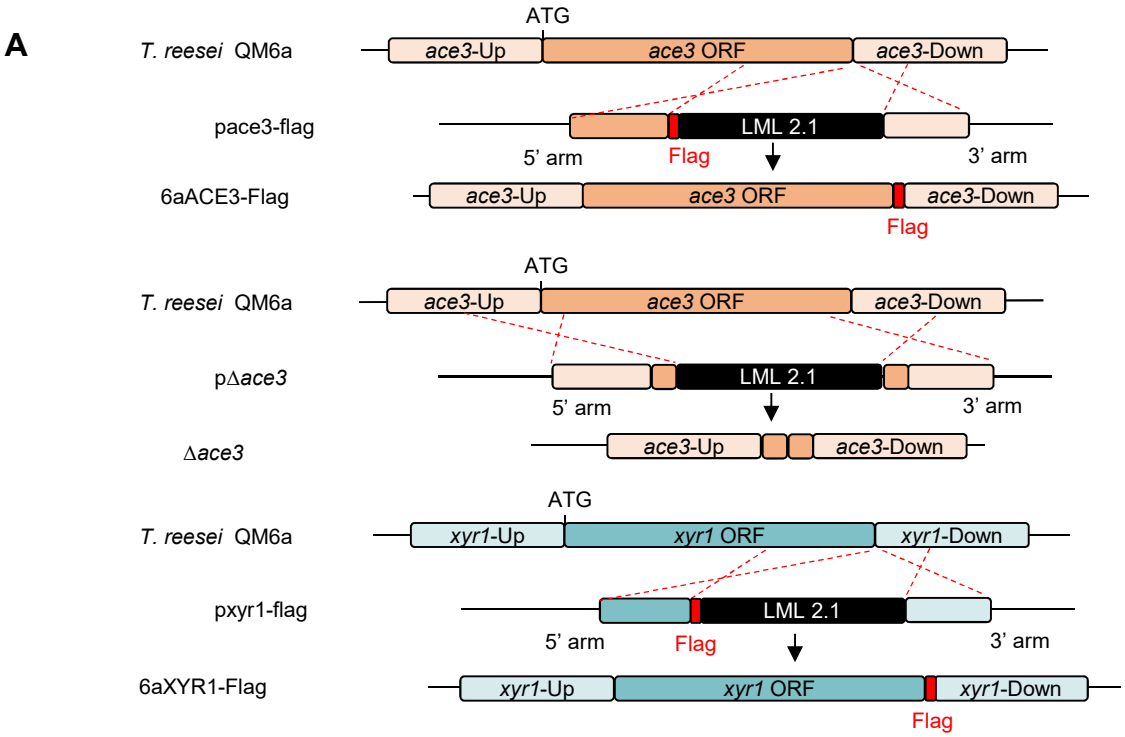

Fig. S1. The addition of a Flag tag to the C-terminus of ACE3 and XYR1 and deletion of *ace3*. (A), Construction of the ACE3-Flag, XYR1-Flag, and Δ*ace3* mutants. (B), *T. reesei* strains cultured on MA medium, using lactose as the sole source of carbon. LML 2.1 is the erasable hygromycin selection marker in *T. reesei*.

