Supplementary Material

Applied Microbiology and Biotechnology

Interplay between pathway-specific and global regulation of the fumonisin gene cluster in the rice pathogen *Fusarium fujikuroi*

Sarah M. Rösler[1,2]

Christian M. K. Sieber[3,4]

Hans-Ulrich Humpf[1]

Bettina Tudzynski[2*]

[1]Institute of Food Chemistry, Westfälische Wilhelms-Universität Münster, Corrensstraße 45, 48149 Münster, Germany

[2]Institute of Plant Biology and Biotechnology, Westfälische Wilhelms-Universität Münster, Schlossplatz 8, 48143 Münster, Germany

[3]Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

[4]Present address: DOE Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598, USA

[*]Corresponding author: Prof. Dr. B. Tudzynski, phone: +49 251 83-24801, fax: +49 251 83-21601, email: bettina.tudzynski@uni-muenster.de

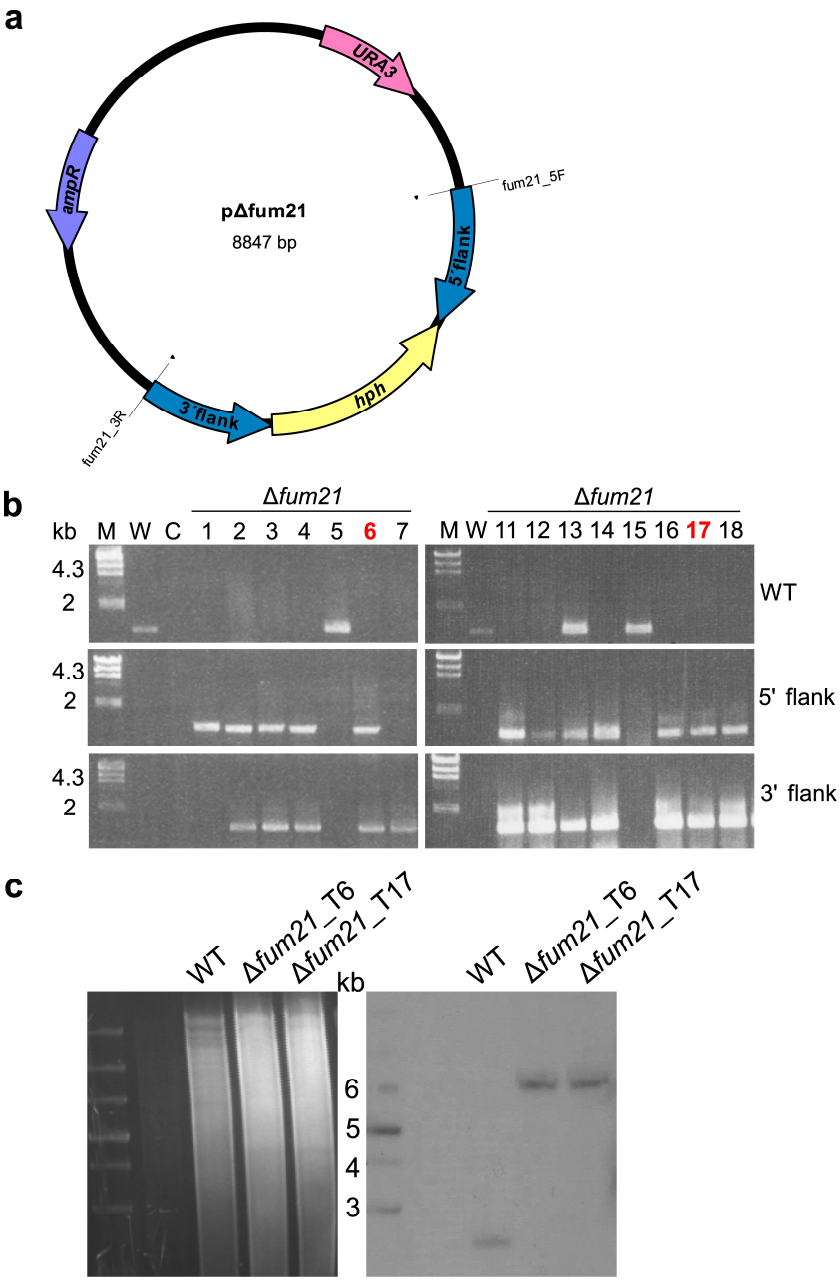


Fig. S1 Deletion of *FUM21* encoding a cluster-specific Zn(II)Cys6 transcription factor. The vector map of p*Δfum21* containing the deletion construct is shown including primers for amplification (a). *URA3* and *ampR* serve for selection in *Saccharomyces cerevisiae* and *Escherichia coli*, respectively. Correct mutants were verified by diagnostic PCR checking for homologous integration at both flanks (3´and 5´) as well as for the wild-type gene (WT) using the WT gDNA (W) and no DNA (C) as controls (b). A λ/*Hin*dIII marker (M) and GeneRuler™ DNA Ladder Mix were used for sizing. Mutants Δ*fum21*_T6 and T17, highlighted in red, were chosen for further experiments. These two mutants were additionally tested in a Southern Blot analysis using *Hin*dIII for digestion and the 5´ flank of *FUM21* as probe (c)

