2-F	CAGATTTCCATTTTCGTTGT
Ta4-rDNA2-R	CTCTGTCGCTCTGCCTTA

a The underlined nucleotide sequences indicate restriction enzyme sites

Supplementary Table 2 The codon-optimized sequences of *FgTRI5*, *TaTRI4*, and *TaTRI11*

Proteins	Encoding sequences
FgTRI5 from <i>F. graminearum</i> (As_FgTRI5)	ATGGAAAACCTTCCCAACTGAATACTTTTTGAACACA TCTGTTAGATTGTTGGAATACATCAGATACAGAGATT CAAACCTACACTAGAGAAGAAAGAATCGAAAATTTG CATTACGCATACAATAAGGCTGCACATCATTTTGCTC AACCAAGACAACAACAAATGTTGAAGGTTGATCCA AAGAGATTGCAAGCATCTTTGCAAACAATCGTTGGT ATGGTTGTTTACTCTTGGGCAAAAGTTTCAAAGGAA TGTATGGCTGATTTGTCAATCCATTACACTTACACAT TGGTTTTAGATGATTCTTCAGATGATCCACATCCAGC TATGTTGAATTACTTTGATGATTTGCAAGCTGGTAGA GAACAAGCACATCCATGGTGGGCTTTGGTTAACGAA CATTTCCCAAACGTTTTGAGACATTTCCGGTCCATTCT GTTCTTTGAATTTGATCAGATCAACTATGGATTTCTT TGAAGGTTGTTGGATCGAACAATACAACCTTCGGTGG TTTCCCAGGTTCTGATGATTACCCACAATTTTTTGAGA AGAATGAATGGTTTAGGTCATTGTGTTGGTGCTTCTT TGTGGCCAAAGGATTTGTTTCGATGAAAGAAAGCATT TCTTGGAAATTACATCAGCTGTTGCACAAATGGAAA ATTGGATGGTTTGGGTAAACGATTTGATGTCTTTCTA CAAGGAATTTGATGATGAAAGAGATCAAATCTCATT GGTTAAAAATTTTCGTTACTTGTCATGAAATCACATTG GATGAAGCATTGGAAAAGTTGACTCAAGAAACATT GCATTCTTCAAAGCAAATGGTTGCTGTTTTCTCTGAT AAAGATCCACAAGTTATGGATACTATCGAATGTTTCA TGCATGGTTACGTTACATGGCATTGTGTGATGCAAG ATACAGATTGCATGAAATCTATGAAAAAGTTAAAGA TCAAGATACTGAAGATGCTAAGAAATTCTGTAAGTT TTTCGAACAAGCTGCAAATGTTGGTGCTGTTGCACC ATCAGAATGGGCTTACCCACAAGTTGCTCAATTAGC AAACGTTAGAGCAAAGGATGATGTTAAGGAAGCTC AAAAGCCAATCTTGTCTTCAATTGAATTAGTTGAATA A

TaTRI4 from *T.*
arundinaceum
(As_TaTRI4)

ATGTTGGATATCAACGCTTTGAAGGAAATCCCACCA
GCTACTGCAGTTGGTGCTGCAGTTGCTGTTGGTGCA
TTGTATTTCTTTTGTCAATGTTTCTACAATTTGTACTT
ACATCCATTAAGAAAAATTCCAGGTCCAAAATTGGC
TGCAATTGGTCCATATTTGGAATTCTACCATGAAGTT
TTGAGAGATGGTCAATATTTGTGGGAAATTGAAAAG
ATGCATCAAAAATACGGTCCAATTGTTAGAGTTAAC
GCTAGAGAAGTTCATGTTAAGGATACTTCTTACTACA
ACACTATCTATACAGCTGGTGCAAGAAAACTAATA
AGGATCCAGCAACAGTTGGTGCTTTTGATGTTCCAA
CTGCAACAGCTGCAACAGTTGATCATGATTTGCATA
GAGCTAGAAGAGGTTATTTGAACCCATACTTCTCTA
AAAGAGCTGTTGCAGGTTTGGAAACCACTATCCATG
AAAGAATCACAAAGTTGTTATCAAGATTCGATCAAC
ATAGAAAGGATGATCAAGTTTTGTCTTTAGATGGTG
CTTTTTCAGCATTAAGTCTGCTGATGTTATCACAGCAAG
ATTCTACGGTGAACATAAGGATTACTTGGATGTTCCA
GATTTCCATTTTCGTTGTTAGAGATGGTTTCCAAGGTT
TGTCTAGAGTTTACCATTTGGGTAGATTTTTACCATC
AGTTGTTGGTGCTTTGAAAGGTTTGCCAAAGTTCTT
GATCAGAATCATCTTCCCACCAATCGCAGAATTGTT
GACTATGAGAGAAGAAATCGAAGCTGGTGGTATCGA
TGAATTCACTAAGTCTAAATCTTCAGGTATTAAATCT
TCAGTTTTGGTTGGTGCTTTATCAGATCCACATATTC
CACCACAAGAAAGAACTGTTGCAAGAATGTTGGAT
GAAGGTACTGTTTTCTTGTTTGCTGGTACAGAACT
ACATCTAGAAGTTTGGGTATCACAATGTTCTATTTGT
TATCAAATCCAGATATTTTGAATAAGTTAAGAGAAGA
ATTGAAATCTTTACCACCATCAGATGATAACATGCAT
TCTTTGGGTCAATTGGAAAATTTGCCATACTTAACAG
GTGTTGTTTCATGAAGGTTTGAGATTATCTTTTGGTCC
AATTTCAAGATCTTCAAGAGTTGCAACTCATGAAGC
TTTGCAATACAAGGAACATACTATTCCAGCAGGTAC
ACCAGTTTCTCAATCAACATACTTCGTTCCATACTGAT
ACAGAAATCTTCCCAGATCCATGGGAATTCAAACCA
GAAAGATGGATTAAAGCTGCAGAAGATGGTGTGCT
TTGAAGAAATACATCACTAACTTCTCTCAAGGTTCA
AGACAATGTATTGGTTACTCTATGTCATTCGCAGAAA
TGTTCTTGACATTGTCTAGAATCATCCCAGCTTTTCA
TTTGGAATTATACGATACTACAAAAGCAGATATTGAT
ATGACTCATGCTAGAATTGTTGGTTACCCAAAGAAA
GTTCCAGGTAAAACAGAATCATTGGGTGAATTAAGA
GTAAAGTTATTAAGAAATTGTAA

