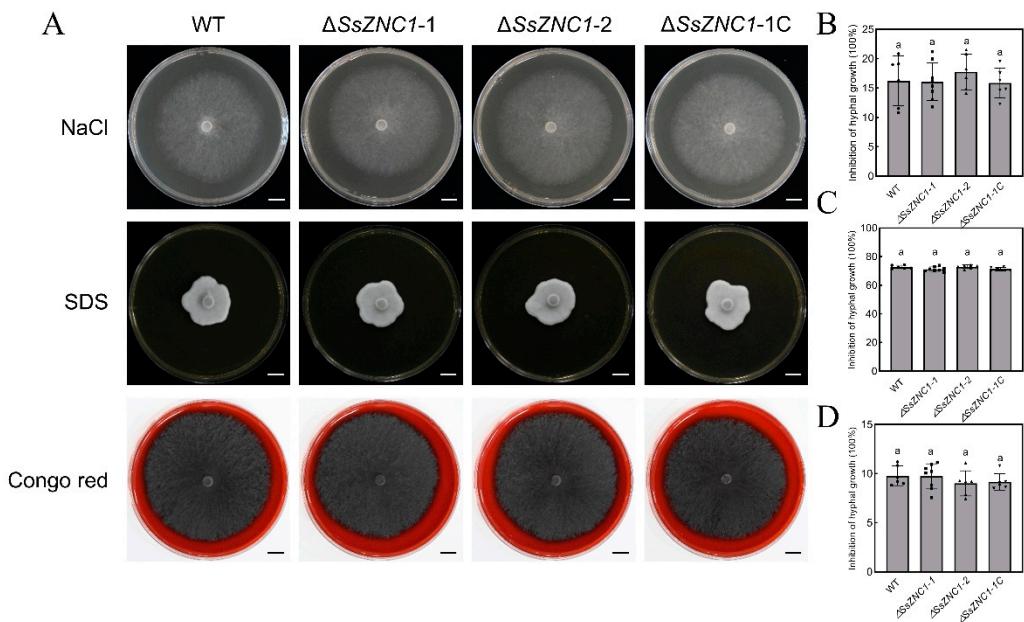
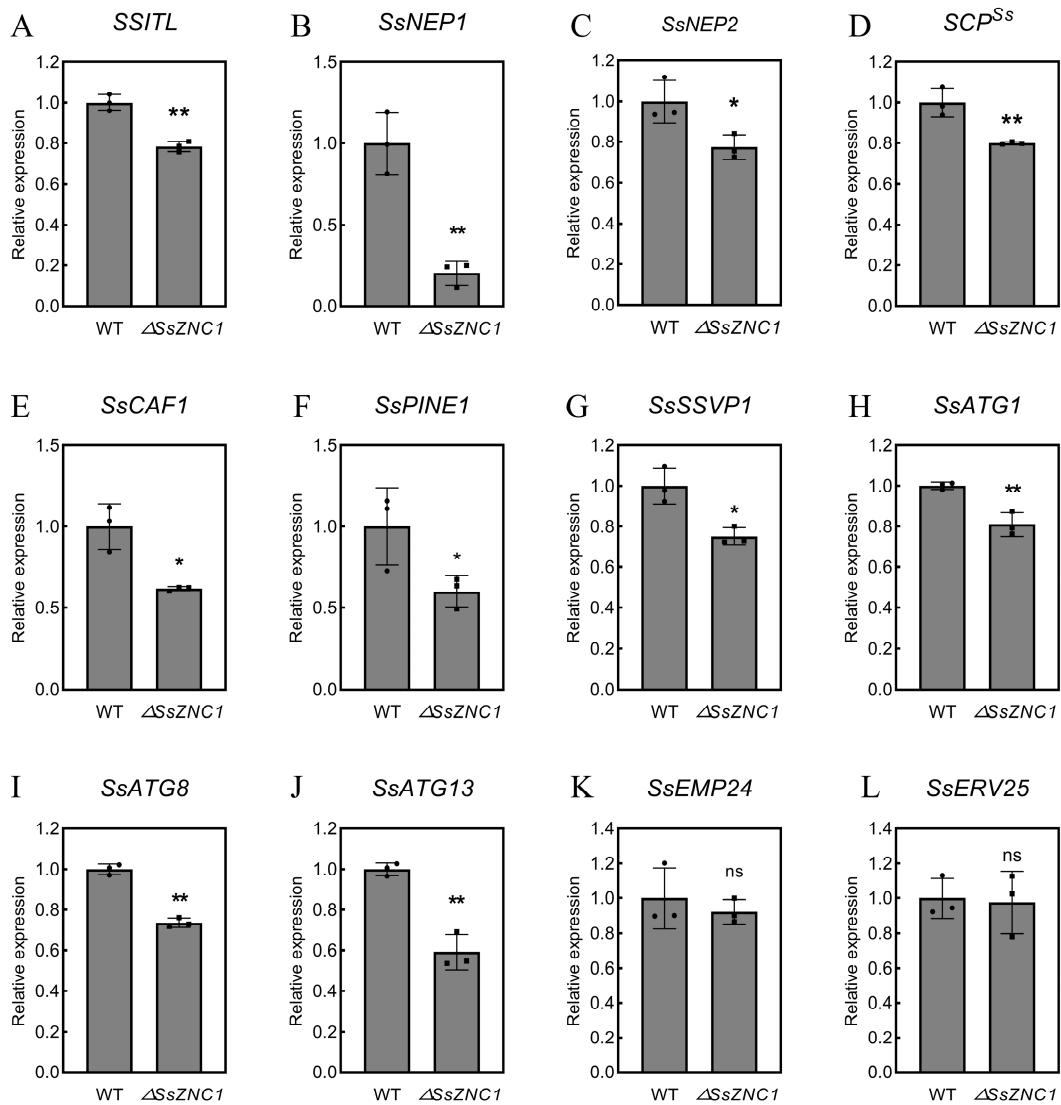


