

Analogous and diverse functions of APSES-type transcription factors in the morphogenesis of the entomopathogenic fungus, *Metarhizium rileyi*

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Table S1 Gene expression in $\Delta MrStuA$ mutants relative to wild-type (WT) during dimorphic transition

Annotation from NCBI Nr protein database	Gene Name	GW1_FPKM	GW2_FPKM	GW3_FPKM	GS1_FPKM	GS2_FPKM	GS3_FPKM	log2FC	Up/Down	FDR
Cellulase	<i>Cel</i>	7.641	5.207	5.092	1.429	0.991	0.967	-2.213	Down	6.32E-05
Cytochrome P450	<i>Cp450</i>	0.609	0.346	0.162	2.113	1.627	1.288	2.357	Up	1.80E-6
Heat shock protein 101	<i>H101</i>	90.944	107.584	84.997	882.414	713.167	717.310	3.214	Up	9.46E-45
GATA transcription factor	<i>NsdD</i>	4.169	4.167	3.989	9.684	9.626	8.946	1.450	Up	1.94E-08
Heat shock protein 90	<i>H90</i>	554.026	352.991	567.606	1395.486	1391.291	1242.497	1.634	Up	2.08E-24
Cell wall beta-glucan synthesis	<i>Cwb</i>	1340.449	1271.608	1278.913	1121.157	1099.003	1045.579	-0.0677	Normal	0.8755
Protein-tyrosine phosphatase	<i>Ptp</i>	65.210	66.009	70.078	43.187	44.949	41.099	-0.454	Normal	0.120
bZIP transcription factor HapX	<i>HapX</i>	106.516	100.291	97.252	43.285	37.952	41.278	-1.125	Down	5.01E-10
MAP kinase kinase EMK1	<i>Emk1</i>	114.815	107.860	100.243	129.258	112.718	119.672	0.3439	Normal	0.181
Cytochrome c peroxidase	<i>Ccp</i>	94.366	99.565	99.157	58.498	88.807	61.365	0.6509	Normal	0.00093
ATPase assembly factor ATP10	<i>Atp10</i>	89.720	83.323	80.491	60.525	63.557	62.765	-0.255	Normal	0.391
Mob1/phocein	<i>Mob</i>	23.610	17.309	22.918	25.562	16.770	18.760	0.6037	Normal	0.2491

DNA-repair protein rad2	<i>Rad2</i>	6.245	10.622	9.246	8.141	9.635	9.040	0.596	Normal	0.221
ChaC-like protein	<i>Clp</i>	56.621	45.068	56.537	52.606	51.923	22.517	0.8753	Normal	0.131

Abbreviation used: RPKM: fragments per kilobase million; FDR: false discovery rate; FC: fold change; Up/Down: up-/down-regulated. GW1, GW2, and GW3 were three samples of the WT strain during dimorphic transition. GS1, GS2, and GS3 were three samples of the $\Delta MrStuA$ mutants during dimorphic transition.

Table S2 Gene expression in $\Delta MrXbp$ mutants relative to WT during dimorphic transition

Annotation from NCBI Nr protein database	Gene Name	GW1_FPKM	GW2_FPKM	GW3_FPKM	GX1_FPKM	GX2_FPKM	GX3_FPKM	log2FC	Up/Down	FDR
Cytochrome P450	<i>Cp450</i>	0.609	0.346	0.162	0.169	0.280	0.161	-0.824	Normal	0.371
GATA transcription factor	<i>NsdD</i>	4.169	4.167	3.989	10.440	12.405	10.568	1.561	Up	7.58E-11
Cellulase	<i>Cel</i>	7.641	5.207	5.092	0	0	0	$-\infty$	Down	7.12E-31
Heat shock protein 101	<i>H101</i>	90.944	107.584	84.997	219.451	335.662	256.553	1.554	Up	3.02E-06
Heat shock protein 90	<i>H90</i>	554.026	352.991	567.606	1395.564	962.506	1596.451	1.452	Up	2.36E-05
Cell wall beta-glucan syntheses	<i>Cwb</i>	1340.449	1271.608	1278.913	3061.632	2841.773	2976.183	1.222	Up	2.99E-20
Protein-tyrosine phosphatase	<i>Ptp</i>	65.210	66.009	70.078	13.032	13.125	11.998	-2.366	Down	1.32E-20
MAP kinase kinase EMK1	<i>Emk1</i>	114.815	107.860	100.243	3061.631	2841.772	2976.182	1.222	Up	2.98E-20
Cytochrome c peroxidase	<i>Ccp</i>	94.366	99.565	99.157	297.820	292.440	299.596	2.050	Up	1.31E-34
ATPase assembly factor ATP10	<i>Atp10</i>	89.720	83.323	80.491	85.463	83.348	78.939	-0.000419	Normal	0.997
Mob1/phocein	<i>Mob</i>	23.610	17.309	22.918	14.267	15.234	14.845	-0.372	Normal	0.1642
DNA-repair protein rad2	<i>Rad2</i>	6.245	10.622	9.246	15.466	16.311	15.420	0.00005	Normal	0.833
ChaC-like protein	<i>Clp</i>	56.621	45.068	56.537	48.013	24.020	46.171	-0.3970	Normal	0.238

bZIP transcription factor <i>HapX</i>	106.516	100.291	97.252	28.235	25.508	23.601	-1.942	Down	1.64E-28
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Abbreviation used: RPKM: fragments per kilobase million; FDR: false discovery rate; FC: fold change; Up/Down: up-/down-regulated. GW1, GW2, and GW3 were three samples of the WT strain during dimorphic transition. GX1, GX2, and GX3 were three samples of the $\Delta MrXbp$ mutants during dimorphic transition.

Table S3 Gene expression in $\Delta MrStuA$ mutants relative to WT during microsclerotium development

Annotation from NCBI Nr protein database	Gene Name	W1_FPKM	W2_FPKM	W3_FPKM M	N1_FPKM	N2_FPKM	N3_FPKM	log2FC	Up/Down	FDR
Heat shock protein 101	<i>H101</i>	298.837	123.399	390.334	3331.313	29995.36	1220.111	2.737	Up	0.00562
Ubiquitin-conjugating enzyme	<i>Uce</i>	16.518	12.835	15.171	0.741	0.399	0.553	-5.098	Down	2.00E-10
Heat shock protein 90	<i>H90</i>	841.821	1072.921	982.204	3700.62	4038.17	3571.412	1.342	Up	0.0307
Mob1/phocein	<i>Mob</i>	268.787	282.770	252.793	88.544	75.437	92.271	-2.099	Down	0.000104
ChaC-like protein	<i>Clp</i>	23.874	44.283	46.679	14.739	13.585	15.763	-1.709	Down	0.000405
MAP kinase kinase EMK1	<i>Emk1</i>	39.946	50.166	55.233	29.705	21.825	30.360	-1.227	Down	0.002
Cell surface protein (Mas1)	<i>Mas1</i>	1.649	1.525	1.871	20.657	25.723	39.557	3.626	Up	1.13E-06
Sodium/calcium exchanger membrane region	<i>Sem</i>	9.694	10.275	11.437	7.801	10.792	11.529	-0.361	Normal	0.629
DNA-repair protein rad2	<i>Rad2</i>	8.583	3.697	2.232	29.018	24.406	37.987	2.30	Up	2.31E-10
Cytochrome c peroxidase	<i>Ccp</i>	193.788	133.213	165.189	145.535	93.391	89.990	-1.513	Down	0.00187
Membrane protein Tapt1	<i>Tapt1</i>	12.885	15.915	11.571	12.765	14.495	12.518	0.619	Normal	0.3194
GATA transcription factor	<i>NsdD</i>	13.459	7.622	9.299	16.071	11.646	9.490	0.312	Normal	0.650

Carbohydrate-binding Wsc	<i>Wsc</i>	25.114	23.851	20.4269	23.877	74.704	52.111	1.243	Up	0.00227
ATPase assembly factor ATP10	<i>Atp10</i>	81.890	135.420	81.929	54.530	40.112	45.331	-1.509	Down	0.000406

Abbreviation used: RPKM: fragments per kilobase million; FDR: false discovery rate; FC: fold change; Up/Down: up-/down-regulated. W1, W2, and W3 were three samples of the WT strain during microsclerotium development. S1, S2, and S3 were three samples of the $\Delta MrStuA$ mutants during microsclerotium development.