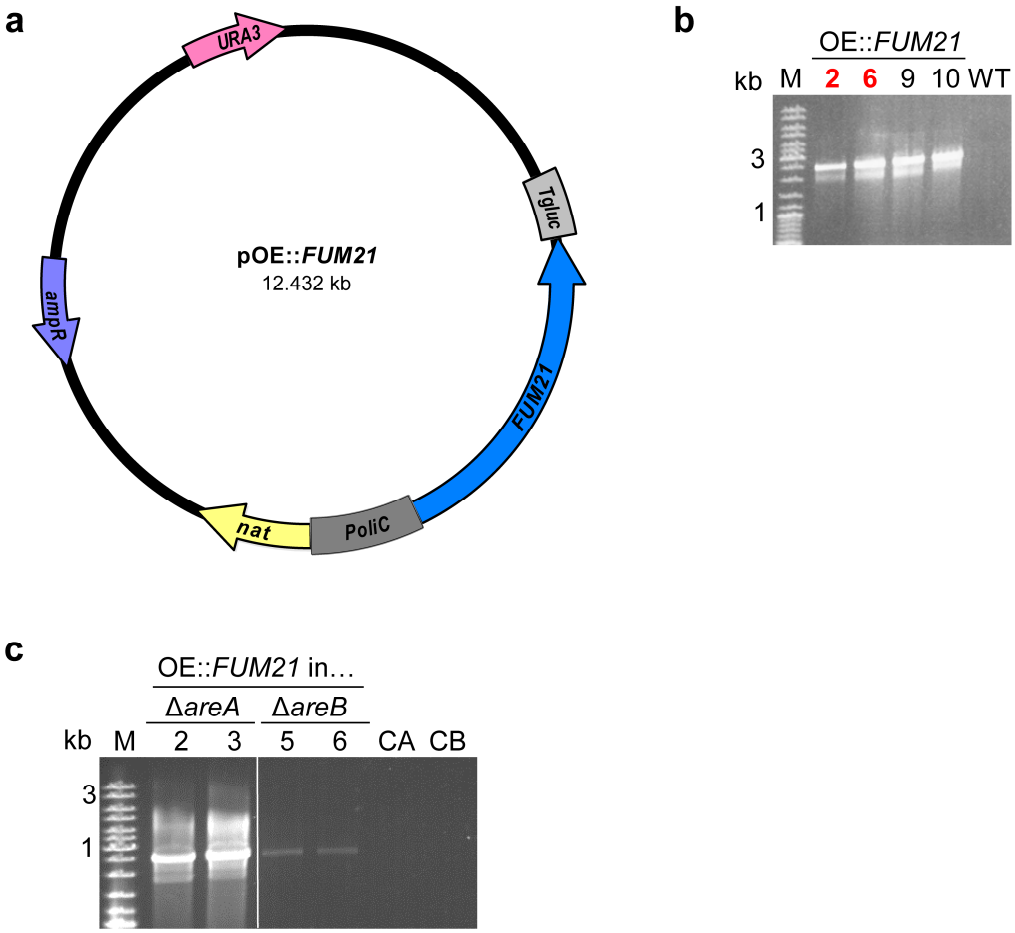


Fig. S2 Over-expression of *FUM21* encoding a cluster specific Zn(II)Cys6 transcription factor. *FUM21* was over-expressed under control of the constitutive *oliC* promoter (PoliC) and the *Tgluc* terminator. The vector map of pOE::*FUM21* is shown (a). *URA3* and *ampR* serve for selection in *Saccharomyces cerevisiae* and *Escherichia coli*, respectively. For selection the nourseothricin resistance cassette (*nat*) was used. Transformants with wild-type (WT) background (b) as well as transformants with Δ*areA* and Δ*areB* background (c), respectively, were verified by diagnostic PCR using the WT, Δ*areA* (CA) and Δ*areB* (CB) gDNA as control. GeneRuler™ DNA Ladder Mix (M) was used for sizing. Transformants OE::*FUM21*_T2 and T6 (highlighted in red), OE::*FUM21*/ Δ*areA*_T2 and T3 as well as OE::*FUM21*/ Δ*areB*_T5 and T6 were chosen for further experiments