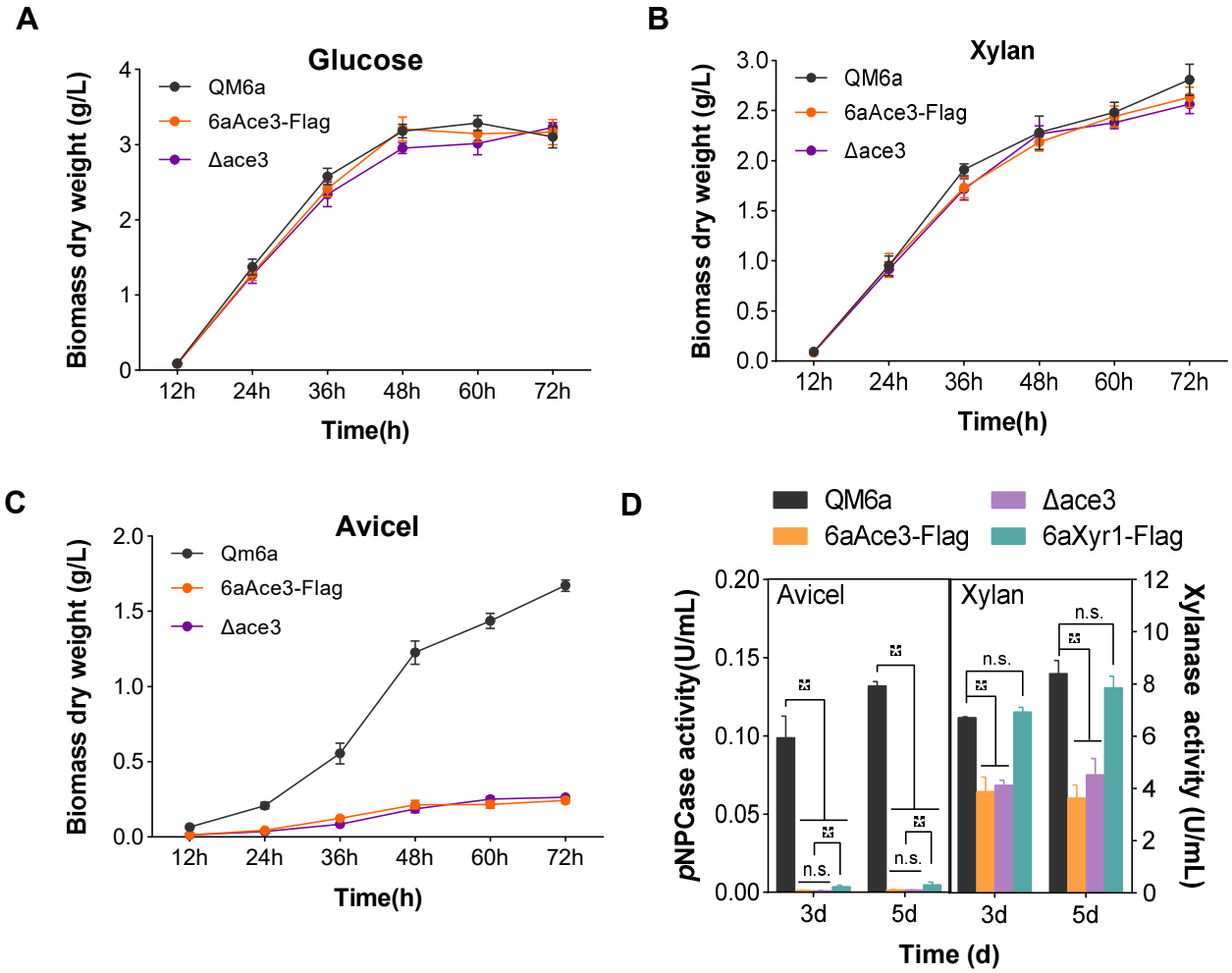

Fig. S2. The addition of a Flag tag to the C-terminus of ACE3 inhibits cellulase production. Biomass production from *T. reesei* strains on MA medium, using 2% glucose (A), xylan (B), or Avicel (C) as the sole source of carbon. (D), Activities of *p*NPCase and xylanase in *T. reesei* strains, in the presence of Avicel and xylan, respectively. Values are the mean ± SD of the results from three independent experiments. Asterisks indicate a significant difference compared to the untreated strain (*$p < 0.05$, Student's *t* test). n.s. indicates no significant difference.

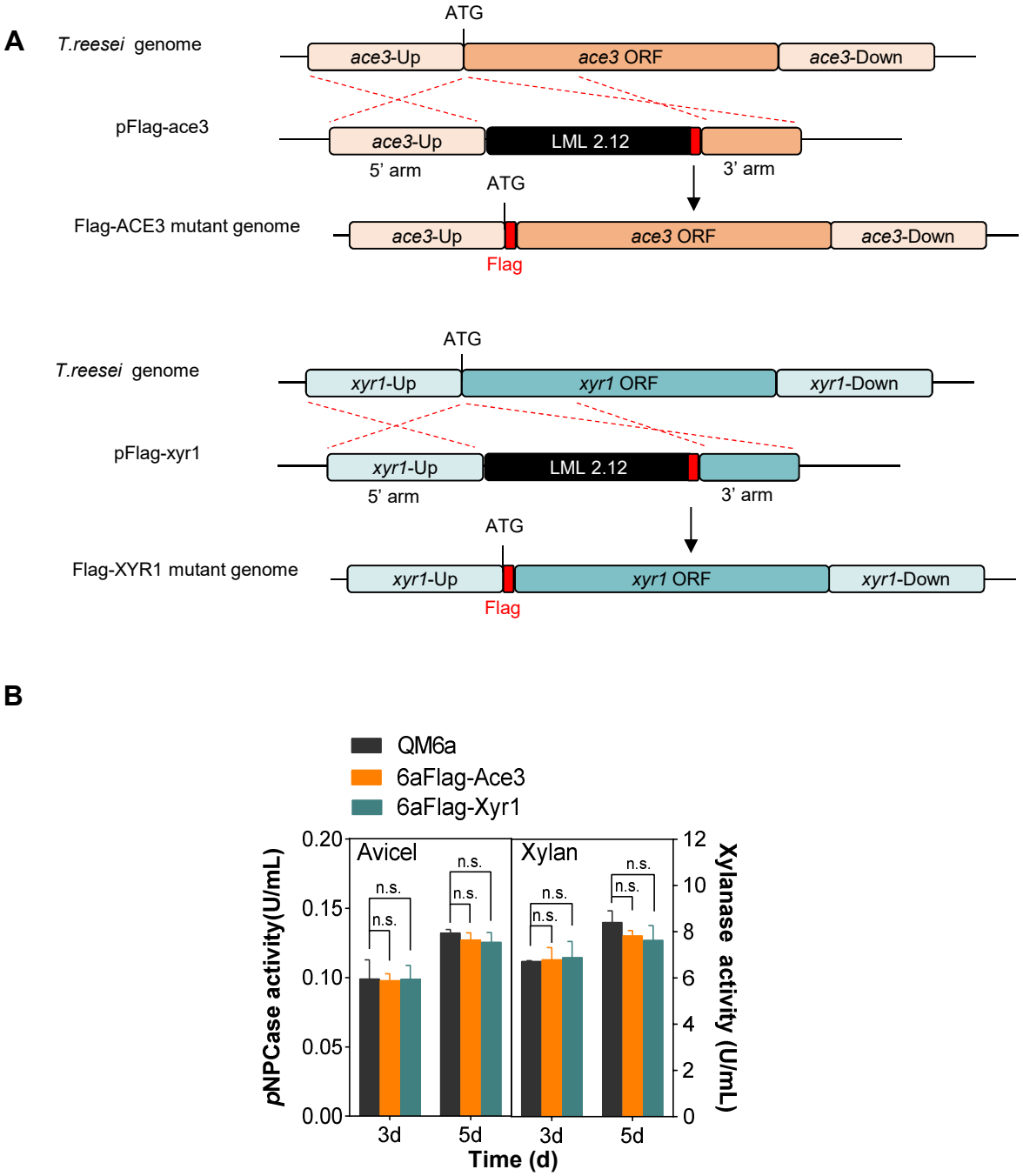

Fig. S3. Adding a Flag tag to the N-terminus of ACE3 or XYR1. (A) Construction of Flag-ACE3 and Flag-XYR1. (B) *p*NPCase and xylanase activities of *T. reesei* strains in Avicel and xylan, respectively. LML 2.12 is the erasable hygromycin selection marker in *T. reesei*. Values are the mean ± SD of the results from three independent experiments. Asterisks indicate a significant difference compared to the untreated strain (*$p < 0.05$, Student's *t* test). n.s. indicates no significant difference.

