Supporting Information

ApWD40a, a Member of the WD40-Repeat Protein Family, Is Crucial for Fungal Development, Toxin Synthesis, and Pathogenicity in the Ginseng Alternaria Leaf Blight Fungus *Alternaria panax*

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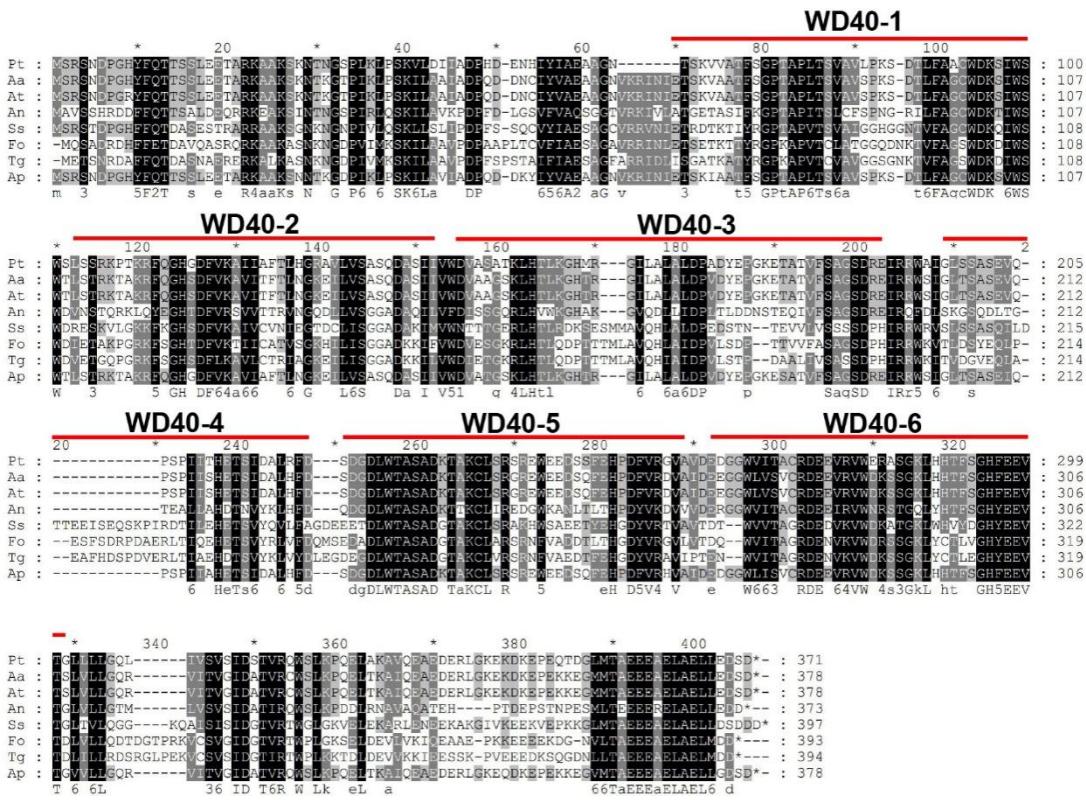
Figure S1. Amino acid sequence alignments of ApWD40a with homologs from different fungal species. Amino