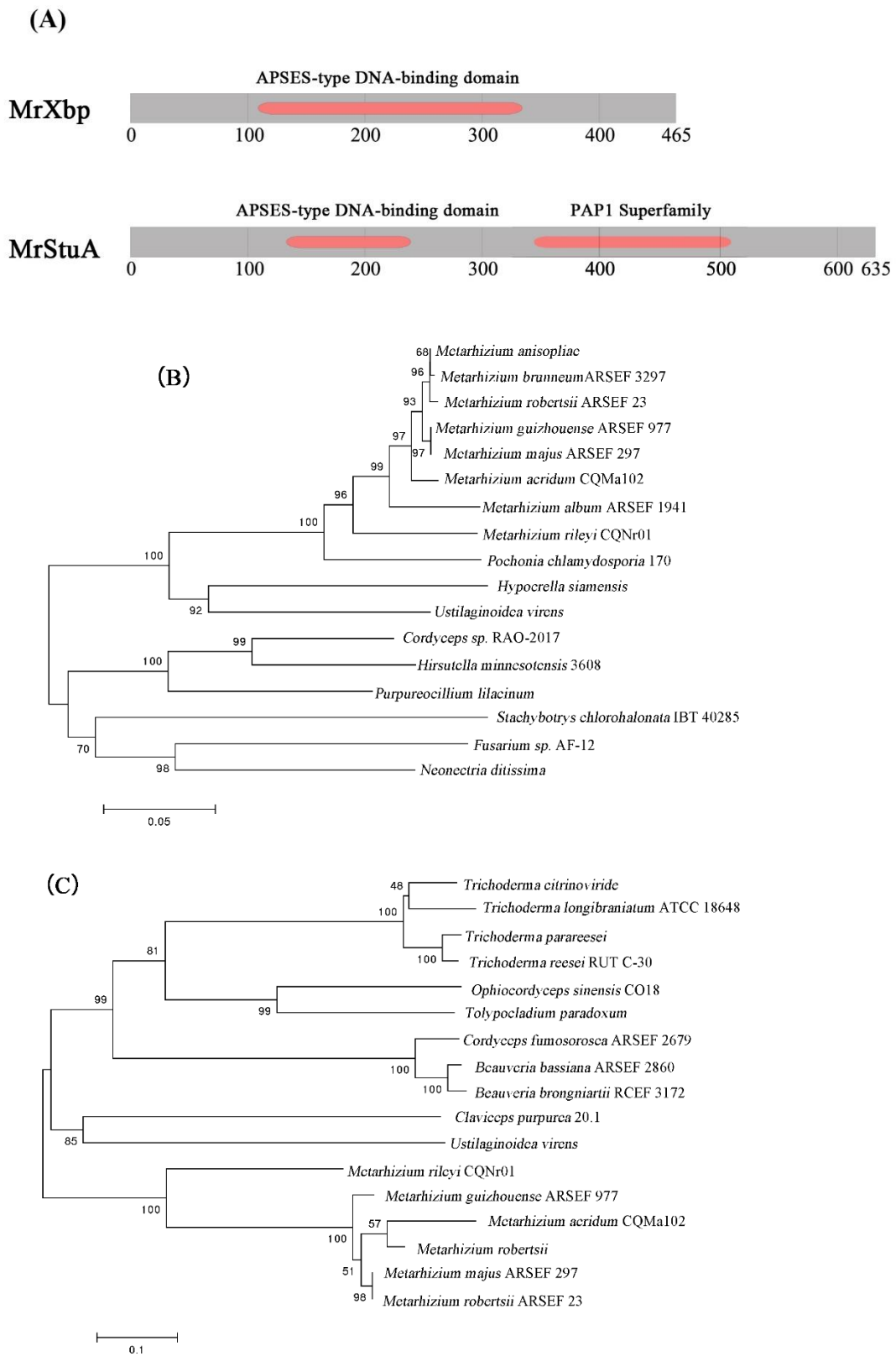
Table S4 Gene expression in $\Delta MrXbp$ mutants relative to WT during microsclerotium development

Annotation from NCBI Nr protein database	Gene Name	W1_FPKM	W2_FPKM	W3_FPKM	N1_FPKM	N2_FPKM	N3_FPKM	log2FC	Up/Down	FDR
Heat shock protein 101	<i>H101</i>	274.482	113.266	355.170	537.836	575.774	318.860	0.752	Normal	0.314
Ubiquitin-conjugating enzyme	<i>Uce</i>	16.518	12.835	15.171	0.741	0.399	0.553	-3.288	Down	0.00616
Heat shock protein 90	<i>H90</i>	841.821	1072.921	982.204	975.138	915.535	3571.412	-0.209	Normal	0.766
Cytochrome c peroxidase	<i>Ccp</i>	158.170	109.121	132.960	302.973	255.471	282.015	0.324	Normal	0.548
Sodium/calcium exchanger membrane region	<i>Sem</i>	9.694	10.275	11.437	44.978	55.074	51.212	2.068	Up	3.46E-11
DNA-repair protein rad2	<i>Rad2</i>	8.583	3.697	2.232	30.8019	30.950	30.974	2.486	Up	2.07E-14
Carbohydrate-binding Wsc	<i>Wsc</i>	25.114	18.687	23.168	61.716	67.843	61.721	1.541	Up	2.12E-06
Mob1/phocein	<i>Mob</i>	268.787	282.770	252.793	99.832	89.361	93.163	-1.693	Down	0.000143
ATPase assembly factor ATP10	<i>Atp10</i>	81.779	135.227	81.833	50.840	50.797	51.808	-1.1207	Down	0.00164
ChaC-like protein	<i>Clp</i>	23.791	44.159	46.555	12.551	9.961	10.557	-1.855	Down	0.00005
MAP kinase kinase EMK1	<i>Emk1</i>	39.892	50.094	55.162	56.886	49.716	44.039	-0.1085	Normal	0.7758
Cell surface protein (Mas1)	<i>Mas1</i>	1.649	1.525	3.355	4.483	3.519	39.557	0.9753	Normal	0.3424
GATA transcription factor	<i>NsdD</i>	13.459	7.622	9.299	16.172	8.558	10.645	0.5103	Normal	0.3257

Membrane protein Tapt1	<i>Tapt1</i>	12.067	15.719	11.571	5.147	6.397	5.439	-1.249	Down	0.00303
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Abbreviation used: RPKM: fragments per kilobase million; FDR: false discovery rate; FC: fold change; Up/Down: up-/down-regulated. W1, W2, and W3 were three samples of the WT strain during microsclerotium development. X1, X2, and X3 were three samples of the $\Delta MrXbp$ mutant during microsclerotium development.