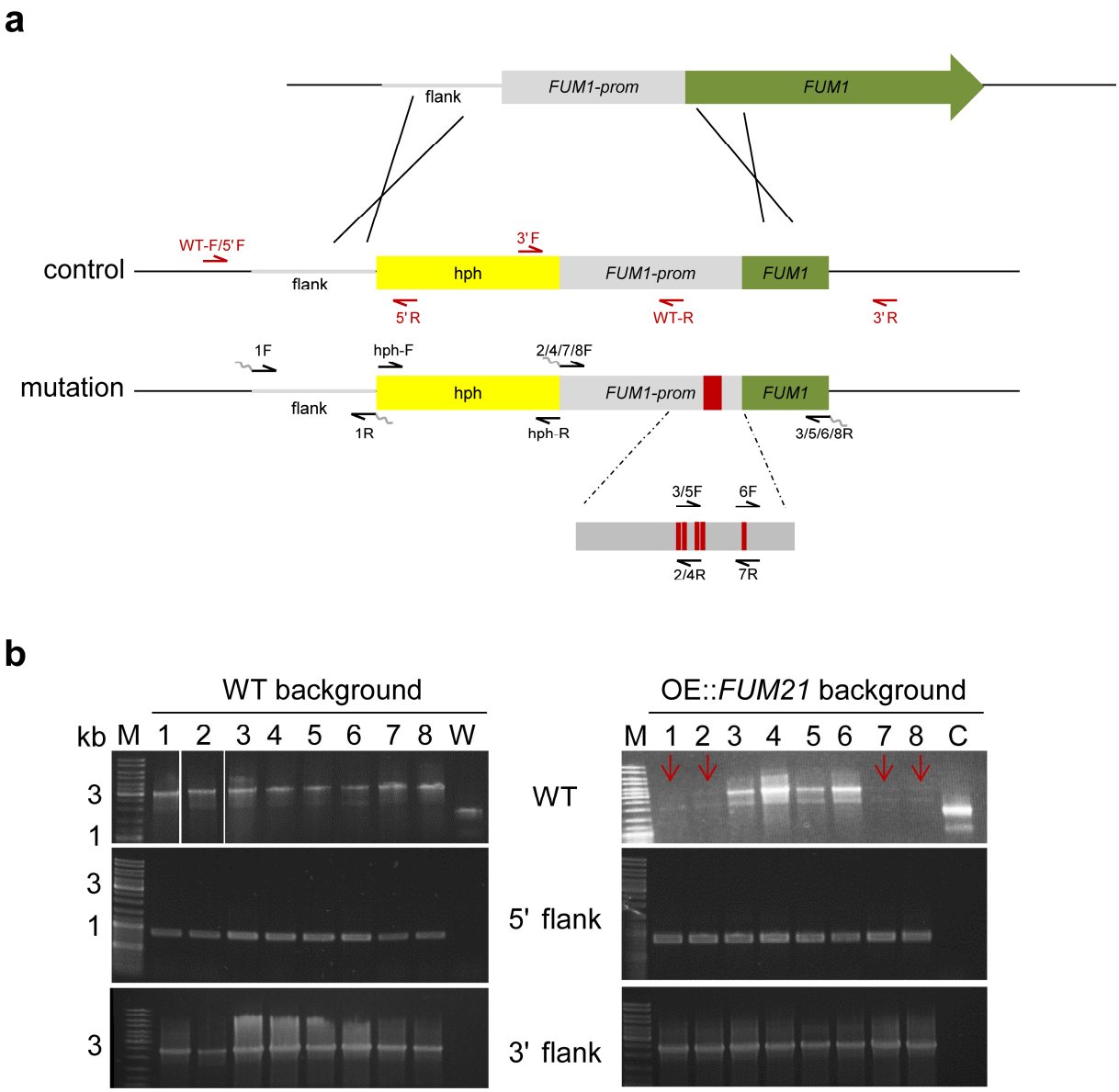


Fig. S3 Directed mutations of *FUM1* promoter motifs. Directed point mutations were inserted by homologous recombination replacing the wild-type (WT) promoter region. The hygromycin resistance cassette was inserted for selection (hph). In case of the control, a non-mutated construct was introduced (a). Primers used for amplification are shown as black arrows while overlaps are implied in grey. Numbers refer to fragments further explained in materials and methods section. Primers for diagnostic PCR of 5´ flank, 3´ flank and WT are depicted in red. Transformants were verified by diagnostic PCR checking for homologous 5´ flank and 3´ flank integration and WT situation (b). For emphasis red arrows are used. Transformants with WT background: 1=mut0_T1, 2=mut0_T2, 3=mut3_T1-3.1, 4=mut3_T3-5, 5=mut2/3_T1, 6=mut2/3_T8, 7=mut_denovo_T3, 8=mut_denovo_T6. Transformants with OE::*FUM21* background: 1=mut0_T1, 2=mut0_T11, 3=mut3_T6-2, 4=mut3_T11-3, 5=mut2/3_T8-2, 6=mut2/3_T7-1, 7=mut_denovo_T6-2, 8=mut_denovo_T15-1