acid sequences of the WD40a proteins used are as follows: Pt: *Pyrenophora teres* EFQ89095.1; Aa: *Alternaria alternata* KAH6846796.1; At: *Alternaria tenuissima* RYN38094.1; An: *Aspergillus nidulans* XP_658660.1; Ss: *Sclerotinia sclerotiorum* XP_001597417.1; Fo: *Fusarium oxysporum* EGU75440.1; Tg: *Trichoderma gamsii* XP_018663274.1; Ap: *Alternaria panax* JY15.
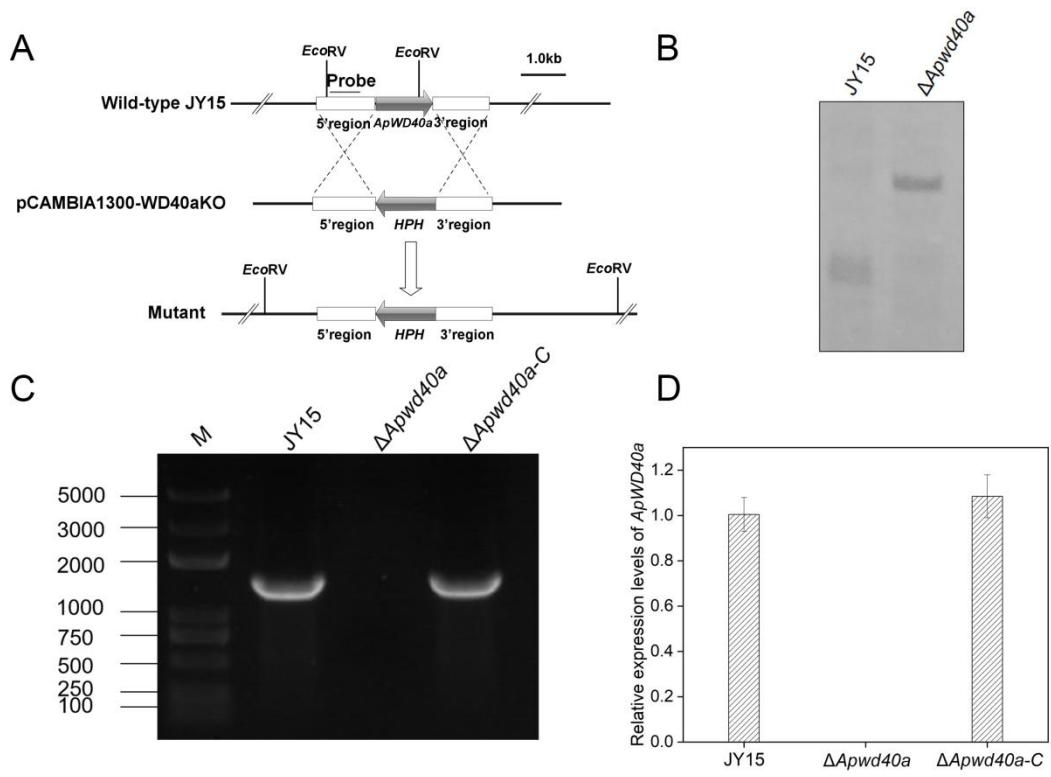