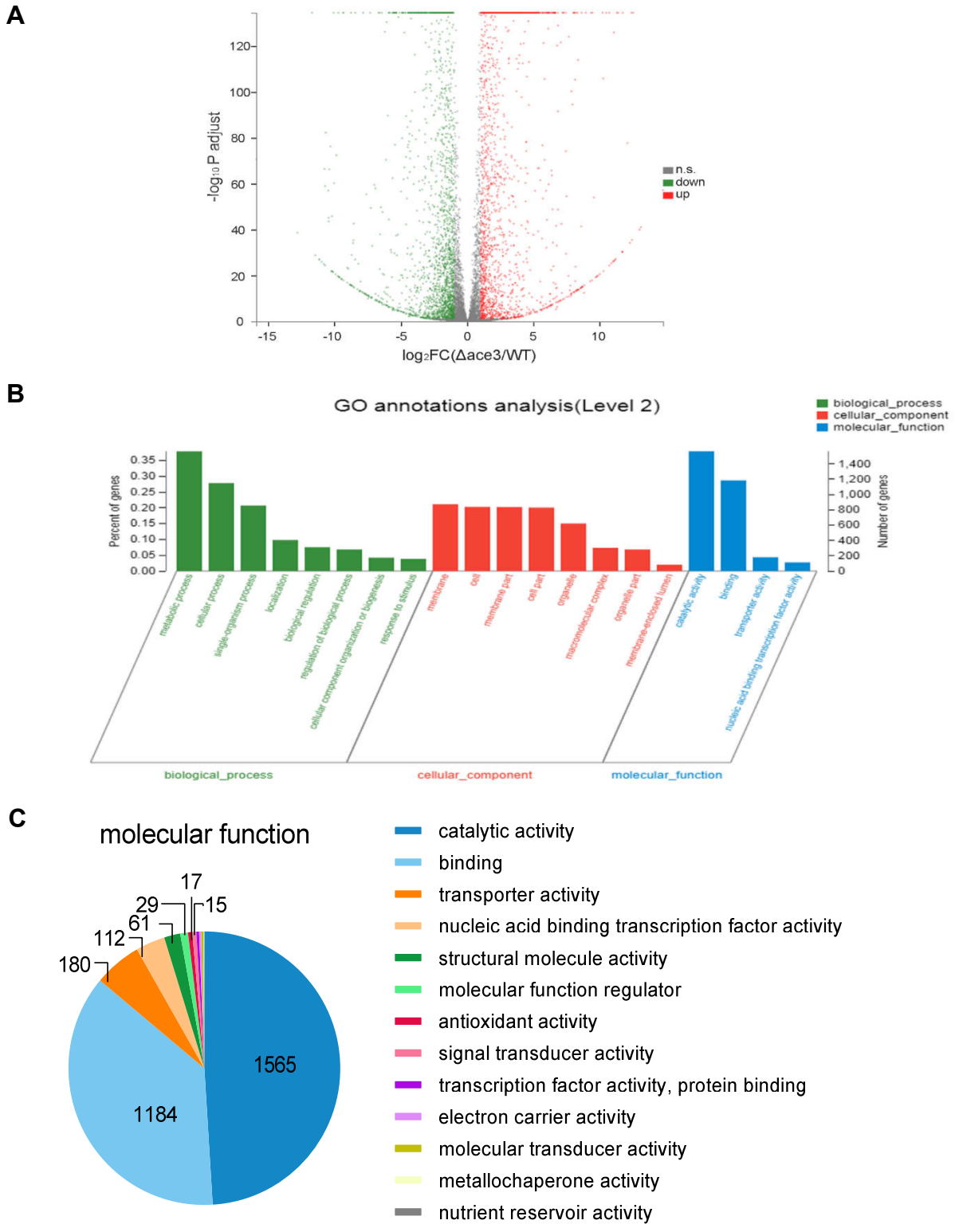

Fig. S4. Differential transcription pattern of the WT and Δ*ace3* strain in response to lactose, as measured by RNA-Seq. (A), Volcano plot analysis of up and downregulated genes in the absence of *ace3* versus that observed for the WT in the presence of lactose. (B), GO annotations analysis of differential genes for the Δ*ace3* and QM6a strain in response to lactose. (C), number of differential genes in each molecular functional category.

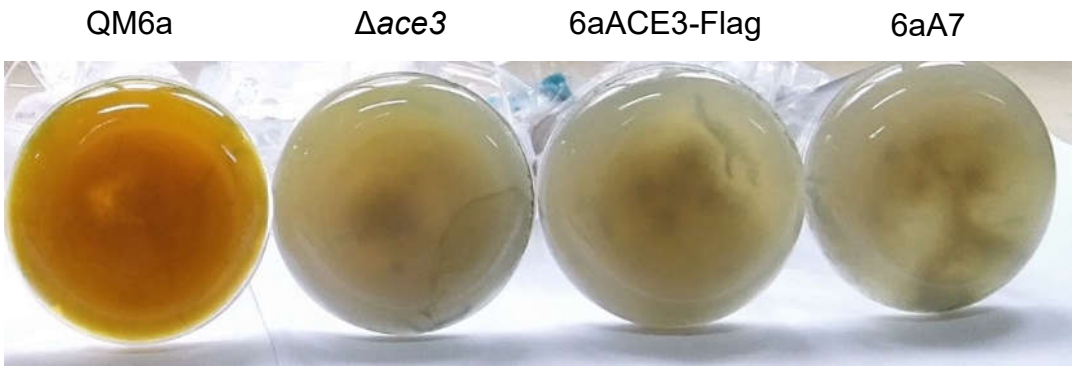

Fig. S5. Phenotypic differences of yellow pigment production in *T. reesei* QM6a, Δ*ace3*, 6aACE3-Flag, and 6aA7 strains.