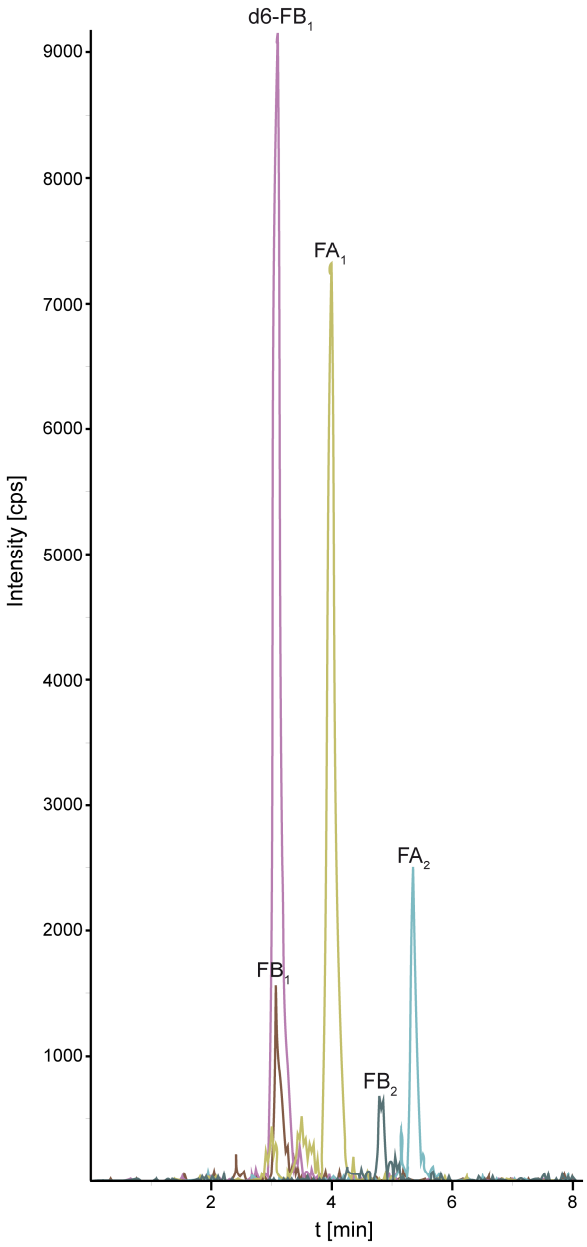


Fig. S4 Exemplary HPLC-MS/MS chromatogram for the analysis of fumonisins in fungal cultures. The *F. fujikuroi* wild type was cultivated for 6 days in liquid ICI with 6 mM glutamine. Fumonisin levels were analyzed by HPLC-MS/MS from the culture supernatant using d_6-FB_1 as