Figure S2. Knockout and complement of the *ApWD40a* gene. A: Strategy of *ApWD40a* gene knockout; B: Southern blot analysis of the wild-type JY15 and the null mutant; C: PCR validation of complement transformants; D: Relative expression of the complement mutants Δ*Apwd40a-C* determined by qRT-PCR.
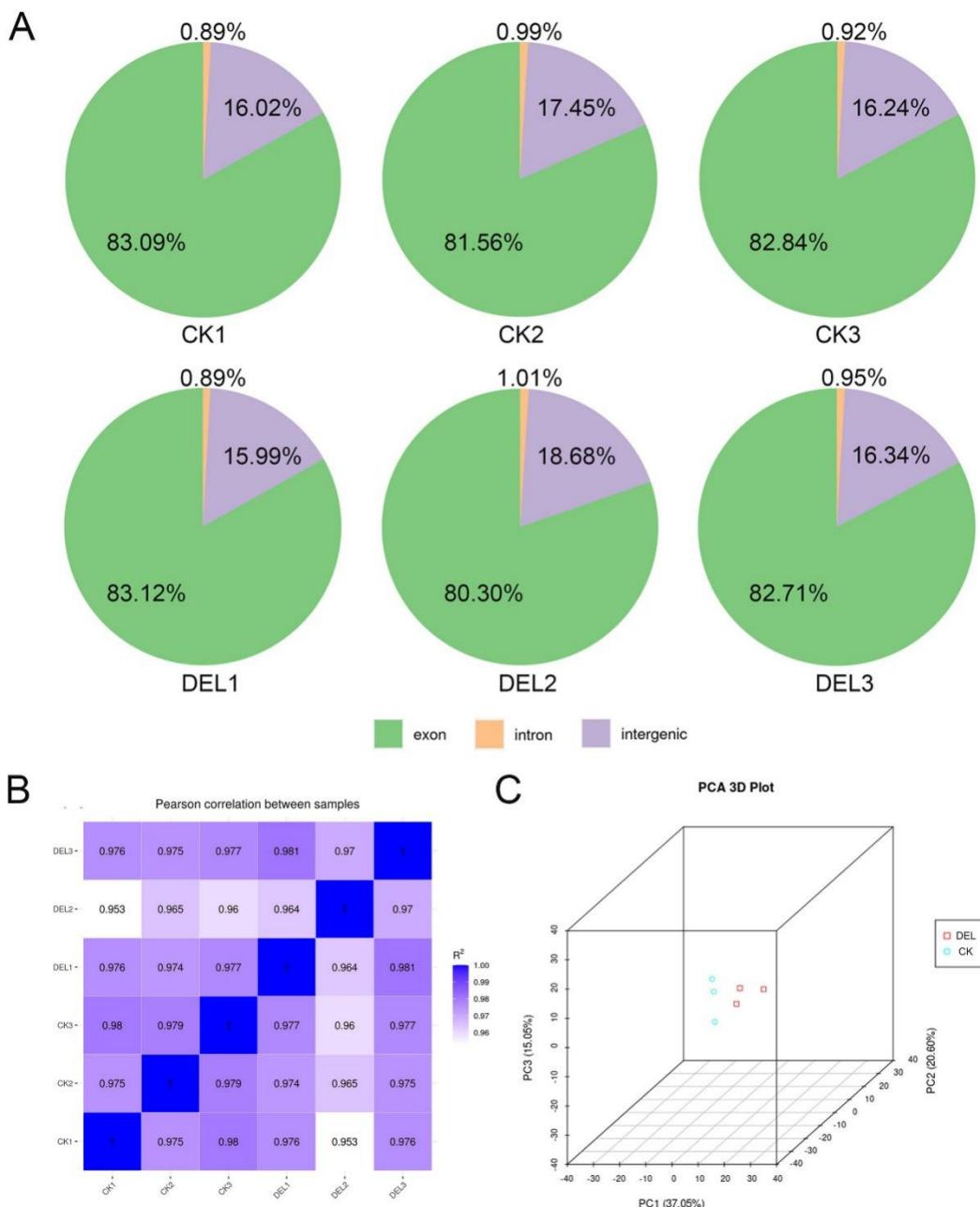
Figure S3. Proportion of the sequencing reads mapped on *Alternaria panax* genome (A) and correlation analysis (B-C)