Fig. S1 Structural and phylogenetic analysis of MrStuA and MrXbp proteins

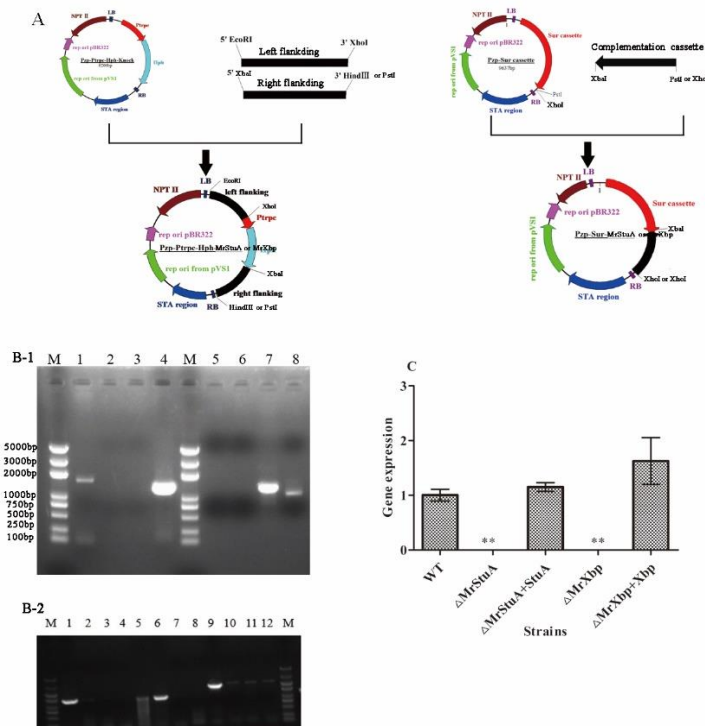


(A) Conserved domains predicted via sequence alignment

(<http://www.ebi.ac.uk/Tools/pfa/iprscan>). (B) Phylogenetic analysis of MrStuA protein. The numbers at the nodes represent the results of bootstrap analyses (1000 replicates) carried out using the neighbor-joining method. The aligned sequences of StuA protein are from *Metarhizium anisopliae* (KFG88133.1); *Metarhizium guizhouense* ARSEF 977 (KID87765.1); *Metarhizium majus* ARSEF 297 (KID98874.1); *Metarhizium brunneum* ARSEF 3297 (XP_014545496.1); *Metarhizium album* ARSEF 1941 (KHN97002.1); *Metarhizium robertsii* ARSEF 23 (XP_007819177.1); *Pochonia chlamydosporia* 170 (XP_018139603.1); *Metarhizium acridum* CQMa 102 (XP_007810169.1); *Ustilagoidea virens* (KDB12946.1); *Hypocrella siamensis* (ANH22586.1); *Fusarium* sp. AF-12 (RMJ13252.1); *Cordyceps* sp. RAO-2017 (PHH78192.1); *Purpureocillium lilacinum* (PWI75332.1); *Hirsutella minnesotensis* 3608 (KJZ71075.1); *Stachybotrys chlorohalonata* IBT 40285 (KFA66336.1); *Neonectria ditissima* (KPM35181.1). (C) Phylogenetic analysis of MrXbp protein. The numbers at the nodes represent the results of bootstrap analyses (1000 replicates) carried out using the neighbor-joining method. The aligned sequences of MrXbp protein are from *Metarhizium majus* ARSEF 297 (KID96819.1); *Metarhizium robertsii* ARSEF 23 (XP_007821239.2); *Metarhizium guizhouense* ARSEF 977 (KID92324.1); *Metarhizium robertsii* (EXV05007.1); *Metarhizium acridum* CQMa 102 (XP_007812764.1); *Tolypocladium paradoxum* (POR39629.1); *Cordyceps fumosorosea* ARSEF 2679 (XP_018703649.1); *Beauveria bassiana* ARSEF 2860 (XP_008593781.1); *Ustilagoidea virens* (KDB17052.1); *Trichoderma parareesei* (OTA01006.1); *Trichoderma citrinoviride* (XP_024754116.1);

Trichoderma longibrachiatum ATCC 18648 (PTB80558.1); *Beauveria brongniartii*
RCEF 3172 (OAA45796.1); *Trichoderma reesei* RUT C-30 (ETS05362.1);
Ophiocordyceps sinensis CO18 (EQL01683.1); *Claviceps purpurea* 20.1
(CCE33631.1).

Fig. S2 Confirmation of gene disruption



(A) Construction of knockout and complementation (CP) vectors. (B) PCR characterization of (B-1) $\Delta MrStuA$ mutants and (B-2) $\Delta MrXbp$ mutants, wild-type (WT), and CP strains. Open reading frame was PCR (Lanes 1-2 in B-1: 1-*MrStuA* of WT, 2- $\Delta MrStuA-1$; Lanes 1-3 in B-2: 1-*MrXbp* of WT, 2- $\Delta MrXbp-1$, 3- $\Delta MrXbp-2$); The *hph* was screened (Lanes 3-4 in B-1: 3-*hph* of WT, 4- $\Delta MrStuA-1$; Lanes 4-6 in B-2: 4-*hph* of WT, 5- $\Delta MrXbp-1$, 6- $\Delta MrXbp-2$). The *hph* and genomic sequence outside the flank regions was PCR (Lanes 5-8 in B-1: 5-Left Frame (LF)-WT, 6-Right Frame (RF)-WT, 7-LF- $\Delta MrStuA-1$, 8-RF- $\Delta MrStuA-1$; Lanes 7-12 in B-2: 7-LF-WT, 8-RF-WT, 9-LF- $\Delta MrXbp-1$, 10-RF- $\Delta MrXbp-1$, 11-LF- $\Delta MrXbp-2$, 12-RF- $\Delta MrXbp-2$). M, DNA molecular size markers (DL 5000, Takara, Beijing). The $\Delta MrStuA-1$ mutants and $\Delta MrXbp-1$ mutants were subjected to further

experiments. (C) qPCR analysis of *MrMid2* in the different strains. Error bars represent standard error. * $P < 0.05$, ** $P < 0.01$, denote significant differences compared with WT in AM cultures.

Fig. S3 Gene ontology (GO) annotation of differentially expressed genes (DEGs) and all genes in the (A) $\Delta MrStuA$ and (B) $\Delta MrXbp$ mutants during dimorphic transition

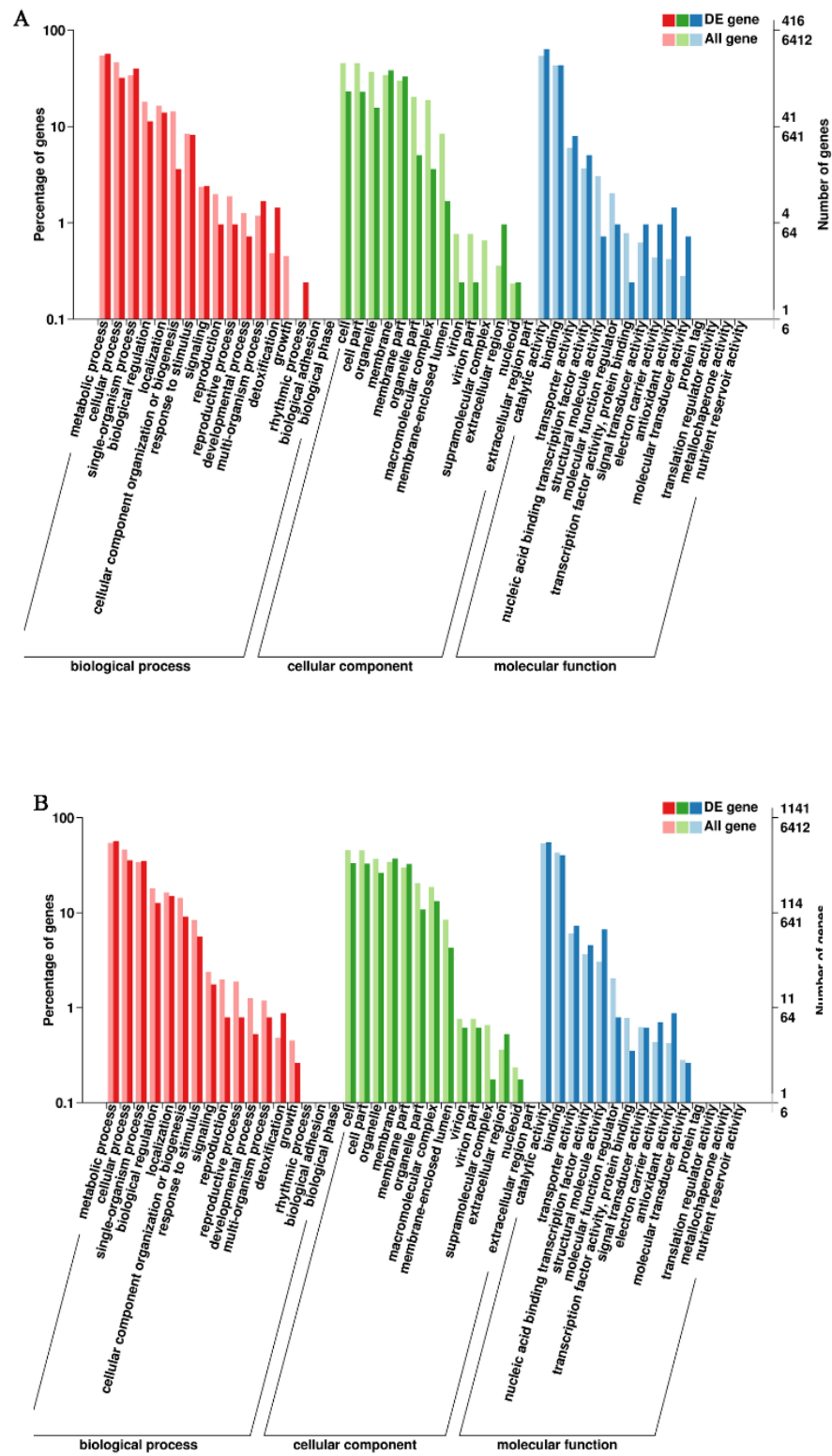


Fig. S4 Clusters of orthologous groups (COG) classifications in the (A) $\Delta MrStuA$ and (B) $\Delta MrXbp$ mutants during dimorphic transition