**Figure S1.** Analysis of *SsZNC1* transformants. **(A)** Schematic diagram of the *SsZNC1* gene knock-out strategy. **(B)** PCR validation of the *SsZNC1* knockout mutants and complemented strain. The verification of *SsZNC1* upstream (Lanes 1, 5, 9, 13, 17, 21); the verification of *SsZNC1* downstream (Lanes 2, 6, 10, 14, 18, and 22); the verification of HYG (Lanes 3, 7, 11, 15, 19, and 23); the verification of the full-length *SsZNC1* gene (Lanes 4, 8, 12, 16, 20, and 24). Lane M, DL5000 marker (3428Q, Takara, China). **(C)** RT-PCR analysis of the transcription of *SsZNC1* in different strains. The  $\beta$ -tubulin gene in *S. sclerotiorum* was used as the reference gene. Lane Marker, DL5000 marker. Primer sequences used were listed in Table S1. **(D)** Detection of protein of *SsZNC1* transformants by western blot. The protein expression of transformants through western blot using FLAG antibody (66008-4-Ig, Proteintech, China).



**Figure S2.** Survivability of the WT and *SsZNC1* transformants in stress media. **(A)** Growth observation of the WT and the *SsZNC1* transformants on PDA medium containing 0.5 M NaCl, 0.02% SDS, and 2.5 mg/mL Congo red. Bar, 1 cm. **(B-D)** The inhibition of hyphal growth of the WT and the *SsZNC1* transformants on PDA medium containing different stress factors. The values represent means derived from three independent replicates, and the error bars indicate  $\pm$  SD of the mean.



**Figure S3.** Validation of the RNA-seq data by RT-qPCR. **(A-G)** The relative expression levels of effectors were verified by RT-qPCR at 3 hpi. **(H-J)** The relative expression levels of autophagy related genes were verified by RT-qPCR at 3 hpi. **(K, L)** The relative expression levels of early secretory pathway related genes were verified by RT-qPCR at 3 hpi. The constitutively expressed  $\beta$ -tubulin gene was used as the reference gene to standardize data (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; ns, no significance).

**Table S1.** Primers used in this study.

Name	Fwd Primer	Rvs Primer	Description of primers
SsZNCI	atgcttaacttgcagaagtaagtgcgtgc	tcaaggggatgacctaacaaggc	Amplification of SsZNCI for sequencing
SsZNCI-promoter	tggaaacaacaagtgaagaac	ctaattgagcgactgagaca	Amplification of SsZNCI promoter
HYG	cagaagatgatattgaaggaggcac	aaagaaggattaccctctaaacaagtgt	Amplification of hygromycin-resistant gene
SsZNCI-upstream	ccaaaccgaacgacaatccg	ccggatcgacaccttggg	Amplification of SsZNCI upstream
SsZNCI-downstream	gctcgagcttgagcttgattg	agtgagtgtccagaagagg	Amplification of SsZNCI downstream
SsZNCI-up-verification	ataaacgcgcgtgtcacgc	cgccgcgcgtactgtacaagt	Amplification of the upstream of SsZNCI overlapped PtrpC for verification of deletion mutant
SsZNCI-down-verification	ctggaaagcaacaccgtgaact	agactgaggaatccgccttgg	Amplification of the downstream of SsZNCI overlapped TrpC for verification of deletion mutant
qPCR-SsTubulin	acctccatccaagaactc	gaactccatctcgatccat	SSIG_04652 ( $\beta$ -Tubulin) for relative biomass
qPCR-AtUBQ10	agatccaggacaaggaaaggatttc	cgcaggaccaagtgaagagtag	AT4G05320 (UBQ10) for relative biomass
qPCR-SsZNCI	tggaaacaacaagtgaagaac	ctaattgagcgactgagaca	SsZNCI expression assay
qPCR-SSIG_09143	cggattggtatcgtagattgt	cgttaagaagtgtgctaatgc	SSIG_09143 expression assay
qPCR-SSIG_04923	gcggaaaggattttggctcg	ggttgagcaggcgaaggata	SSIG_04923 expression assay
qPCR-SSIG_01229	tccgcactggagttgaacat	agcattggagcagggtggtag	SSIG_01229 expression assay
qPCR-SSIG_11895	ctctcatctgcgcgtatgg	tgattcctccgtggtatctc	SSIG_11895 expression assay
qPCR-SSIG_10071	cacatcggtcgtaacacatt	caggtacgagtaatcgagaa	SSIG_10071 expression assay
qPCR-SSIG_12937	gatggtggaatgtcgaaat	ccgtggcggttggtagaaatc	SSIG_12937 expression assay
qPCR-SSIG_13036	accttccacagccaaactatga	ttactagcaccgtttctccaa	SSIG_13036 expression assay