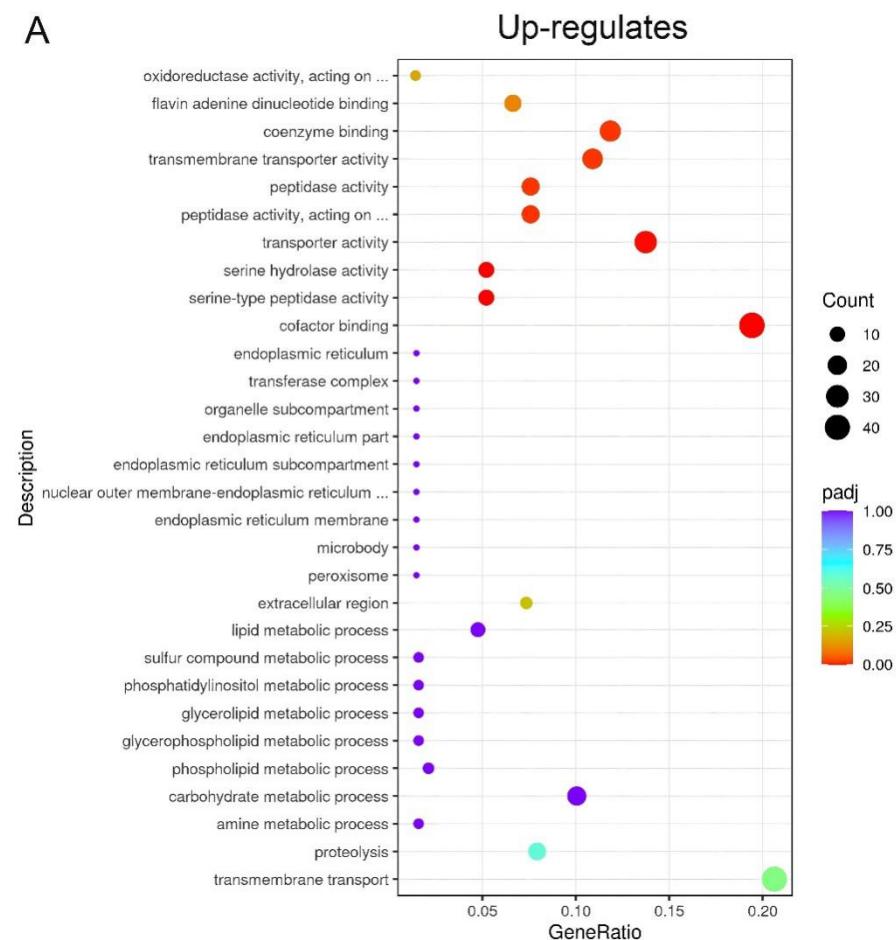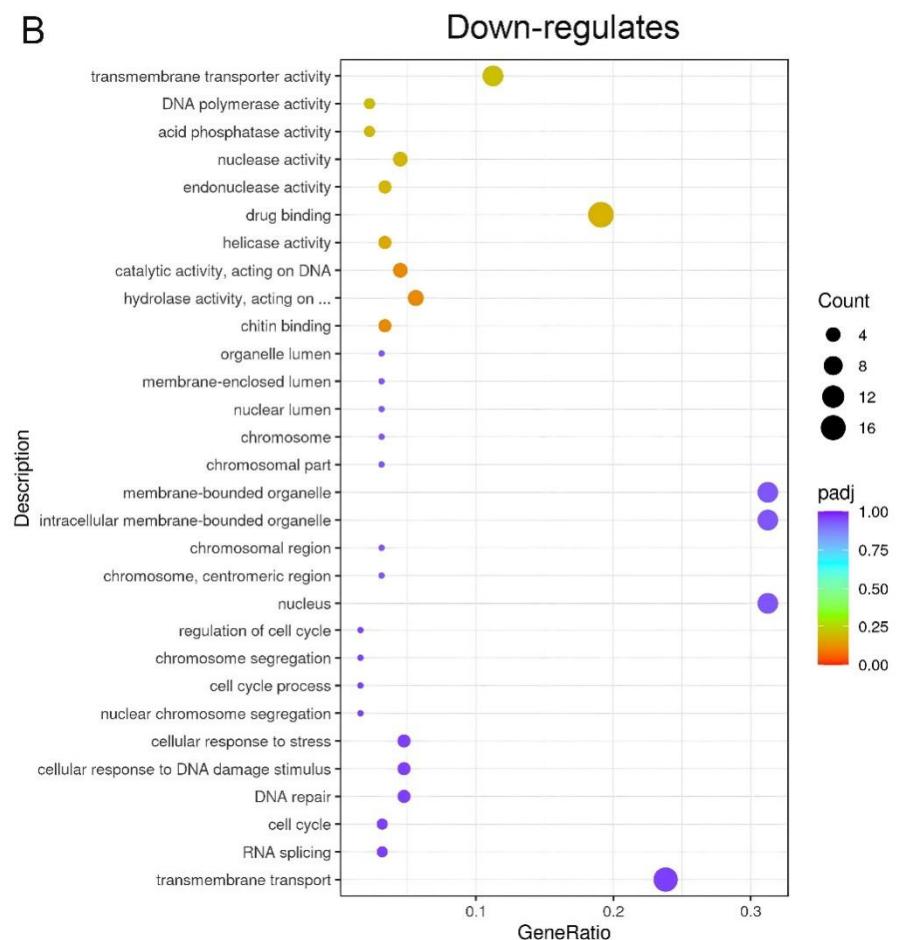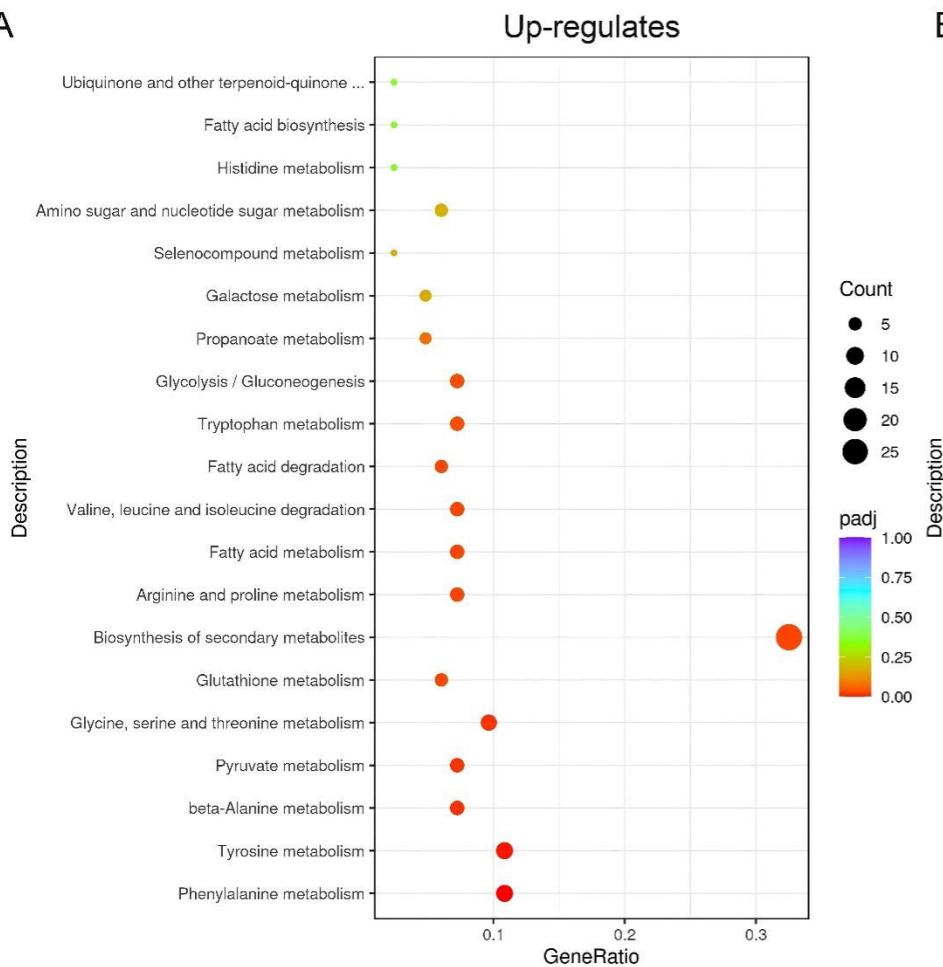
Figure S4. Enrichment of GO terms for the upregulated (A) and downregulated (B) DEGs in Δ*Apwd40a*. The size of each circle represents the number of genes number involved in the corresponding pathway.
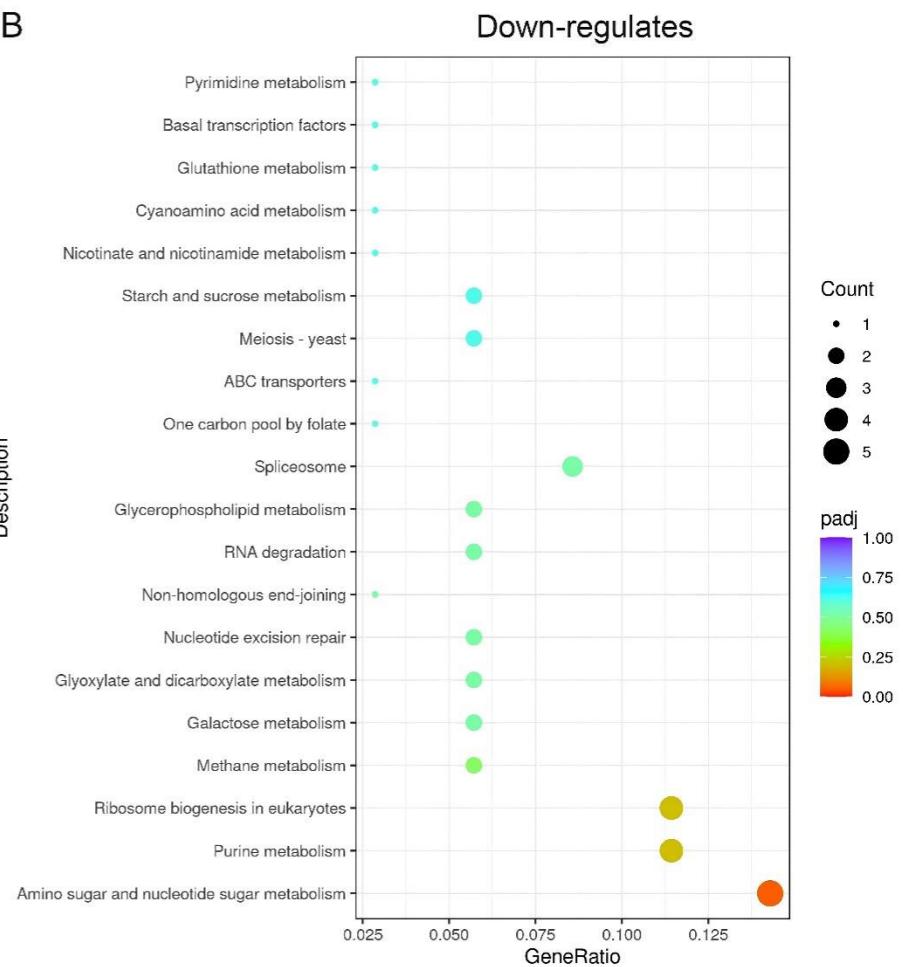