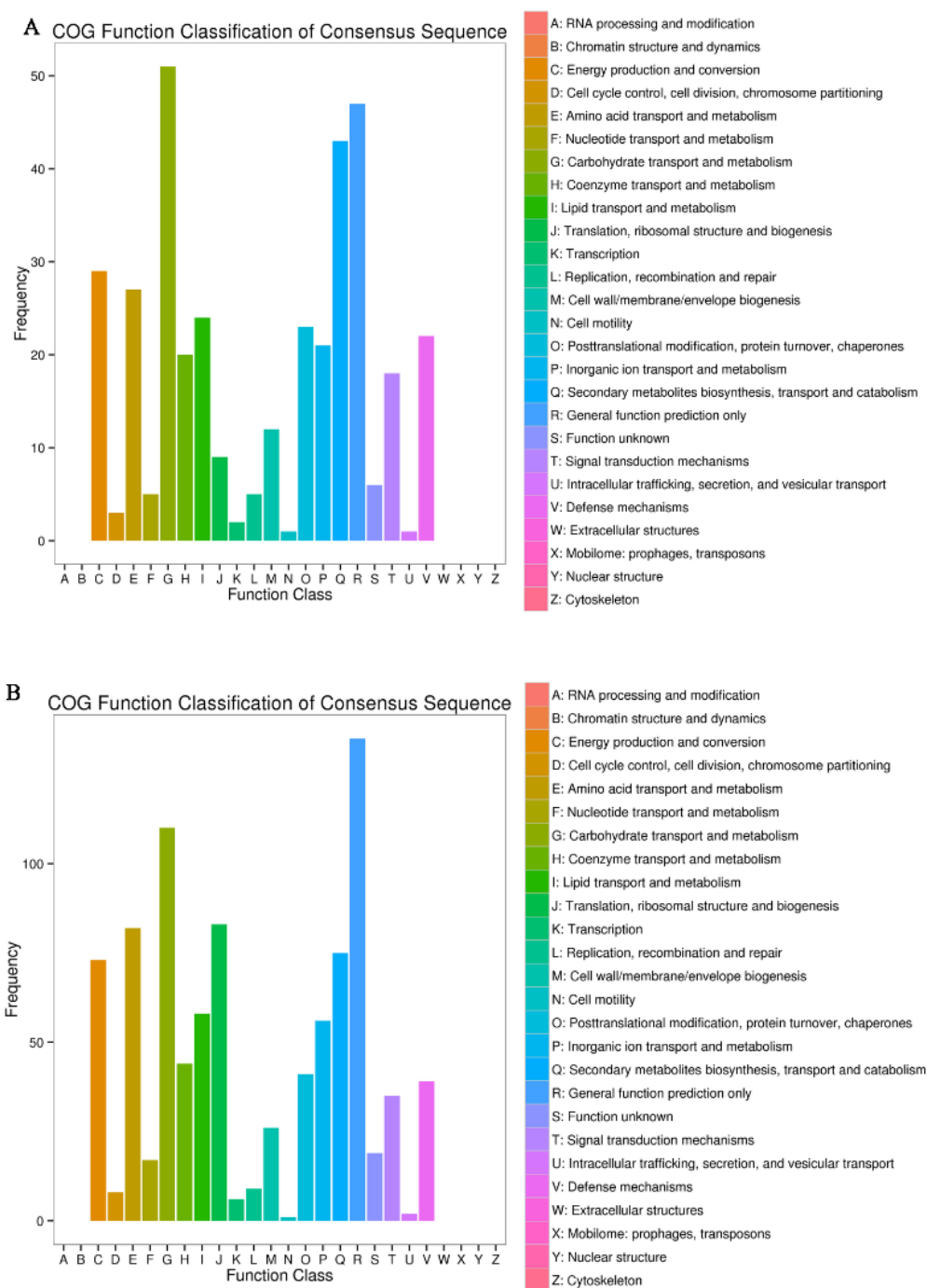


Fig. S5 Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathways of DEGs in the (A) $\Delta MrStuA$ and (B) $\Delta MrXbp$ mutants during dimorphic transition

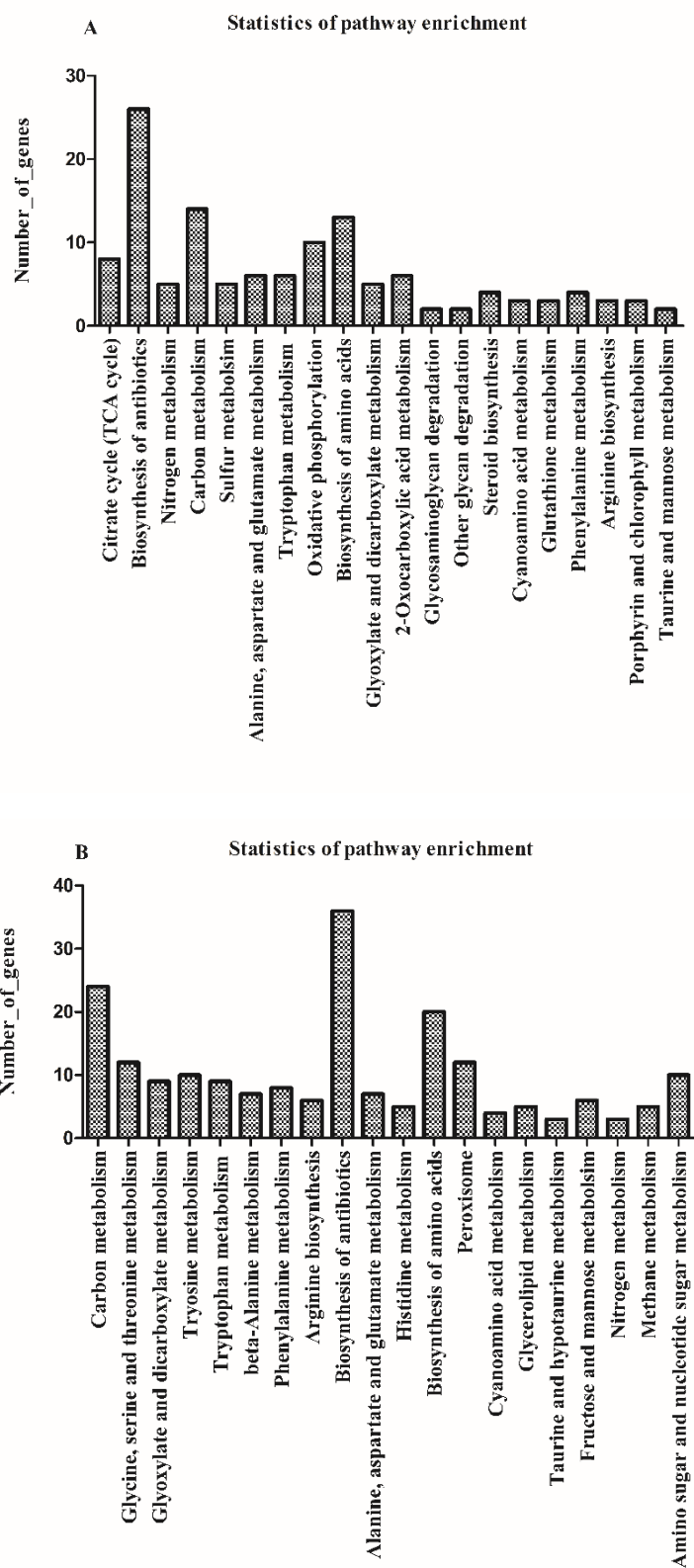


Fig. S6 GO annotation of DEGs and all genes in the (A) $\Delta MrStuA$ and (B) $\Delta MrXbp$ mutants during microsclerotium development

