

Figure S1. To investigate gliotoxin production in the *ZafA* deletion mutant, the indicated strains were cultured in Czapek-Dox medium for 3 days at 37 °C, and gliotoxin was extracted from the culture medium. The produced gliotoxin was measured with HPLC. RP-HPLC with a UV detector and a polar C18 RP-HPLC column (Agilent Eclipse XDB-C18 (5 μm) 4.6 mm × 250 mm) were used in this HPLC analysis. The flow rate was 1 ml/min, and the mobile phase was methanol:water (50:50).

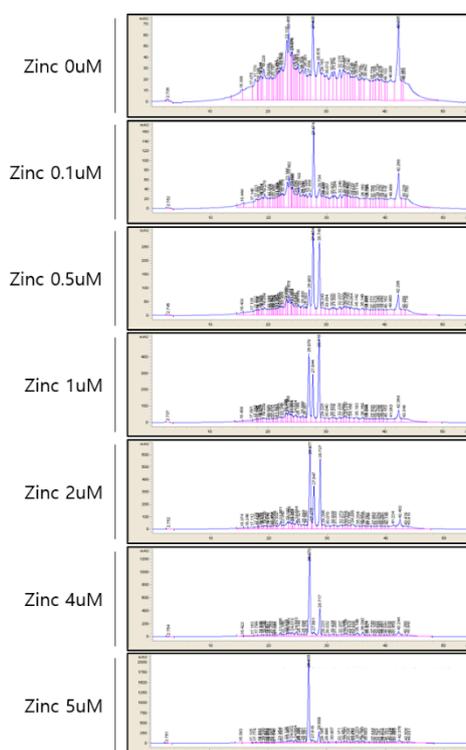


Figure S2. The effect of zinc on gliotoxin production was investigated. Wild-type cells of *A. fumigatus* were cultured in Czapek-Dox medium with the indicated concentration of zinc for 3 days at 37 °C, and gliotoxin was extracted from the culture medium. The produced gliotoxin was measured with HPLC. The analysis condition is as S1.

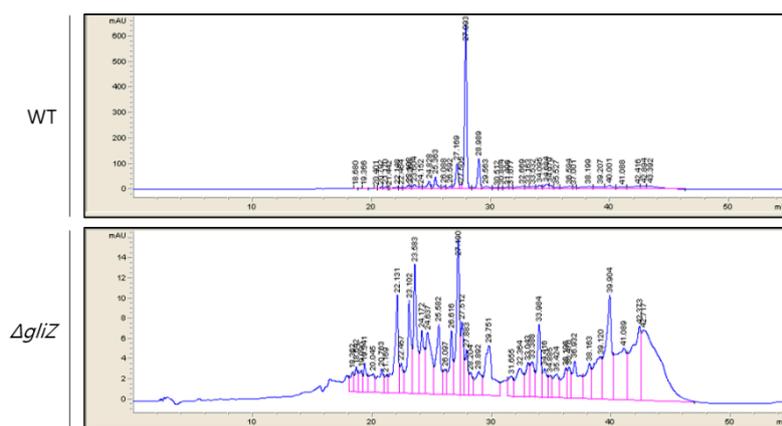


Figure S3. The *GliZ* deletion mutant was used as a control for gliotoxin production to identify the function of *GliZ* in gliotoxin production. The analysis condition is as S1.

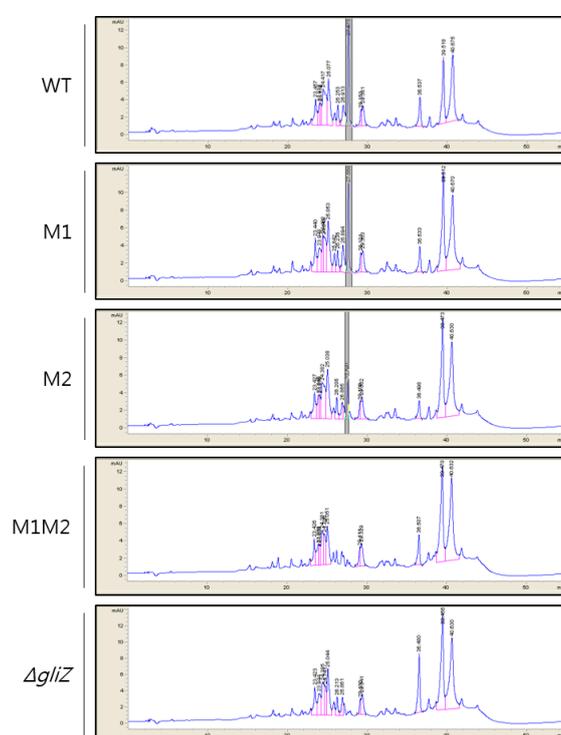


Figure S4. The wild-type and each mutant strain were cultured in Czapek-Dox medium for 3 days at 37°C, and gliotoxin was extracted from the culture medium. The produced gliotoxin was measured with HPLC. The *GliZ* deletion mutant was used as a control. The analysis condition is as S1.