internal standard: FB_1 (1.2e4), FB_2 (5.3e3), FA_1 (6.9e4), FA_2 (1.7e4), and d6-FB_1 (9e4), giving the peak areas in brackets. External calibration was used to calculate produced FB_1 amounts of 6.5 ng/mL (400 ng/g fungal dry weight)

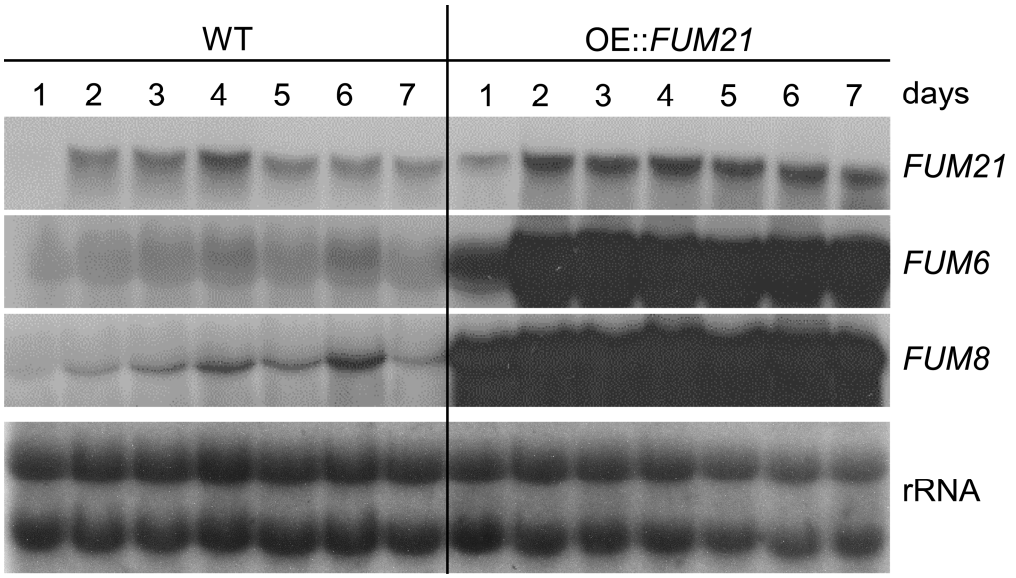
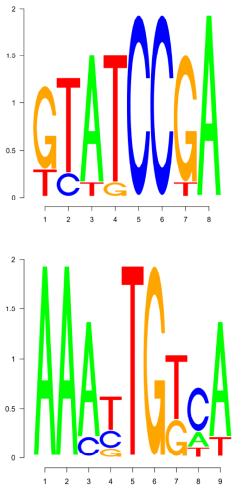