Coils output for ACE3

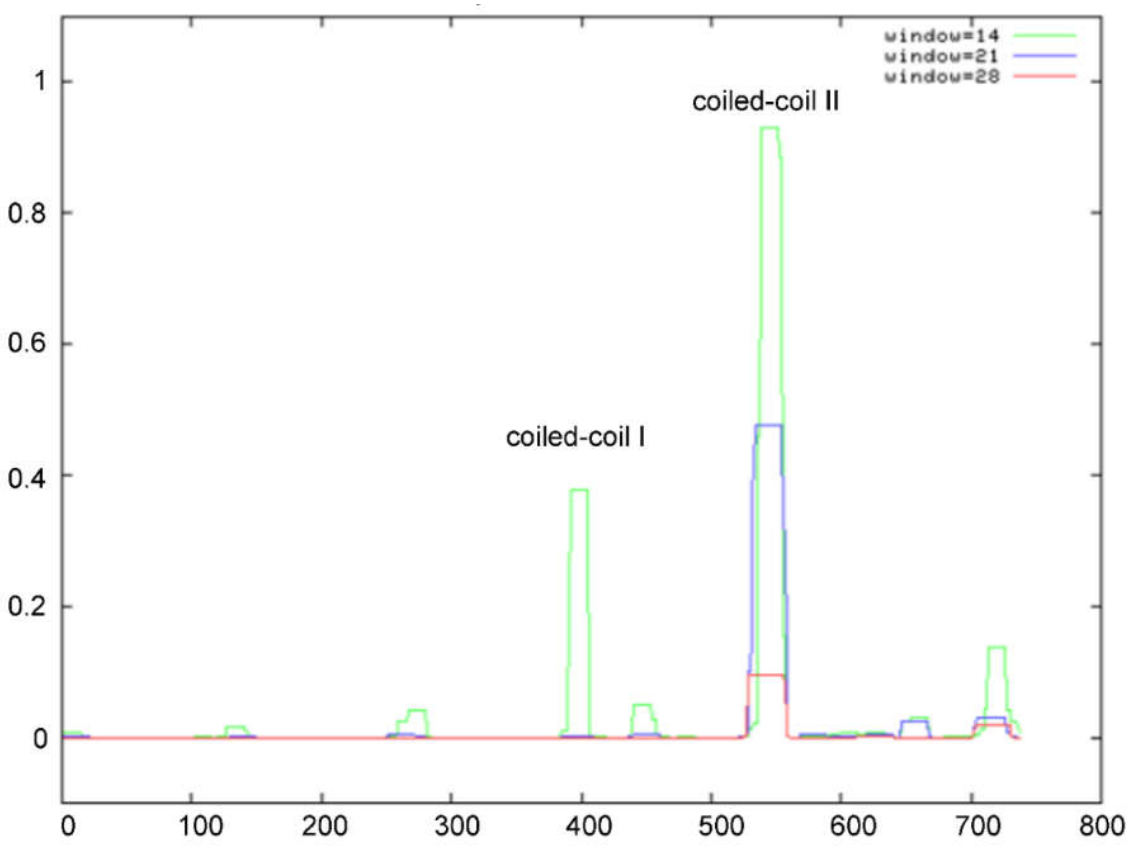

Fig. S6. Predicted coiled-coil domains in the complete ACE3 sequence using COILS.

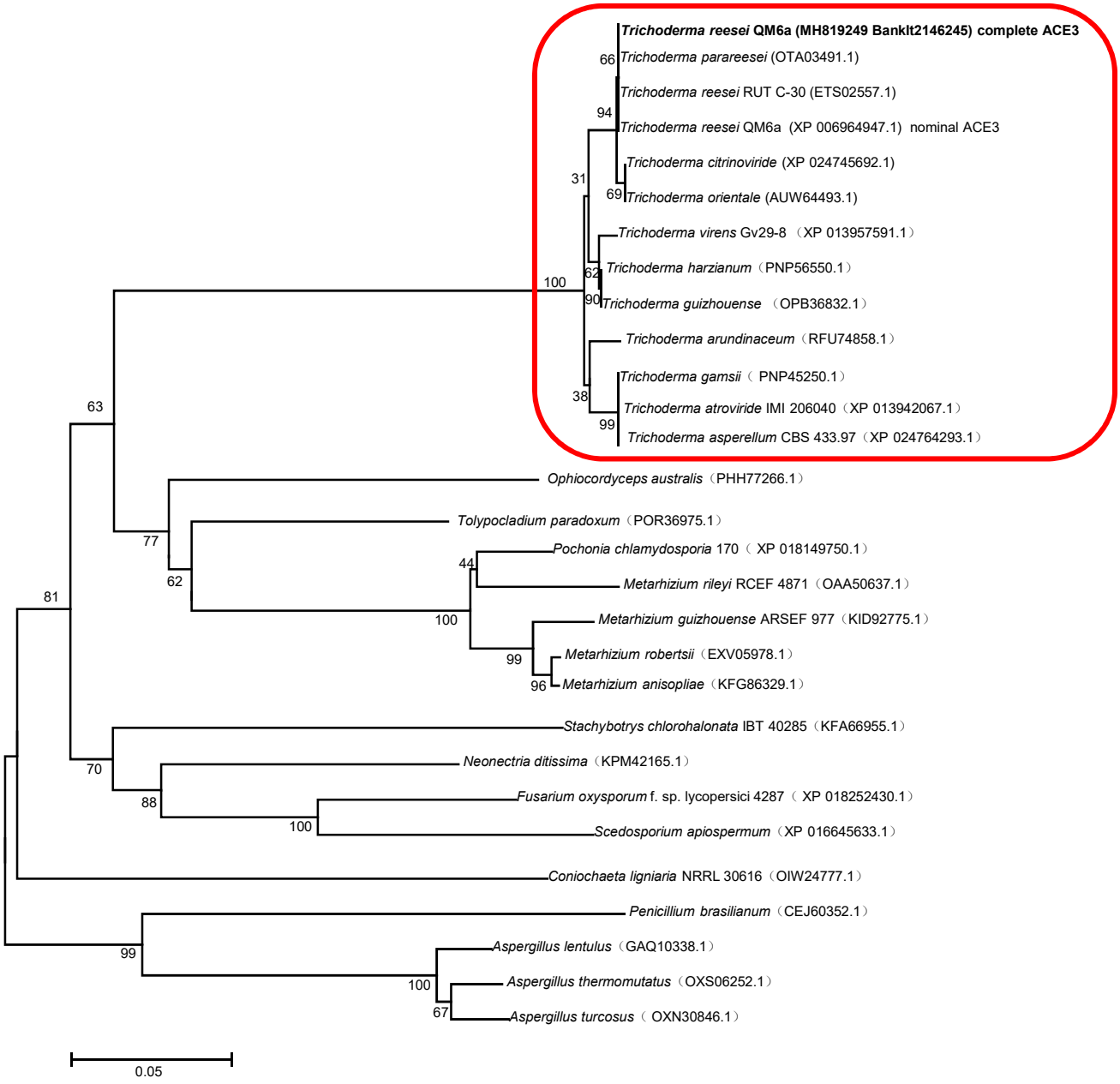

Fig. S7. Phylogenetic analysis of ACE3 and its homologs. Sequence alignments were performed with ClustalW, and the neighbor-joining tree was generated with MEGA 6.0. Numbers on the tree branches represent the bootstrap support calculated per 1000 bootstrap replicates. ACE3 sequences belonging to the *Trichoderma* clade are indicated by boxes.