**Figure S5. Enrichment of KEGG terms for the upregulated (A) and downregulated (B) DEGs in Δ*Apwd40a*. The size of each circle represents the number of genes number involved in the corresponding pathway.**
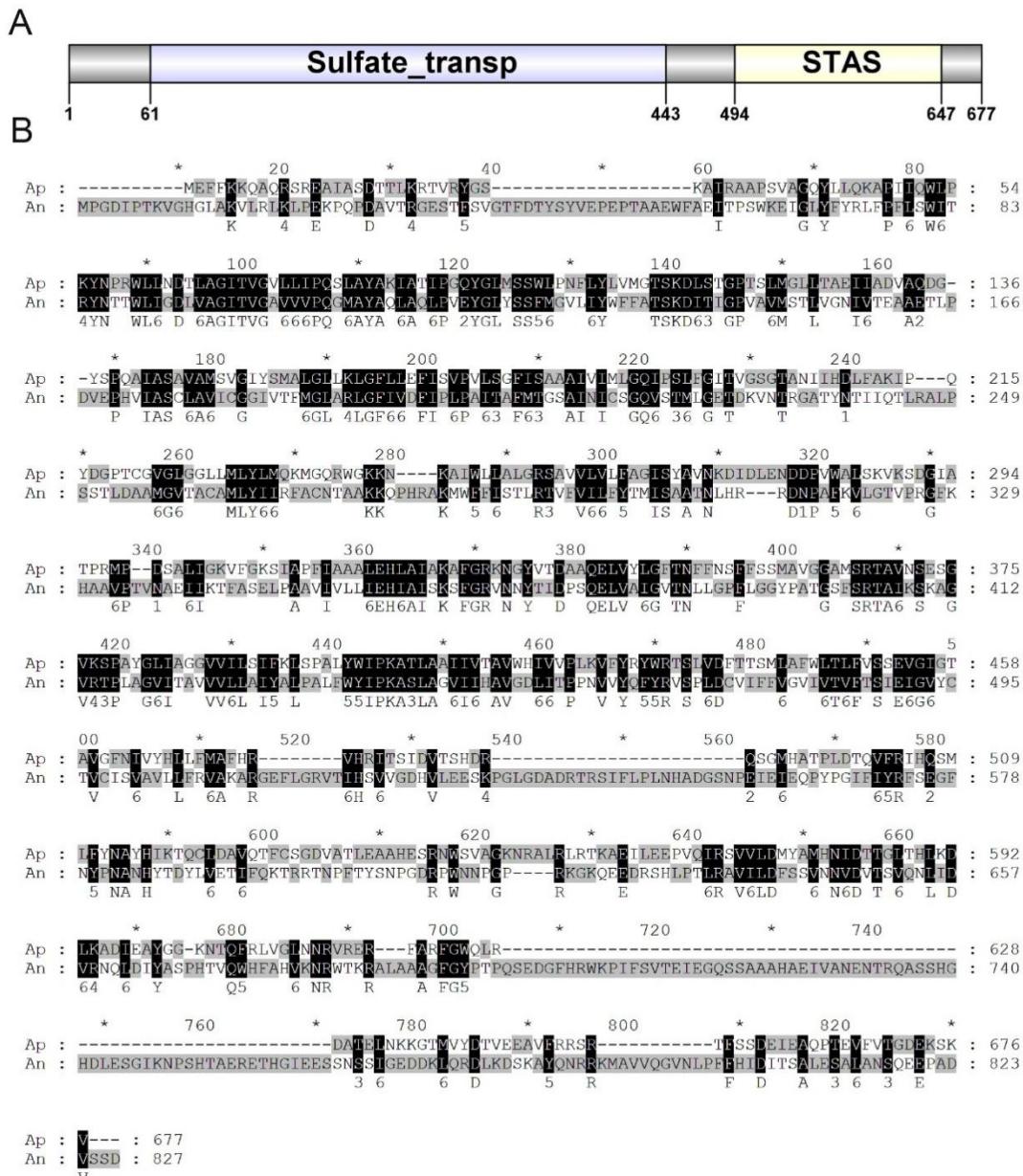
Figure S6. Sequence alignment of ApSulp2 with *Aspergillus nidulans* Sulp2 proteins. The Sulp2 protein sequence used is as follows: *Aspergillus nidulans* FGSC A4 AnSulp2 (XP_660334.1).