Fig. S5 Time-course expression of *FUM* genes. The *F. fujikuroi* wild type (WT) and OE::*FUM21* were cultivated for 1 to 7 days in liquid ICI medium (6 mM Gln). The expression of *FUM21* itself and the essential cluster genes *FUM6* (P450 monooxygenase) and *FUM8* (aminotransferase) was detected in a Northern blot analysis

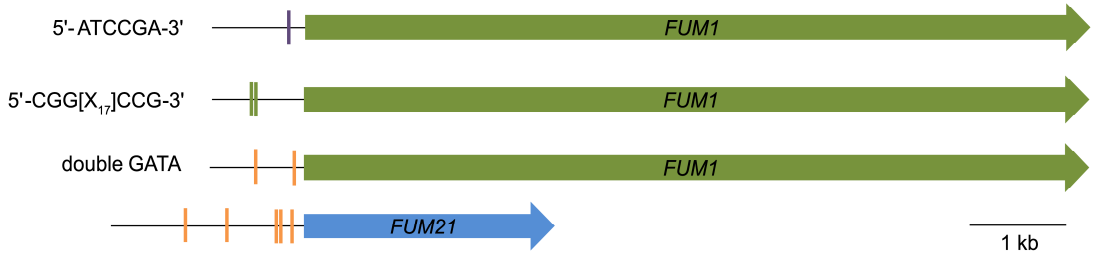
a



b

	F. fujikuroi	5'-ATCCGA-3'	5'-CGG[X$_{17}$]CCG-3'	motif3	*F. verticillioides*	5'-ATCCGA-3'	5'-CGG[X$_{17}$]CCG-3'
FUM21	FFUJ_09240	-996, -407	-834	-490, -264	FVEG_00315	-141	
FUM1	FFUJ_09241	-196	-479, -430	-749	FVEG_00316	-388, -145	-702, -494
FUM6	FFUJ_09242	-355		-458, -34	FVEG_00317	-363, -318	
FUM7	FFUJ_09243	-184		-506, -82	FVEG_00319	-211	
FUM8	FFUJ_09244	-230		-652, -618	FVEG_00318	-230	
FUM3	FFUJ_09245	-273, -234		-401, -24	FVEG_00320	-188	
FUM10	FFUJ_09246	-213,-174		-424, -47	FVEG_00321		-531
FUM11	FFUJ_09247	-275, -156		-354	FVEG_00322		
FUM2	FFUJ_09248	-709, -590		-512	FVEG_00323		
FUM13	FFUJ_09249	-455, -413	-425	-41	FVEG_00324	-414, -363, -19	-487
FUM14	FFUJ_09250	-394, -283	-98		FVEG_00325	-878	-826
FUM15	FFUJ_09251	-193, -82	-393		FVEG_14636		
FUM16	FFUJ_09252	-294, -141		-12	FVEG_00326	-725, -664, -286	
FUM18	FFUJ_09253	-388, -106	-157	-315, -286	FVEG_00328	-174	-165
FUM19	FFUJ_09254	-440, -158	-404	-261, -232	FVEG_00329	-159	-197