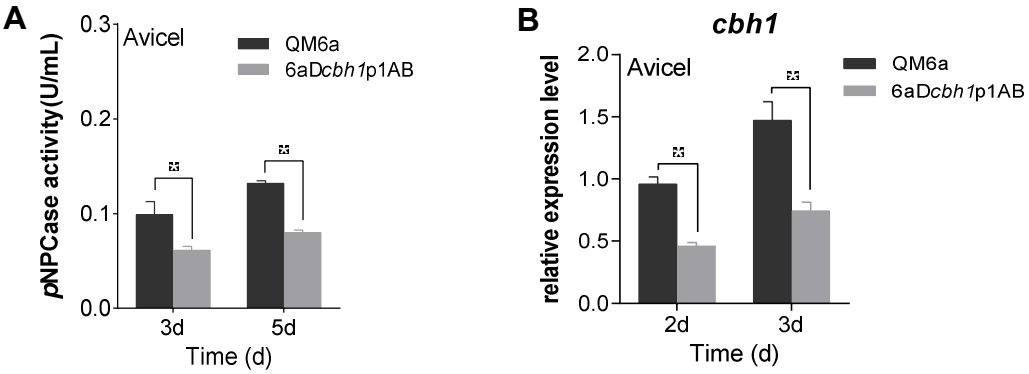

Fig. S8. *p*NPCase activities (A) and relative transcriptional levels of *cbh1* (B) of *T. reesei* strains QM6a and 6aD*cbh1*p1AB in Avicel. Values are the mean ± SD of the results from three independent experiments. Asterisks indicate a significant difference compared to the untreated strain (*$p < 0.05$, Student's *t* test).

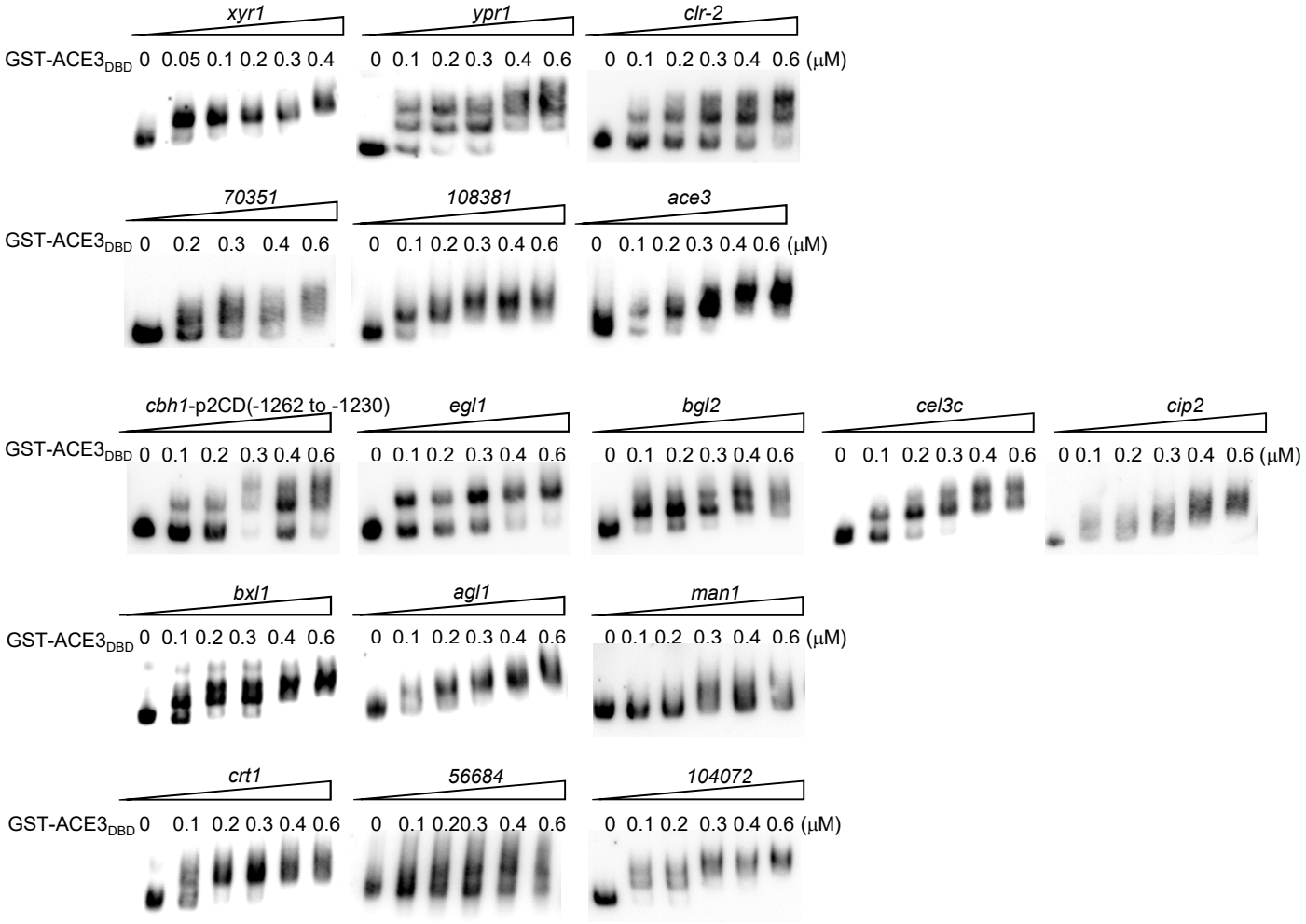

Fig. S9. EMSAs of ACE3 bound to the promoter regions of selected genes.