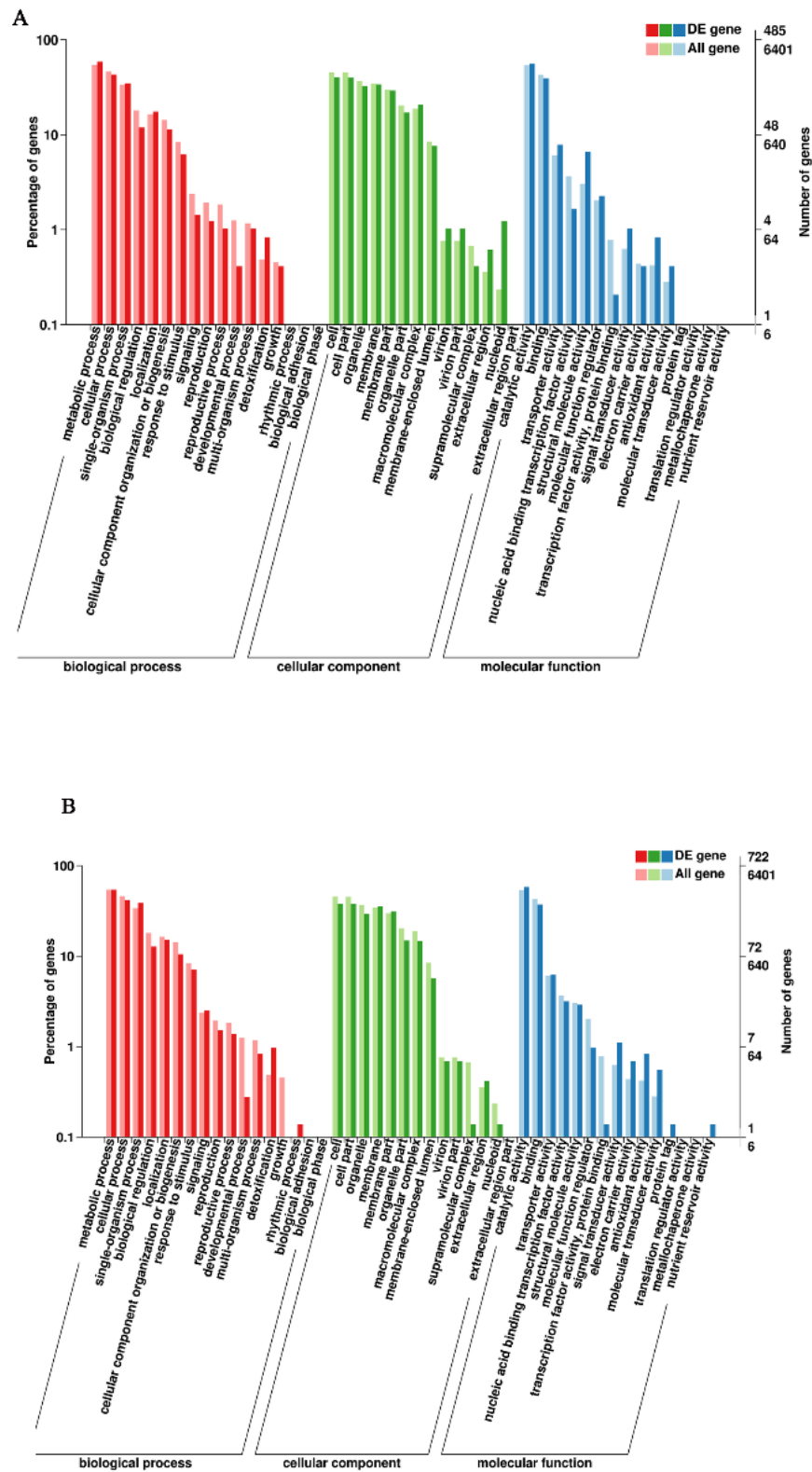


Fig. S7 COG classifications in the (A) $\Delta MrStuA$ and (B) $\Delta MrXbp$ mutants during microsclerotium development

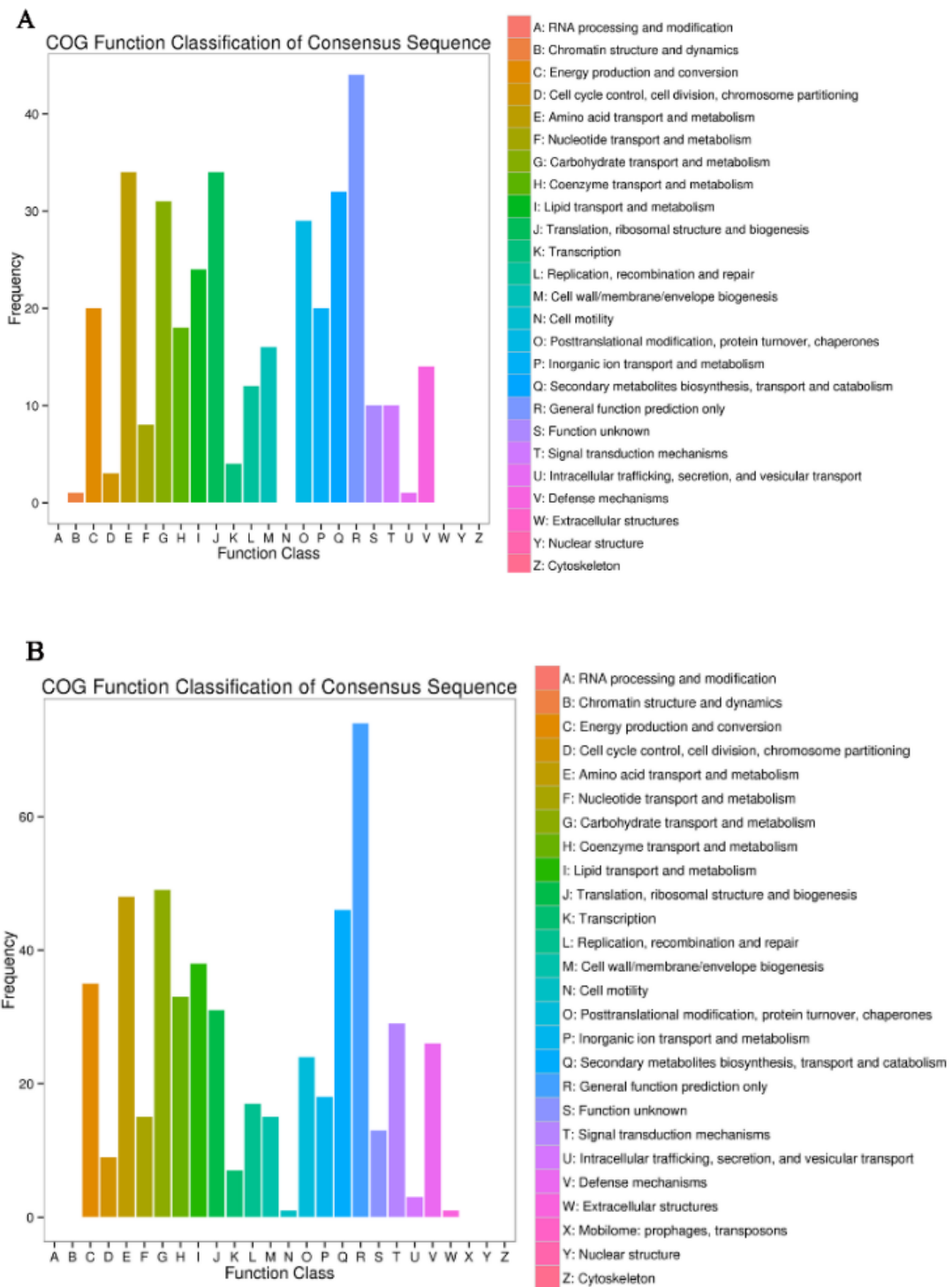


Fig. S8 KEGG enrichment pathways of DEGs in the (A) $\Delta MrStuA$ and (B) $\Delta MrXbp$ mutants during microsclerotium development