c



d

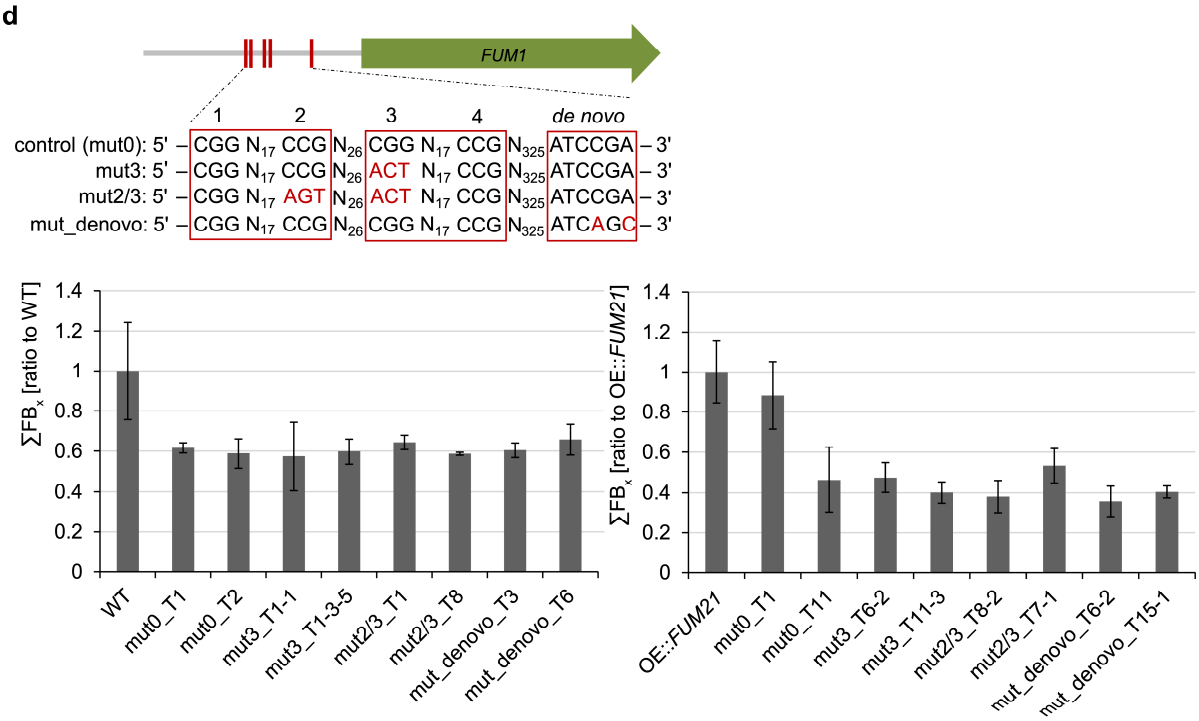


Fig. S6 Promoter motifs of *FUM* genes. Promoter analysis revealed the over-represented consensus sequence 5´-ATCCGA-3´ and a motif with the consensus 5´-AAATTGTCA-3´ in *F. fujikuroi FUM* genes (a). Additionally the palindromic 5´-CGG[X_{17}]CCG-3´ motif was identified in 7 promoters, including the *FUM1* promoter. Both motifs are present in *F. fujikuroi*, as well as in *F. verticillioides* (b). Binding sites are listed with distance from ATG. Furthermore, the promoter regions of *FUM1* and *FUM21* contain several double GATA motifs that might enable AreA and/or AreB binding (c). The motifs 5´-ATCCGA-3´ and 5´-CGG[X_{17}]CCG-3´ were mutated in wild-type and OE::*FUM21* background (d). *Loci* in the promoter of *FUM1* and directed nucleotide exchanges are highlighted in red. Two independent mutants carrying each mutation construct and the corresponding parental strain were cultivated for 7 days in fumonisin-inducing liquid medium (ICI, 6 mM Gln) and fumonisin levels (FB_1+FB_2 = ΣFB_x) were analyzed in triplicate via HPLC-MS/MS from the culture supernatant using d_6-FB_1 as internal standard

Table S1 Oligonucleotides used in this study. Overlapping regions for cloning are printed in lowercase letters. Changes to original sequence, creating directed mutations, are highlighted in red