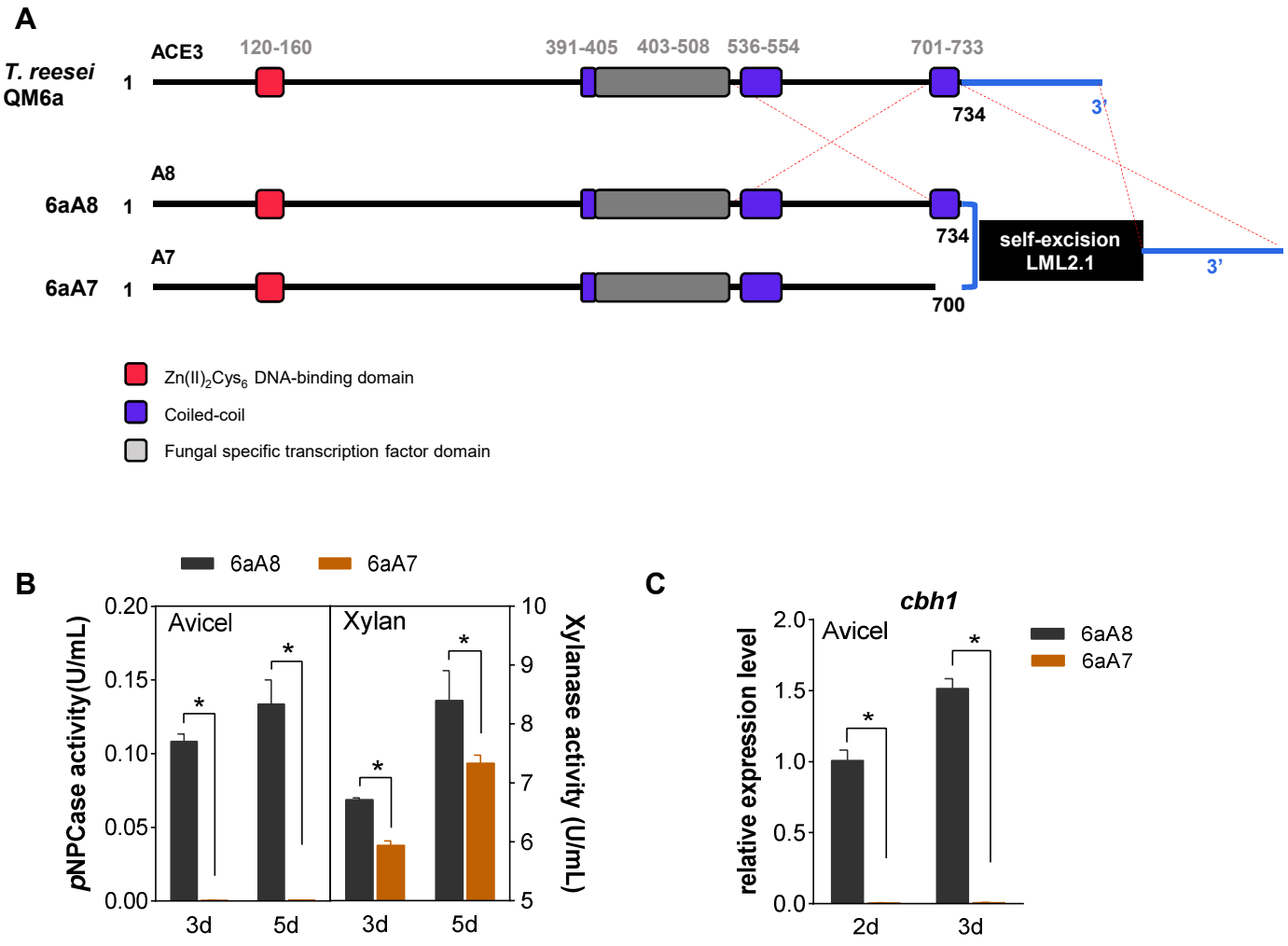

Fig. S10. Truncation of the C-terminus of ACE3 terminates its function. (A), Construction of *T. reesei* 6aA8 and 6aA7. The native ACE3 cassette named A8 that contains all 734 amino acids of ACE3, is used as the positive control. The A7 cassette (amino acids 1-700 of ACE3) is constructed by the deletion of the C-terminal 34 amino acids of ACE3. (B), *p*NPCase and xylanase activities of *T. reesei* strains 6aA8 and 6aA7 in Avicel and xylan, respectively. (C), Relative transcriptional levels of *cbh1* in *T. reesei* strains 6aA8 and 6aA7, in Avicel. LML 2.1 is the erasable hygromycin selection marker in *T. reesei*. Values are the mean ± SD of the results from three independent experiments. Asterisks indicate a significant difference compared to the untreated strain (*$p < 0.05$, Student's *t* test).

