

Supplementary materials for the manuscript submitted to Frontiers in

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**Engineering the effector domain of the artificial zinc finger transcription factor
to improve cellulase production by *Trichoderma reesei***

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Supporting tables

Table S1 List of primers used in this work *

Primer name	Sequence (5'-3')	Purpose
pUG6-F	cttctcgtgcttgacaccaggttcggtgatcagatc cactagtggcc	Amplification of pUG6 fragment
pUG6-R	caacgcacaccacaaatggatcccggtcccgcttct atagtgcacct	
xyn3-upstream-F	aggtgacactatagaacgcgggaaccgggatccattt gtggtgtgcgttg	Amplification of xyn3- upstream
xyn3 upstream-R	gatggatgattgtacccagctgcgatgcattggcctta attaaattgtc	
pyr4-F	gacaatttaattaaggccaatgcatcgacgtgggta caatcatccatc	Amplification of <i>pyr4</i> gene
pyr4-R	catatactgactctagagataccgcgtctccctcattac taccctctcg	
xyn3-downstream-F	cgagagggtagtaatgaggagacgcggtatctctag agtcaagtatatg	Amplification of xyn3- downstream
xyn3 downstream-R	ggccactagtggatctgatcacccgaacctggtctgc aagcacgagaag	
AZFP-F	atgcaccatcatcatcatcaagctatgggtgctcct cc	Amplification of AZFP _{M2} -Gal4 DBD
AZFP-R	cgccaaagacacggggagagcagccgcttttcacc ggtgt	
xyl1- overlap-F	tgtctctcccgtgtctttggcgtg	Amplification of Xyl1 _{AD}
xyl1-R	acgttaagtggatcctctagattagaggccagaccg gttc	
xyn3-AZFP-inf-F	caattgaggcggacaatttaatgcaccatcatcatc atcaagctatgggtgctcctcc	Amplification of AZFPs expression cassette
xyn3-TtrpC-inf-R	ttgtacccagctgcgatgcatgagtgagatgtggag tgggcgc	
xyn3-protoplast-F	gagtgcgggctaacaagtcatg	Fragment amplification for protoplast transformation
xyn3-protoplast-R	tggtctgcaagcacgagaagc	
Xyn3-probe-F	aacctccctacaagcatccac	Amplification of probe fragment for southern blot verification
Xyn3-probe-F	gaacgggctgacaacatcg	

Table S2. Primers for the RT-qPCR analysis

Primer	Sequence (5'-3')
cre1-F	CCTTACTTTGGCCAGGGTGT
cre1-R	AGTTGGGCCTTGACCTCTTG
ace1-F	ACCAAGACCAACGGCAAGA
ace1-R	CGTGGAGGAAGGCGTAGACA
ace2-F	GCCTCAATGCTGCTCTCTGTT
ace2-R	GACGAACGACCTTTGCTTCTCT
ace3-F	ATTGTGCGAGACATGCTGAG
ace3-R	GATGGCCAGCAAAGTAGCTC
xyl1-F	ACAGTGGAGCGGTAACAGACA
xyl1-R	CACGAATCCTTCCGACGAG
vib-1-F	TGACCTGCTACCGAAGAAACC
vib-1-R	CCACGGGATGACAATAAGACG
bglr-F	GCAAGGTCAAGTGCATGG
bglr-R	CTGTTGATGCGGTTGTGGA
ctf1-F	TCAACCAAAAGCCAAAGGAG
ctf1-R	GGGTCAAAGTCGGTGTGTG
cbh1-F	ACGAGTTCTCTTTCGATGTTGATG
cbh1-R	CGGTGTTGGTGGGATACTTG
cbh2-F	TCCTGGTTATTGAGCCTGAC
cbh2-R	GCAACATTTGGAAGGTTTCAG
egl1-F	CTCAGATGGACGAGAACGGG
egl1-R	CTGGTGGCTAGTGTTGAGGG
egl2-F	AACAAGTCCGTGGCTCCATT
egl2-R	TCCGCTCCAACCAATACCTC
xyn1-F	AAACTACCAAAGTGGCGG
xyn1-R	TTGATGGGAGCAGAAGATCC
xyn2-F	CGGCTACTTCTACTCGTACTG
xyn2-R	TTGATGACCTTGTTCTTGGTG
bgl1-F	CCGAGTGATCTGTTCCAGAATGT
bgl1-R	CTGGGTGCTGAAGATGGGTAG
CIP1-F	TCCACCGTCACTCTGCCTAC
CIP1-R	CCAGCGTCGTTTGGATTG
CIP2-F	CGCAAGAATAGACACCACCAAG
CIP2-R	AAATCCTCCAGCACGCAGA
Cel61a-F	TCAACTACATCATCCCTGGACCT
Cel61a-R	CCGTTGTCGTGGTTCTGCT

Swol-F	GCTTCCACCTACACAACCACA
Swol-R	TGGGCAGCAAACATTATCCA
tefl α -F	CTGGGTGTCAAGCAGCTCA
tefl α -R	GAGATGGGGACGAAAGCAAC

Supporting figures

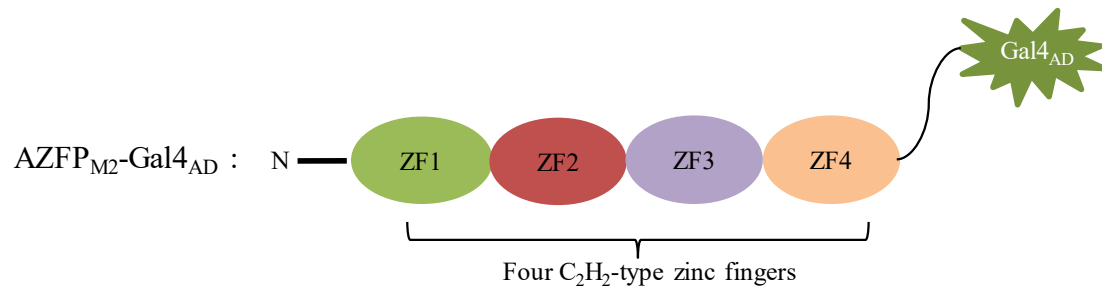


Figure S1 Schematic diagram of the AZFP_{M2}-Gal4_{AD} in *T. reesei* M2. ZF represent a C₂H₂-type zinc finger.

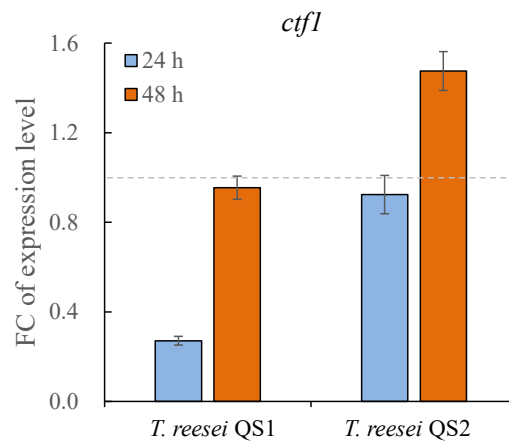


Figure S2 Gene expression of cellulase repressor encoding gene *ctfl* analyzed by quantitative RT-PCR in *T. reesei* QS1, QS2 and the parent strain TU-6. Strains were cultured at 28 °C and 180 rpm in flasks using minimal medium supplemented with 2% cellulose and 2% wheat bran as a carbon source for 24 h and 48 h, respectively. Expression levels of the reference gene *tefl* were used as an endogenous control. The value is the mean of three biological replicates with SD as the error bars. FC represents fold change of the transcription levels with *ctfl* detected in the mutants over that detected in the control.