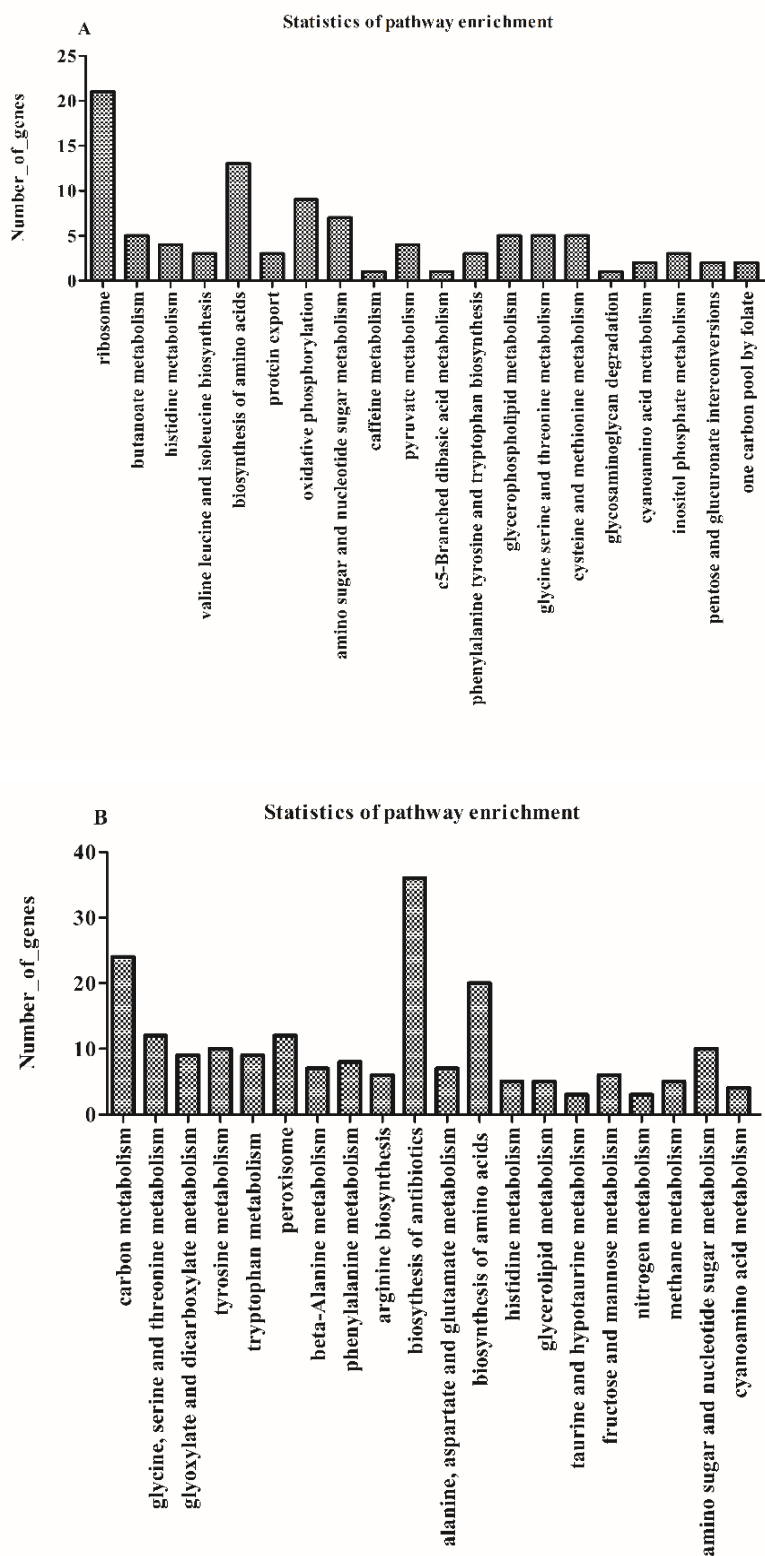
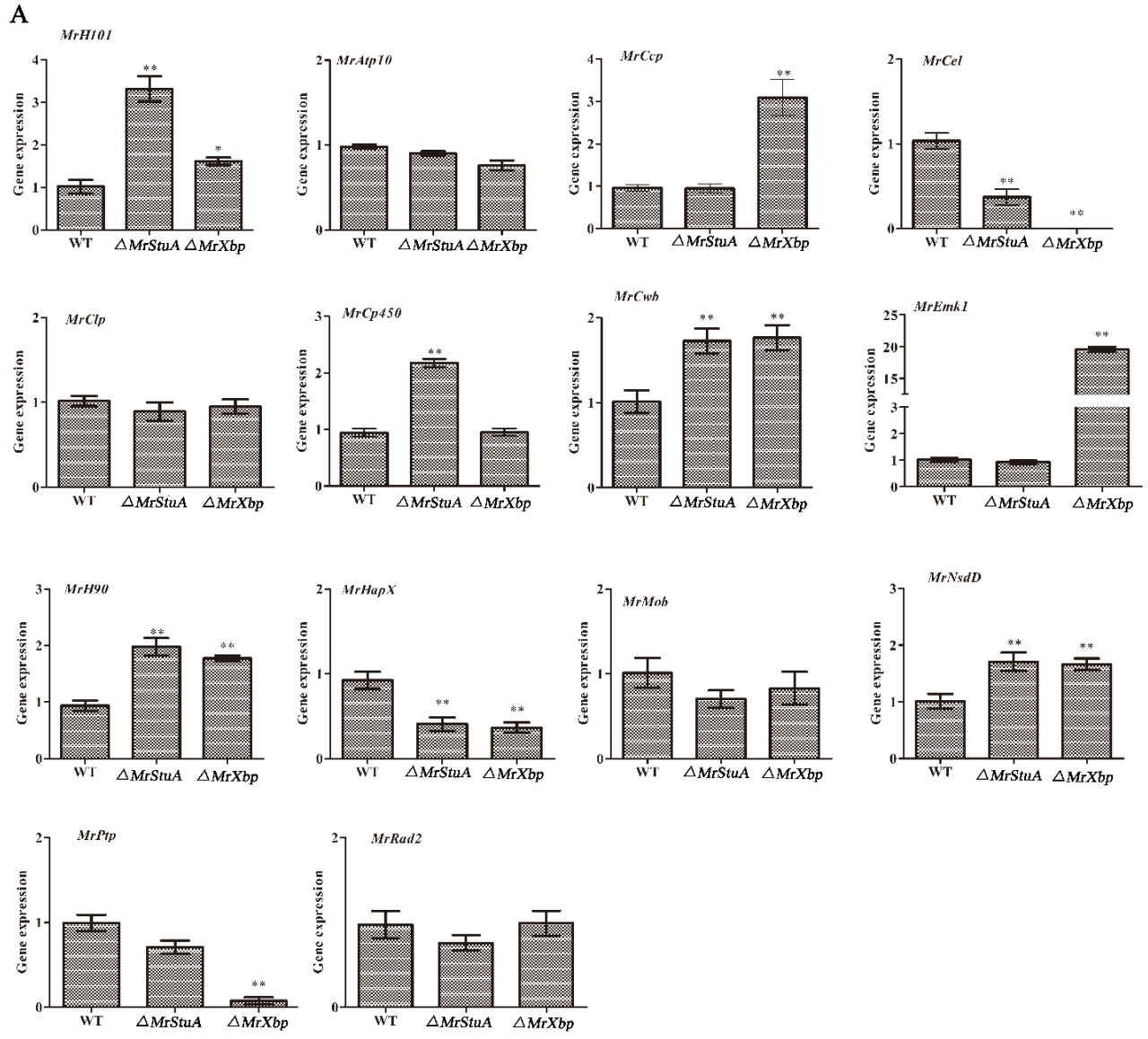


Fig. S9 RT-qPCR of WT, $\Delta MrStuA$ and $\Delta MrXbp$ mutants during (A) dimorphic transition and (B) microsclerotium development