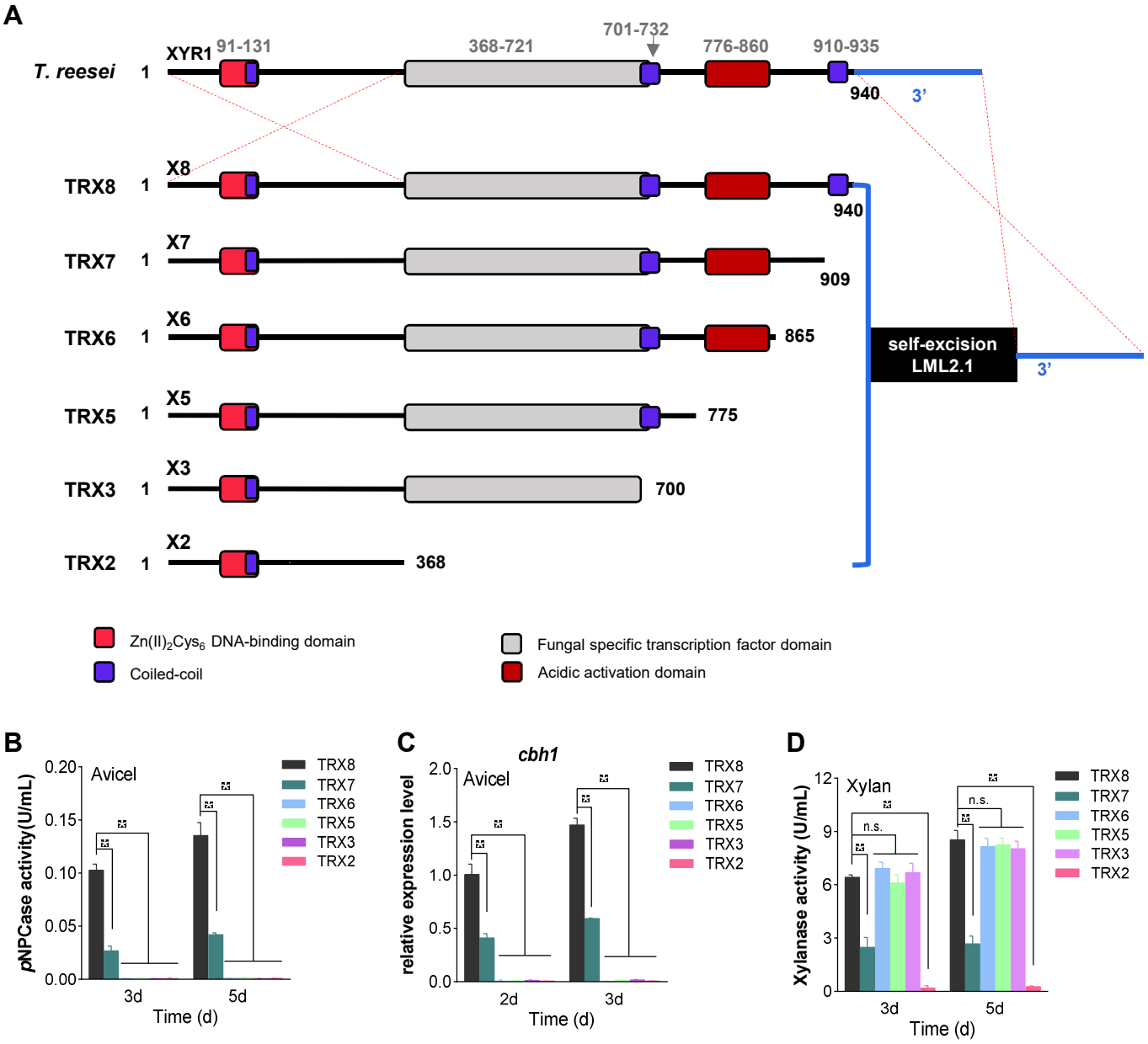

Fig. S11. Truncation of the C-terminus of XYR1 terminates its function. (A), Construction of *T. reesei* TRX8, TRX7, TRX6, TRX5, TRX3, and TRX2. The native XYR1 cassette named X8, which contains all 940 amino acids of XYR1, is used as the positive control. XYR1 truncated analogues X7 (amino acids 1-909 of XYR1), X6 (amino acids 1-865 of XYR1), X5 (amino acids 1-775 of XYR1), X3 (amino acids 1-700 of XYR1), and X2 (amino acids 1-368 of XYR1) were constructed by deleting the corresponding amino acids. (B), *p*NPCase activities of *T. reesei* strains TRX8, TRX7, TRX6, TRX5, TRX3, and TRX2 in Avicel. (C), Relative transcriptional levels of *cbh1* in *T. reesei* strains TRX8, TRX7, TRX6, TRX5, TRX3, and TRX2 in Avicel. (D), Xylanase activities of *T. reesei* strains TRX8, TRX7, TRX6, TRX5, TRX3, and TRX2 in xylan. LML 2.1 is the erasable hygromycin selection marker in *T. reesei*. Values are the mean ± SD of the results from three independent experiments. Asterisks indicate a significant difference compared to the untreated strain (*$p < 0.05$, Student's *t* test).

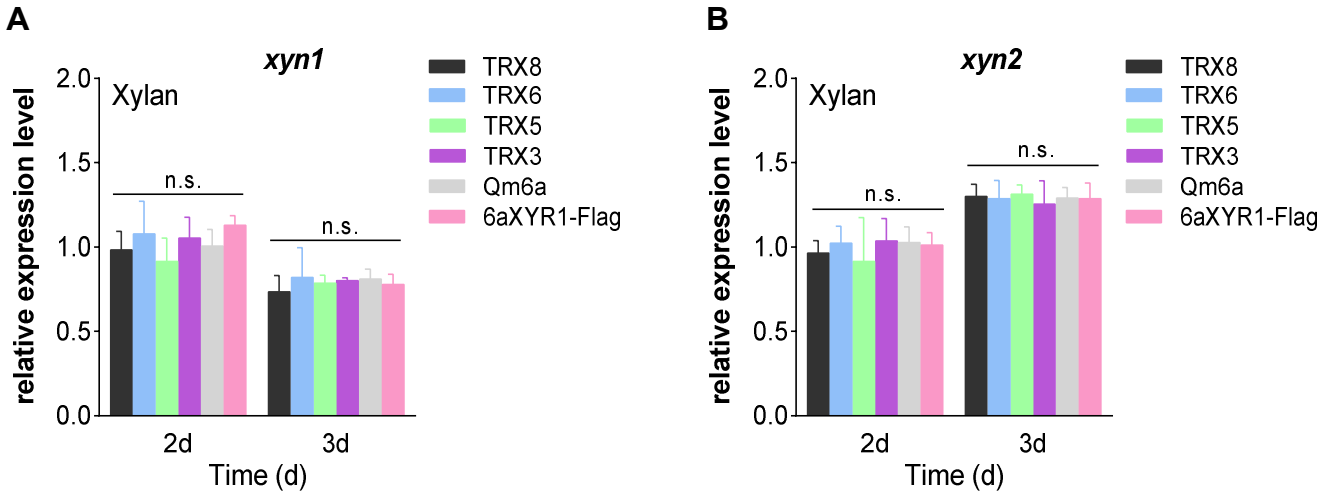

Fig. S12. Relative transcriptional levels of *xyn1* and *xyn2* in *T. reesei* QM6a, TRX3, TRX5, TRX6, TRX8, and 6aXYR1-Flag in Xylan. (A) *xyn1*. (B) *xyn2*. Values are represented as mean ± SD of the results from three independent experiments. n.s. indicates no significant difference.