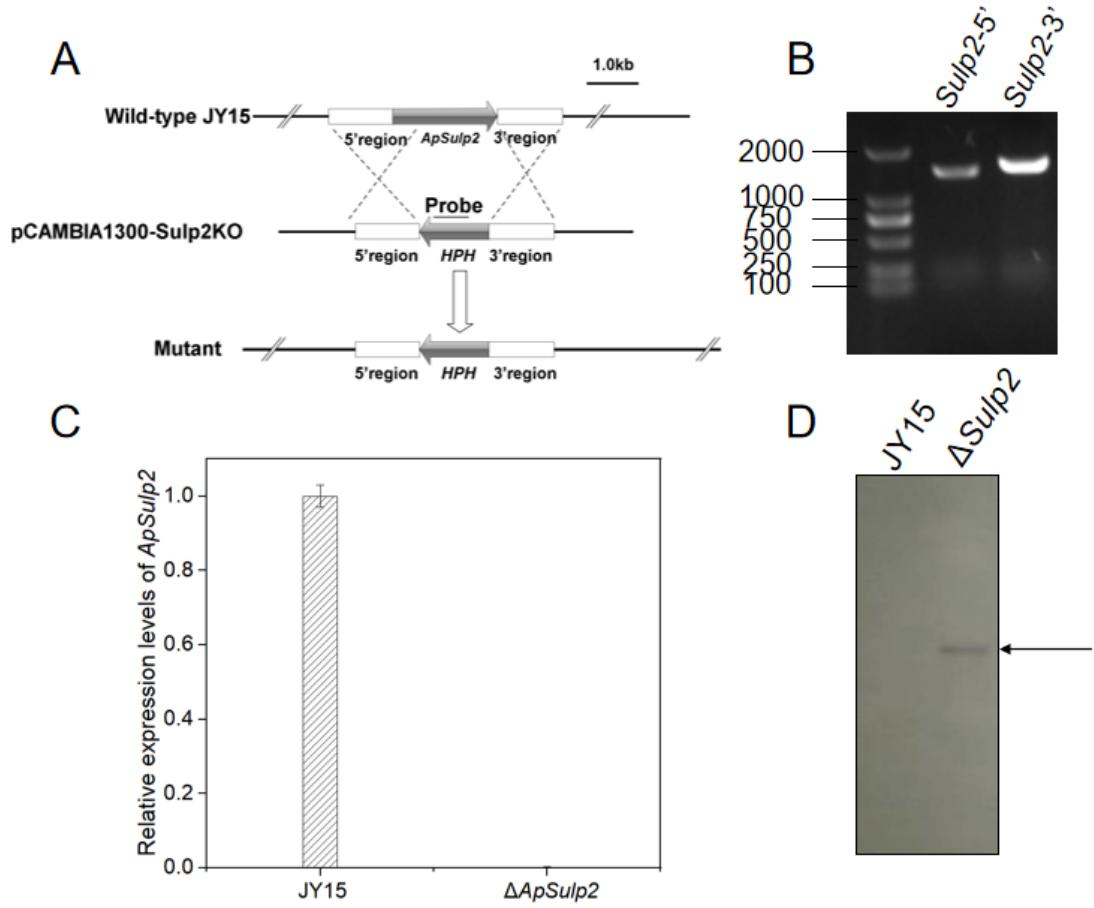
Figure S7. Knockout of the *ApSulp2* gene. A: Strategy of *ApSulp2* gene knockout; B: 5′ and 3′ flanking PCR validation of knockout transformants; C: Relative expression level of the target gene *ApSulp2* in the wild-type JY15 and the Δ*Apsulp2* mutant; D : Single copy analysis of the *Hyg* gene in the null mutant of *ApSulp2*.