B

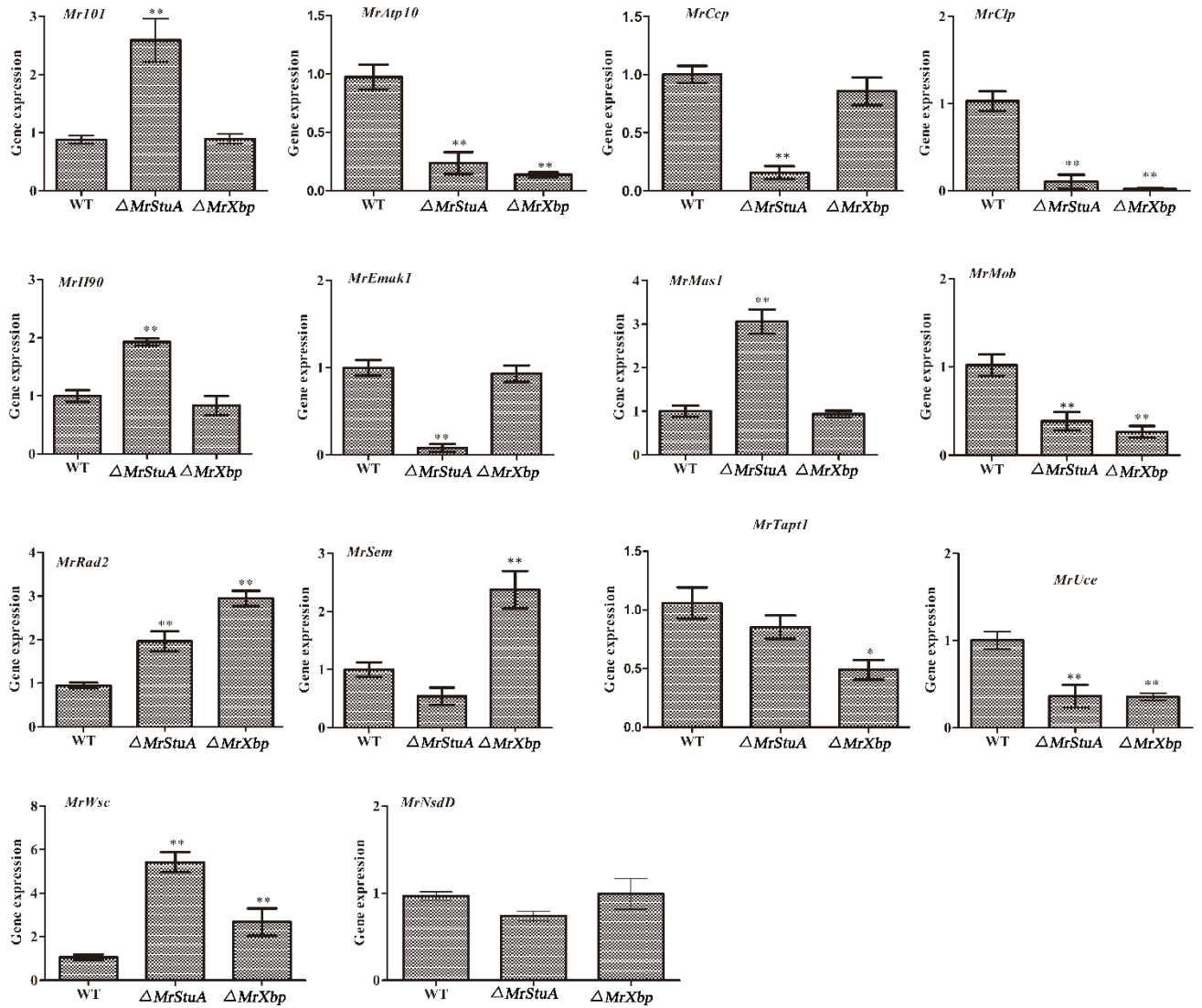


Fig. S10 GO annotation of DGEs and all genes in the two morphogenesis in the (A)

$\Delta MrStuA$ and (B) $\Delta MrXbp$ mutants

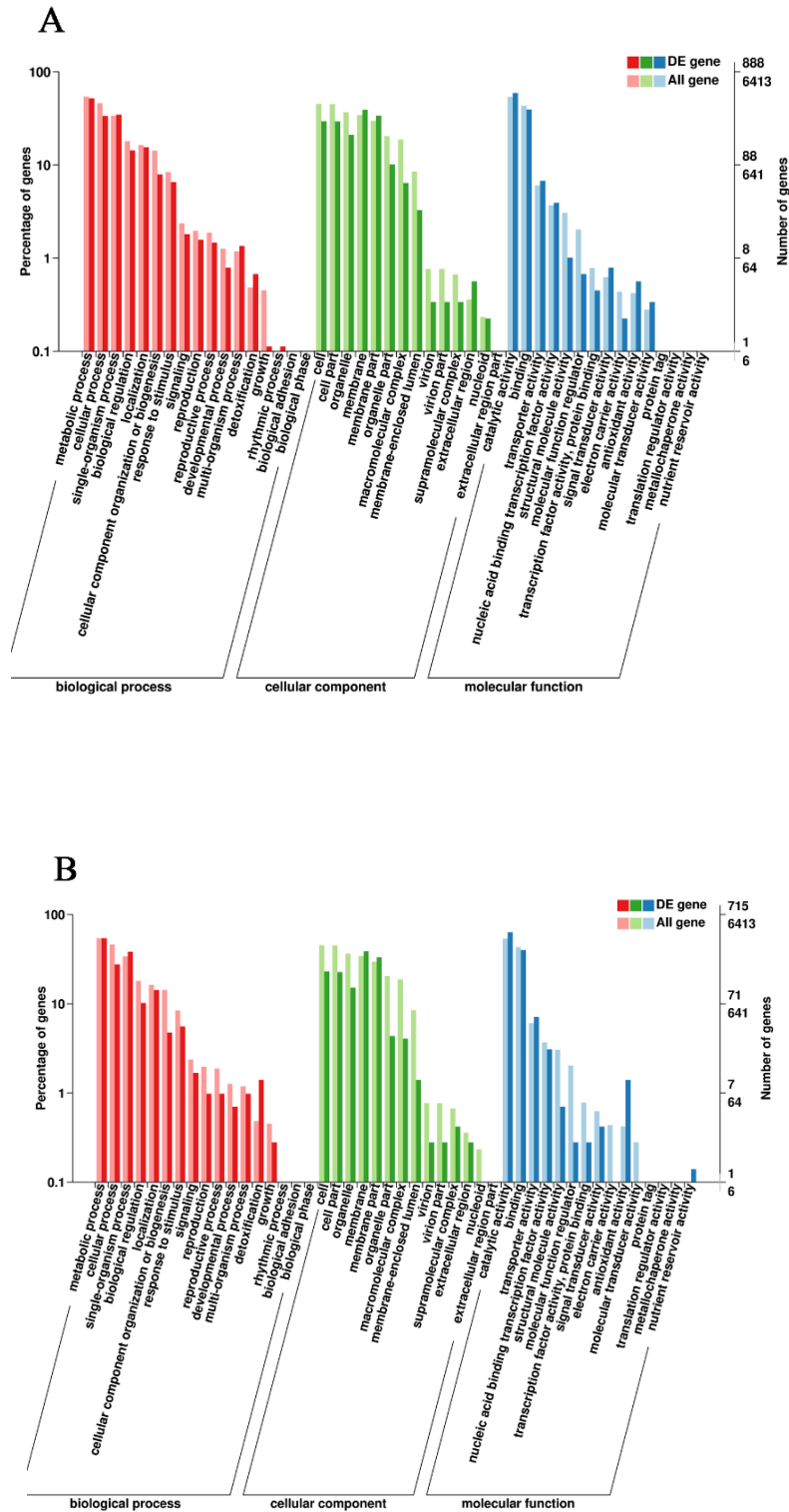


Fig. S11 COG classifications of consensus sequence in the two morphogenesis in the