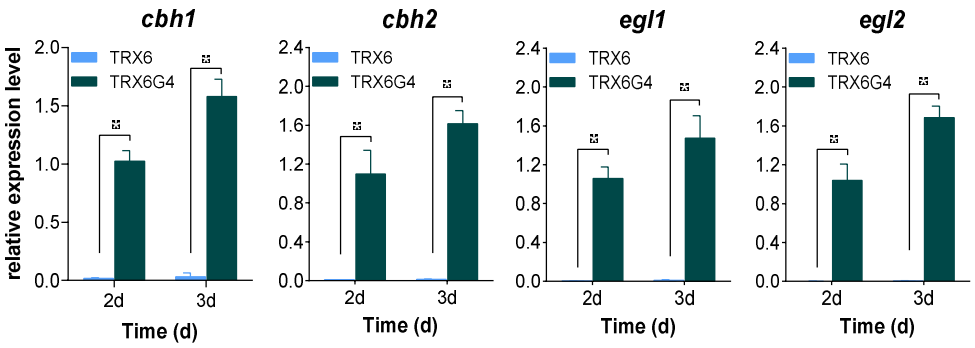

Fig. S13. Relative transcriptional levels of *cbh1*, *cbh2*, *egl1*, and *egl2* in *T. reesei* strains TRX6G4 and TRX6 in Avicel. Values are the mean ± SD of the results from three independent experiments. Asterisks indicate a significant difference compared to the untreated strain (*$p < 0.05$, Student's *t* test).

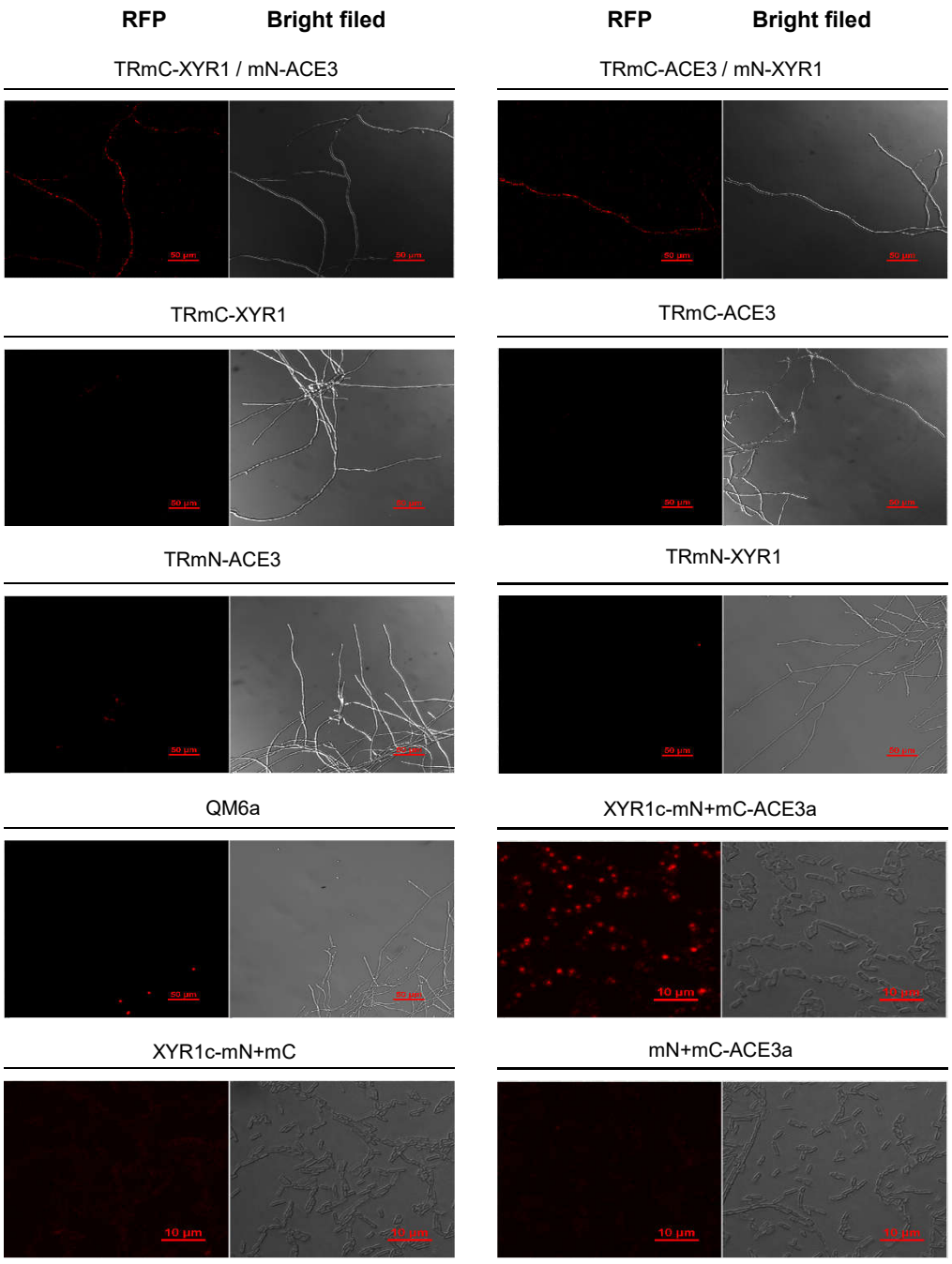

Fig. S14. BiFC assay for studying the protein-protein interactions of XYR1-ACE3 and XYR1c-ACE3a domains, respectively. The two domains of mCherry (mN, N-terminal aa 1–159 of mCherry; mC, C-terminal aa 160–237 of mCherry) were fused with the N-terminus of XYR1 or ACE3. The mCherry fused XYR1 and ACE3 were under the control of the *pdc* promoter and were transformed successively into *T. reesei*. Positive interactions in the co-expressed strains TRmC-XYR1/mN-ACE3 and TRmC-ACE3/mN-XYR1 resulted in red fluorescence. TRmC-XYR1, TRmN-ACE3, TRmC-ACE3, TRmN-XYR1, and QM6a are used as negative controls. The BiFC assay results revealed the protein-protein interactions of the XYR1c and ACE3a domains in *E. coli*. Strains expressing protein partners fused with mN or mC are indicated on each panel. Negative controls for the BiFC assay of the interaction of the XYR1c-ACE3a domains were performed (XYR1c-mN + mC and mN + mC-ACE3a). Positive interactions resulted in fluorescence.