Table S1. Primers used in this study

| Primers	Sequences (5'- 3')
Construction and verification of deletion mutants	
WD40a-5F	CGAGCTCGGTACCCGGggatccCTTCGCCGCCGCCAGTG
WD40a-5R	GATGTGTTGACCTCCCGGTGACGCCCAGCAAGATA
WD40a-3F	AAAGGAATAGAGTAGTCTTCGACGAGCGGACCACTACC
WD40a-3R	ACGACGGCCAGTGCCaagcttGCCCAGCCATCAACGAGGAAACTA
WD40a-Hyg-F	TGCTGGGCGTCACCGGGAGGTCAACACATCAATGCT
WD40a-Hyg-R	TCCGCTCGTCGAAGACTACTCTATTCCTTTGCCCTC
Sulp2-5F	CGAGCTCGGTACCCGGggatccTCCAAGTACCCGCTTCATAGC
Sulp2-5R	GATGTGTTGACCTCCTCAACCGCAAGATAGCCTCAT
Sulp2-3F	AAAGGAATAGAGTAGGCCGTCTGCTCTGCGACACT
Sulp2-3R	ACGACGGCCAGTGCCaagcttCCTCCGAAGAATTAAGTCCAGTT
Sulp2-Hyg-F	AGGCTATCTTGCGGTTGATAGGAGGTCAACACATCAATGCT
Sulp2-Hyg-R	GTCGCAGAGCAGACGGCCTACTCTATTCCTTTGCCCTC
1300F1	ATTCGAGCTCGGTACCCGGG
*Hyg*R1	CCAAAATAGCATTGATGTGTTGAC
*Hyg*F2	CGAGGGCAAAGGAATAGAGT
1300R2	CTAATAAACGCTCTTTTCTCTTA
RPB2-F	TGTCACAGTTGTGCAGGAGCT
RPB2-R	TTTCGCAATGGGTGTAGGC
WD40a-F	GTCAAGGCCGTCATCGCATTCAC
WD40a-R	GCCATCCGCCGTCTTCGTCA
WD40a-upF	ATCCATGCCAACCAAACCCACCAA
WD40a-upR	TGCACCAAGCAGCAGATGA
WD40a-downF	TCCGAGGGCAAAGGAATAGA
WD40a-downR	CCCCCTTGTAGCTCGGTCTGG
Sulp2-F	TGGTGGTGTCGTCATCCTCA
Sulp2-R	CGCTCCAGTTACGGCTTTCG
Sulp2-upF	AACCAAGTTGTCGCATCCAC
Sulp2-upR	GTCCTCGTTCCTGTCTGCTAAT
Sulp2-downF	ACTCGCCGATAGTGGAAACC
Sulp2-downR	GTGGACCGCTCGGAGACAT
Hyg-probe-F	GGAGGTCAACACATCAATG
Hyg-probe-R	CTACTCTATTCCTTTGCCCTCG
WD40a-probe-F	TGTTATGATGGGCGGTGCG
WD40a-probe-R	TAGGGTGGGAGGAGCGGTGA
Construction and verification of complement mutants	
WD40a-ProF	GTACCCGGGGATCCTtctagaCAAACCCTGACGACCCAAAT
WD40a-ProR	GTTTGAGCGAGACATGGTGACGCCCAGCAAGATA
HB*WD40a*-F	TGCTGGGCGTCACCATGTCTCGCTCAAACGATCCA
HB*WD40a-R*	TCCGCTCGTCGAAGAATCGCTATCTCCCAGAAGCTC
WD40a-TerF	CTGGGAGATAGCGATTCTTCGACGAGCGGACCACTA
WD40a-TerR	ACGACGGCCAGTGCCaagcttGAATGAAGCCAGGAATCTGT
RT-qPCR assays	
q*Hyg*-F	GCGTTTGTCAAGCAAGGTAAG
q*Hyg*-R	GATCGAGGCTTGGGTAGAATAG
q*RPB2*-F	TTCACTCCAACGCTCAACAG
q*RPB2*-R	GGTCACGAATGTCACGAATCA

q*WD40a*-F	CAAACGATCCAGGTCACTACTT
q*WD40a*-R	CCTTGGTGTTGTTGGACTTTG
q*Sulp2*-F	TGGCTACCAAACTTTCTCTACC
q*Sulp2*-R	CCTGAGCAACATCTGCAATTATC
qG6011_00826F[a]	GAATTTCCGGCCTTGAATGTATC
qG6011_00826R	GACGATGTTGGGATGAGATAGG
qG6011_01906F	CTGATCCCACATGGTCCATAAC
qG6011_01906R	CCGTCAAGACAGTCGCAATAA
qG6011_10931F	CACGTCCGACGAGAAGTATTG
qG6011_10931R	CCCTCCACACGTAGTTTGTT
qG6011_02567F	TGCCACCTACAAGAACAATGA
qG6011_02567R	GAGAGATGAGATGGTTGGGATG
qG6011_02791F	CGCTTACCGCATACCTACTATC
qG6011_02791R	CATCAACCCATTGCCGTATTT
qG6011_03601F	CACACACTCTGGGATGACTTT
qG6011_03601R	TACCAACGCGTGGTGATATG
qG6011_03829F	CATGTCCGACGAAGAACTTACT
qG6011_03829R	GTGTGAGATACGACCCAATCC
qG6011_04126F	TCCCTCGCCATGGTTTATTC
qG6011_04126R	CGACAGCTTGTAGTGCTTCT
qG6011_04138F	ATCTCCGACCACCCTACAA
qG6011_04138R	TACTCTCGAGCTTCGCAATTC
qG6011_04402F	GGATGCTACCTACAACGTCTAC
qG6011_04402R	CTCACTAGCTTTGGGAGAGAAG
qG6011_04785F	TGGCTACCAAACTTTCTCTACC
qG6011_04785R	CCTGAGCAACATCTGCAATTATC
qG6011_07253F	CCAGCTCATGTACGATGTCA
qG6011_07253R	GGTATGGTCGTCGAAAGAGTAG
qG6011_07538F	TTGCAGCCTATGTCGTTCTC
qG6011_07538R	CCGGACTTCAGGCATATCTTT
qG6011_08141F	CATACTTCCAGATGCCGACTAC
qG6011_08141R	GTCCTGTTCCCATAGCTGATAC
qG6011_09959F	CGAACGACAGCGTGGATAA
qG6011_09959R	CTCGGTGTCAGTCCAAGAAA

[a]Notes:

G6011_00826, GenBank accession number: KAG9195705.1, gene name: *NEK2*;