(A) $\Delta MrStuA$ and (B) $\Delta MrXbp$ mutants

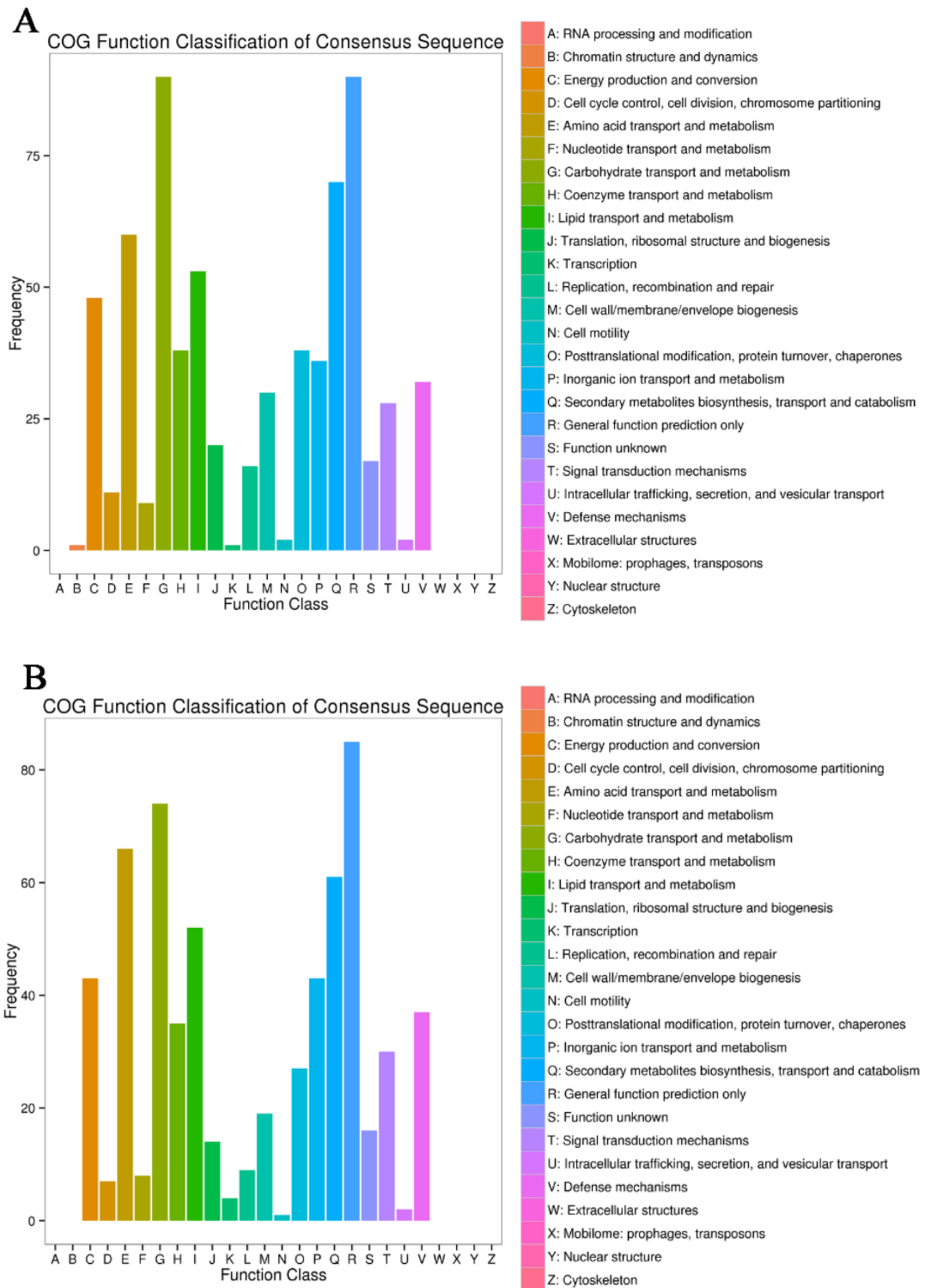


Fig. S12 KEGG enrichment pathways of DGEs in the two morphogenesis in the (A) $\Delta MrStuA$ and (B) $\Delta MrXbp$ mutants

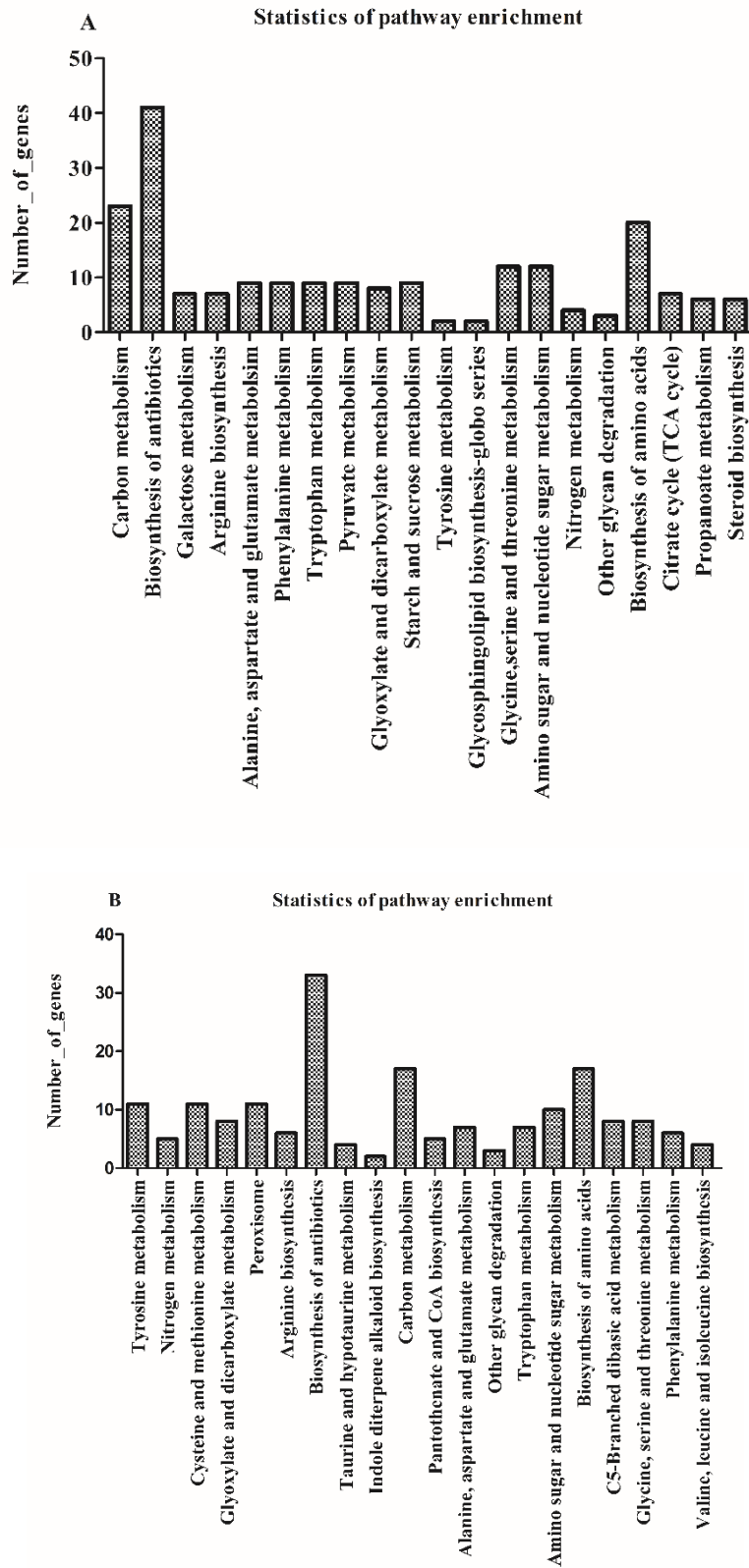
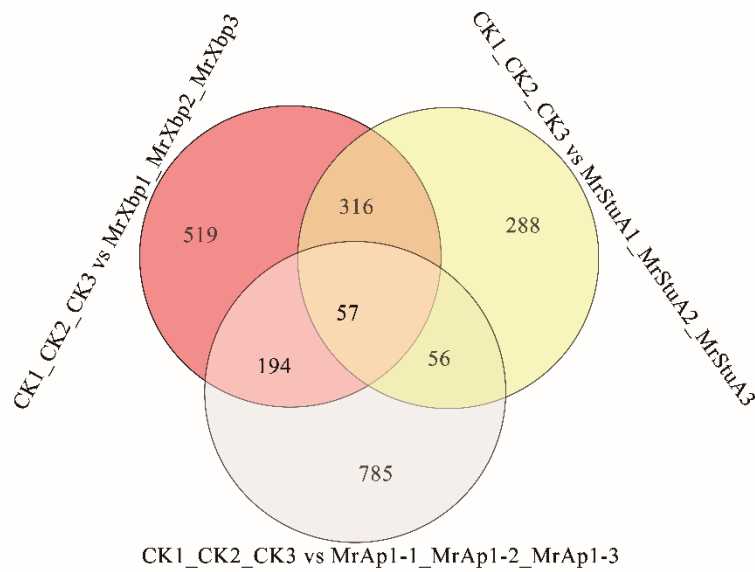


Fig. S13 Venn diagram showing the number of shared differentially expressed genes in the three fungal transcription factors



The distribution of shared upregulated and downregulated genes in wild-type (WT), $\Delta MrXbp$, $\Delta MrStuA$, and $\Delta MrAp1$ mutants during microsclerotium development. CK1, CK2, and CK3 were three replicates of the WT strain. *MrXbp 1*, *MrXbp2*, and *MrXbp3* were three replicates of the $\Delta MrXbp$ mutants. *MrStuA*, *MrStuA2*, and *MrStuA3* were three replicates of the $\Delta MrStuA$ mutants. *MrAp1-1*, *MrAp1-2*, and *MrAp1-3* were three replicates of the $\Delta MrAp1$ mutants.