TaTRI11 from *T.*
arundinaceum
(As_TaTRI11)

ATGGCTAATGCAATTTCTGTTGGTGTTCAGTTCAAT
TGGTTTTGACTGTTTTGTTGGCTTCAATCCCATTGAG
AGTTATTTGGAATTTGTTTTCCATCCATTGTCTTACA
TCCCAGGTCCAAGATTGTGGATCGCTTTCCCAATTTT
TAGACAAATCGCATCTATTAGAGGTGTTTTCGATGCT
CAAATGTGTGAATACCATAGAAAGTACGGTAACGCA
GTTAGATTTTCTCCAAACGAAGTTTCTTTTATTACTG
AACAAGCTTGGAGAGATATCTATGATCATAGACCAA
ACCAATTGGAAAGATTCATTTTGTCAACTACAAGAA
GACCAGATATCTTCGATGCTAACGAAATCGATCATGC
AAGATATAGAAAGGCTATGTTACCAGCATTTTCTCCA
AAAGGTTTGCAAGAACAAGAACCAATTGTTAGAGG
TTACATCGATACTTTTATTGAAAGATTGAGAGAAGTT
TCTGCAACTGGTGAATCAACAGATATGGTTAAGTGG
TACAACTTCACTACATTTCGATATTATTGGTGACTTGG
CTTTTGGTGAATCATTGTTGGTGGTTTGAGAAATAGAG
AATACCATTTTACTATCTCTTTTACATTCTGAAGCTTTT
AAATTGTTATCATATTGGAAGCTGGTGCTGCATACC
CATTGTTGTTGAAGATCTTGATGGCTTTTACTCCACA
ATCTTTGATTGAAGCAAGAGATAAGAAAGAAGAAC
ATGCTGAAACTACAGTTAGAAAGAGATTGGATAACA
GAGCTTTGCATGGTCGTGGTGACTTCATGGATTATTT
GTTGAGAAACAGAGGTGAAAAGCAAGGTTTGAACG
ATAAGGAATTGGTTGCTAACGCATCTACTTTGATCAC
AGCTGGTTCAGAACTACAGCAACTATCTTGTCAGG
TATCACATACTGGTTGTTGCAAACCTCCAAACGTTTTG
CAAAAGGTTACTGAAGAAGTTAGATCTGCTTTCCAA
TCAGAAGCAGATATCACTTTTACATCTGCTACATCAC
AATTACCATATATGTTGGCTTGTTTTCAAGAAGCTTT
TAGACATTACCCACCAGTTCCAACCTGGTATGCCAAG
AGTTACTCCATCTCATGGTATCACAAAGATCTCTGGT
TACGATATCTCACCAAACACAAAAGTTTCTGTTTCATC
AATTAGCTGCATACTCACATCCAGATAACTTCCATAG
ACCAAGAGAATTTCGTTCCAGAAAGATGGTTGCCAG
ATGCTAAAATAATCCATCTTCACCATGGTATAATGAT
AGAAGAGAAACAGTTCAACCTTTTAATGTTGGTCCA
AGAAATTGTGTTGGTAGAAATTTGGCAGAACAAGA
AATCAGAGTTATGTTGGCTAGAGTTTTGTGGAACCTT
CGATTTGGAATTGGCTCCAGAATCTAAAAATTGGAC
TGATCAAAAGACTCATTCTTGTGGGAAAAAGGTGC
ATTGATGTGTAAGTTGCATGATAGATTTGCTTCTAAA
TAA

Supplementary Table 3 Genes related to terpenoid metabolism and ABC transporters in TD5

Gene	Gene ID	Characterization
Terpenoids metabolism		
RAM1	851468	Subunit of the CAAX farnesyltransferase
STE14	852019	Farnesyl cysteine-carboxyl methyltransferase
MVD1	855779	Mevalonate pyrophosphate decarboxylase
ABC transporters		
PDR5	854324	ABC transporter
PDR11	854802	ABC transporter
PDR12	856049	ABC transporter
YOR1	853198	ABC transporter
SNQ2	851574	ABC transporter
PDR1	852871	Drug-responsive transcription factor
PDR3	852278	Drug-responsive transcription factor
STB5	856583	Transcription factor