G6011_01906, GenBank accession number: KAG9187983.1, gene name: *GalE*;

G6011_10931, GenBank accession number: KAG9192197.1, gene name: *EIF2α*;

G6011_02567, GenBank accession number: KAG9186011.1, hypothetical protein;

G6011_02791, GenBank accession number:KAG9186235.1, gene name: *IDO1*;

G6011_03601, GenBank accession number:KAG9193566.1, gene name: *TYRP1*;

G6011_03829, GenBank accession number:KAG9193794.1, gene name: *Pr1*;

G6011_04126, GenBank accession number:KAG9194091.1, gene name: *PTR2*;

G6011_04138, GenBank accession number:KAG9194103.1, hypothetical protein;

G6011_04402, GenBank accession number:KAG9194367.1, gene name: *MEP2*;

G6011_04785, GenBank accession number:KAG9194750.1, gene name: *SulP2*;

G6011_07253, GenBank accession number:KAG9185922.1, hypothetical protein;

G6011_07538, GenBank accession number:KAG9188833.1, gene name: *TRI101*;

G6011_08141, GenBank accession number:KAG9190053.1, gene name: *DHX16*;

G6011_09959, GenBank accession number:KAG9186851.1, gene name: *Pol4.*

Table S2. Data quality control (Table S2A) and statistics of RNA-Seq reads

mapped on *Alternaria panax* genome (Table S2B)

A.

Sample_ Name	Clean_ reads	Clean_ bases	Error_ rate	Q20	Q30	CG
CK1	42747474	6.41G	0.03	97%	91.88%	56%
CK2	45209334	6.78G	0.03	96.76%	92.45%	55.7%
CK3	43375358	6.51G	0.03	97.66%	93.79%	56%
DEL1	44805016	6.72G	0.03	97.3%	92.79%	55.87%
DEL2	41996816	6.3G	0.03	97.32%	92.92%	55.63%
DEL3	44268042	6.64G	0.03	97.18%	92.52%	55.94%

B.

Sample	Total_reads	Total_map	Unique_map	Multiple_map
CK1	42747474	41120074(96.19%)	40446835(94.62%)	673239(1.57%)
CK2	45209334	43139665(95.42%)	42368714(93.72%)	770951(1.71%)
CK3	43375358	41623240(95.96%)	40832566(94.14%)	790674(1.82%)
DEL1	44805016	43032559(96.04%)	42264560(94.33%)	767999(1.71%)
DEL2	41996816	40235439(95.81%)	39280314(93.53%)	955125(2.27%)
DEL3	44268042	42583370(96.19%)	41902141(94.66%)	681229(1.54%)

Table S3. Enrichment of GO terms of the differentially expressed genes in

ΔApwd40a

Category	GOID	Description	padj
BP	GO:0055085	transmembrane transport	0.032000948
MF	GO:0005215	transporter activity	0.001211536
MF	GO:0048037	cofactor binding	0.001211536
MF	GO:0022857	transmembrane transporter activity	0.003186695
MF	GO:0008236	serine-type peptidase activity	0.007926748
MF	GO:0017171	serine hydrolase activity	0.007926748
MF	GO:0050662	coenzyme binding	0.032838847

Note: padj represents the corrected *p*-value. The padj value < 0.05 means a most enriched GO term.

BP represent biological process, MF represent molecular function.

Table S4. Enrichment of KEGG pathways of the differentially expressed genes in Δ*Apwd40a*

KEGG ID	Description	GeneRatio	BgRatio	*p*-value	padj
aalt00360	Phenylalanine metabolism	9/118	35/2252	4.89E-05	0.003375103
aalt00350	Tyrosine metabolism	10/118	56/2252	0.000488644	0.013034596
aalt00520	Amino sugar and nucleotide sugar metabolism	10/118	57/2252	0.000566722	0.013034596
aalt00480	Glutathione metabolism	6/118	29/2252	0.003206746	0.052173861
aalt00260	Glycine, serine and threonine metabolism	9/118	61/2252	0.003780715	0.052173861
aalt00410	beta-Alanine metabolism	6/118	31/2252	0.004560904	0.052450399
aalt00620	Pyruvate metabolism	6/118	35/2252	0.008469627	0.083486325
aalt00052	Galactose metabolism	6/118	38/2252	0.012671	0.109287379
aalt00330	Arginine and proline metabolism	6/118	43/2252	0.02261348	0.157414778
aalt00071	Fatty acid degradation	5/118	32/2252	0.023431276	0.157414778
aalt01212	Fatty acid metabolism	6/118	44/2252	0.02509511	0.157414778
aalt00280	Valine, leucine and isoleucine degradation	6/118	45/2252	0.027752879	0.159579054
aalt00680	Methane metabolism	4/118	24/2252	0.033730313	0.179030121
aalt00380	Tryptophan metabolism	6/118	48/2252	0.036824591	0.181492628
aalt00010	Glycolysis / Gluconeogenesis	6/118	49/2252	0.040226548	0.185042119
aalt01110	Biosynthesis of secondary metabolites	31/118	446/2252	0.048631299	0.209722476

Table S5. Expression of the *ApSulp2* gene in the Δ*Apwd40a*

Description	Gene_ID	log$_2$FoldChange	padj
Sulp2	G6011_04785	2.212936241	0.000268382