Table S1. List of media and buffer solutions.

Media	Composition	Per liter
AMM	Glucose	10 g
	20X salt mix solution (-MgSO ₄)	50 ml
	200X MgSO ₄ solution	5 ml
	1000X Hunter's trace element solution	1 ml
CM	Glucose	10 g
	Yeast extract	1.5 g
	Casamino acid	1.5 g
	100X vitamin solution	10 ml
	20X salt mix solution	50 ml

	200X MgSO ₄ solution (-MgSO ₄)	5 ml
	1000X Hunter's TE solution	1 ml
100X vitamin solution	Biotin	0.1 g
	Pyridoxin-HCl	0.1 g
	Thiamin-HCl	0.1 g
	Riboflavin	0.1 g
	p-Aminobenzoic acid	0.1 g
	Nicotinic acid	0.1 g
20X salt mix (-MgSO ₄)	NaNO ₃ (sodium nitrate)	120 g
	KCl (potassium chloride)	10.4 g
	KH ₂ PO ₄ (potassium phosphate, monobasic)	16.3 g
	K ₂ HPO ₄ (potassium phosphate, dibasic)	20.9 g
200X MgSO ₄ solution	MgSO ₄ ·7H ₂ O (magnesium sulfate)	104 g
	FeSO ₄ ·7H ₂ O (ferrous sulfate)	5 g
	EDTA	50 g
	ZnSO ₄ ·7H ₂ O (zinc sulfate)	22 g
	H ₃ BO ₄ (boric acid)	11 g
1000X Hunter's trace element solution	MnCl ₂ ·4H ₂ O (manganous chloride)	5 g
	CoCl ₂ ·6H ₂ O (cobaltous chloride)	1.6 g
	CuSO ₄ ·5H ₂ O (cupric sulfate)	1.6 g
	(NH ₄) ₆ Mo ₇ O ₂ ·4H ₂ O (ammonium molybdate)	1.1 g

Table S2. List of primers used in this study.

Gene name	Primer name	Primer sequence (5' → 3')
<i>ZafA</i>	Afu.1g10080(<i>ZafA</i>) north_F	AAGATGATTTCTGCCTCGAA
	Afu.1g10080(<i>ZafA</i>) north_R	CAGCATTGAGTCTAGATTGT
<i>GliZ</i>	Afu.6g09630(<i>GliZ</i>) north_F	TGCTGCTGCTGCACCCAAGC
	Afu.6g09630(<i>GliZ</i>) north_R	CGATGTAGCCGGGAGTGAGG
<i>GliT</i>	Afu.6g09740(<i>GliT</i>) north_F	CAGTCGTCTTCGACTCTGGCGTC
	Afu.6g09740(<i>GliT</i>) north_R	TTGCGACCGTACCAGCTGTGG
<i>GliN</i>	Afu.6g09720(<i>GliN</i>) north_F	AAGACGCCTCGACCCCTCCTC
	Afu.6g09720(<i>GliN</i>) north_R	TGAGTCGGTACAGCGCCTGC
<i>GliC</i>	Afu.6g09670(<i>GliC</i>) north_F	GTTCTTCCGCAACTCGCACC
	Afu.6g09670(<i>GliC</i>) north_R	GCTCAGATGAGGCGAGCAGG
<i>GliM</i>	Afu.6g09680(<i>GliM</i>) north_F	GCCTGAGGTTTCAGTCCTGGCTG
	Afu.6g09680(<i>GliM</i>) north_R	AAGGAGACGGACGGCACGAG
<i>GliA</i>	Afu.6g09710(<i>GliA</i>) north_F	TCAGTGTCATCATGGCCGGTCTG
	Afu.6g09710(<i>GliA</i>) north_R	GTCACGGAGTTTTTGGCGACG
<i>TmtA/GtmA</i>	Afu.2g11120(<i>GtmA</i>) north_F	CACCGAAAAGCTCACGGGAC
	Afu.2g11120(<i>GtmA</i>) north_R	TTGGAGTCTCCGCTTGGTGG
<i>GliZ</i>	Afu.6g09630(<i>GliZ</i>) xho1_F	ctcgagGCGGTTGACTGATATCCCTA
	Afu.6g09630(<i>GliZ</i>) hind3_R	aagcttCGCTGACGAGTAGTTTGCTC
	<i>GliZ</i> _1st CAAGGT knockout F	CTTTCCCGCCCCGCTGTCCGAGCC
	<i>GliZ</i> _1st CAAGGT knockout R	GGCTCGACAGGCGGGGCGGAAAG
	<i>GliZ</i> _2nd CAAGGT knockout F	ACCTCGATCTAACCTCAGCAGGCG
	<i>GliZ</i> _2nd CAAGGT knockout R	CGCCTGCTGAGGTTAGATCGAGGT