Name	Fwd Primer	Rvs Primer	Description of primers
qPCR- <i>SSIG_02760</i>	cctccaccatctccaccatc	gccttcatacgccgaacac	<i>SSIG_02760</i> expression assay
qPCR- <i>SSIG_13736</i>	ttcatcggtcgcttcagttg	ctccaggtagtcgtcggtgt	<i>SSIG_13736</i> expression assay
qPCR- <i>SSIG_00891</i>	ctcagttcaggcagtggttac	cggtagtatggcttgttcc	<i>SSIG_00891</i> expression assay
qPCR- <i>SSIG_02334</i>	ttgctgagatggatgttgatg	tgaccgttgatgaacttgatg	<i>SSIG_02334</i> expression assay
qPCR- <i>SSIG_14362</i>	ggacacctgaagaataccaagt	ccatcggttacatccatatac	<i>SSIG_14362</i> expression assay
qPCR- <i>SCP<sup>Ss</sup></i>	aatctccatcgccatccaa	cgcagcacaggtaaagc	<i>SCP<sup>Ss</sup></i> expression assay
qPCR- <i>SsCAF1</i>	tggcttgtaggattgg	cgaagttgtctatgtatgt	<i>SsCAF1</i> expression assay
qPCR- <i>SsITL</i>	ctcaggaagataacggaaaca	catcaccatcatagtcatacac	<i>SsITL</i> expression assay
qPCR- <i>SsNEP1</i>	gtcaacctccataccaaca	acagtttagcatcaccgaat	<i>SsNEP1</i> expression assay
qPCR- <i>SsNEP2</i>	cacactctatgaagectaca	ccattgctactgccactt	<i>SsNEP2</i> expression assay
qPCR- <i>SsPINE1</i>	attctcgcagcatcaact	atccgtcaactaatccatca	<i>SsPINE1</i> expression assay
qPCR- <i>SsVPI</i>	atggcaatctgtcaagtt	ggttttttatgcacatatac	<i>SsVPI</i> expression assay
qPCR- <i>SsATG1</i>	tcatacactaccgcattag	taacctctccaccatcaca	<i>SsATG1</i> expression assay
qPCR- <i>SsATG13</i>	acactccagccataccctt	catttcttaacctgcctct	<i>SsATG13</i> expression assay
qPCR- <i>SsATG8</i>	ggtcaattcgctatgtcatc	ggtttctctaaggcttca	<i>SsATG8</i> expression assay
qPCR- <i>SsEMP24</i>	ttcacggcattgtctatgt	cttcaccaaccagaactaac	<i>SsEMP24</i> expression assay
qPCR- <i>SsERV25</i>	cattgtaggcgagaagagaa	gttgtgaatacggcttgc	<i>SsERV25</i> expression assay

**Table S2.** The information of genes used for RNA-Seq analysis.

Gene ID	Locus tag	Chromosomal location	SignalP4	TM	Gene function description
SSIG_01229	Sscl01g010410	Chromosome 1 (3541576..3544299)	YES	No TM	Glycoside hydrolase family 55 protein (FCW)
SSIG_12937	Sscl02g011730	Chromosome 2 (47473..48702)	YES	No TM	Glycosyl hydrolase 76 (mannans degradation)
SSIG_10071	Sscl016g107500	Chromosome 16 (46815..48014)	YES	No TM	Pectin lyase (pectin degradation)
SSIG_02760	Sscl04g036990	Chromosome 4 (1709458..1710439)	YES	TM	Carbohydrate esterase family 5 protein (hemicellulose degradation)
SSIG_09143	Sscl015g107260	Chromosome 15 (1740933..1742517)	YES	No TM	Amidohydrolase 2
SSIG_13736	Sscl010g079780	Chromosome 10 (1741791..1743368)	YES	No TM	Stress response protein rds1(ferritin 2)
SSIG_13036	Sscl02g021570	Chromosome 2 (3422968..3425247)	YES	No TM	Cu-oxidase 3
SSIG_11895	Sscl012g090620	Chromosome 12 (1405870..1406372)	YES	No TM	Hydrophobin class I
SSIG_04923	Sscl008g062810	Chromosome 8 (258861..260207)	YES	No TM	Hypothetical protein
SSIG_00891	Sscl003g023660	Chromosome 3 (604987..606652)	NO	No TM	Cellulose catabolic process and cellulose binding
SSIG_02334	Sscl004g033820	Chromosome 4 (604987..606652)	YES	No TM	Cellulose catabolic process and cellulose binding
SSIG_14362	Sscl016g110770	Chromosome 16(1227771..1230138)	NO	No TM	Methyltransferase activity