Primer identifier	Primer sequence
Generation of plasmid for targeted gene deletion and verification of knock-out mutants	
fum21_5F	gtaacgccagggttttcccagtcacgacgTTAGCTATCATGTCACTGG
fum21_5R	atccacttaacgttactgaaatctccaacAACTGTAGGGAGGTGATACTG
fum21_3F	ctccttcaatatcatcttctgtctGCTTATCATAAGATACTAC
fum21_3R	gcggataacaatttcacacaggaaacagcAACGAGTATAAGGTTACGG
hph_F	GTCGGAGACAGAAGATGATATTGAAGGAGC
hph_R	GTTGGAGATTTCAGTAACGTTAAGTGGAT
fum21_5F_dia	AGTCATAGCTCCTTTCAGG
fum21_3R_dia	AAGGTCATCACAGGTATGG
pCSN44_trpC_P2	GTGATCCGCCTGGACGACTAAACC
pCSN44_trpC_T	GGAATAGAGTAGATGCCGACCGG
fum21_seq1	AGTATGATCAAGCTACAG
fum21_seq2	AGCTCTATACATGAGACAG
Generation of plasmids for FUM21 overexpression and verification of transformants	
OEfum21_F	ccatcacatcacaatcgatccaaccATGACGGAACCTATAGTGTTC
OEfum21_R	taatcatacatcttatctacatacgTTACTGACATTTCATTATCT
PoliC_seqF2	GGGAGACGTATTTAGGTGCTAGGG
Tgluc_seqR2	CCGCCCTCTTTTGTCTTCCGC
fum21_seq3	TGGAAACAGCGAGACAATAC
fum21_seq4	AACTGTTACGCTACCAAGCC
Generation of promotor point-mutation constructs and verification of transformants	
MutPFum1_5´F	gtaacgccagggttttcccagtcacgacgTGACACTCCCTCATAGCAGC
MutPFum1_5´R_hph	atccacttaacgttactgaaatctccaacTTACTGACATTTCATTATC
MutPFum1_PromF_hph	tccttcaatatcatcttctgtctccgacTGCTTATCATAAGATACTACC
Mut2u3_F	GACAAAGTTCTCCGTGCAGGTCAAAATACGGCTTACTCATCTA
Mut2u3_R	TAGATGAGTAAGCCGTATTTTGACCTGCACGGAGAACTTTGTC
MutPFum1_3´R	gcggataacaatttcacacaggaaacagcAGCTTGGCATCGACATACC
Mut3_F	GACAACCGTCTCCGTGCAGGTCAAAATACGGCTTACTCATCTA
Mut3_R	TAGATGAGTAAGCCGTATTTTGACCTGCACGGAGACGGTTGTC
Mut_F1	ATTGTGCATCAGCGTGCGGCGTGTTC
Mut_R1	ACGCCGCACGCTGATGCACAATAATC

fum1_probe_R	ATTCGGCGTGAAGAA
fum21_probe_F	CTCTGGTTCTTGAACTCACTTGAGAAGC
PromFum1_seq1	TATCCATTGCTCTCTTCTGC
PFum1_Mut_seqF1	TCACCTCAGTGTTCCGCTAAG
PFum1_Mut_seqR1	TACGGTGTATGAGCTACG
PFum1_Mut_seqF2	TGTAGTGCATATACCCGTGG
PFum1_Mut_seqR2	ACTGAACTGCAACCACCATC
Mut_seq1	AATCGGACATCCGAAGCCTG
Primers used for amplification of probe templates	
fum1_F	TGATAGCTCTTCATGAAGC
fum1_R	TGAAGCACCCTCGCTATGTC
fum6_F	ACTGCATTATTTACGAGACG
fum6_R	TGTCGACGGGATTCTGTCG
fum8_F	AGTGGTGGCAAGATTGTGG
fum8_R	ATCGTCGAGGTATTGCTTCG
fum21_F2	ACTGGTTTGCCATCAACGC
fum21_R2	ACCTCATCTACAGGAACG