

**Supplementary Figure 1.** Workflow diagram of quantitative proteomics analysis using TMT-based profiling. The  $\Delta 3\beta G$  strain was grown on Vogel's salts with sucrose for 16 h, followed by shifting to Vogel's salts containing cellobiose, cellulose (Avicel), sucrose, or no carbon supplementation. The resulting cultures were kept shaking for additional 4 h. Total proteins were extracted from the cultures and the protein extracts were digested with trypsin. The resulting peptide mixture was labeled by multiplexed isobaric tandem mass tag (TMT). The labeled peptides were mixed together, followed by SPS-MS3 analysis.

**Supplementary Figure 2.** Protein sequence alignment of CWH43 from the filamentous fungus *N. crassa* and the fission yeast *Schizosaccharomyces pombe*

**Supplementary Table 1.** Overlapped targets between the direct regulons of CLR-1, CLR-2, or XLR-1 and the proteomes up-regulated by cellobiose and/or Avicel

Fig. S1

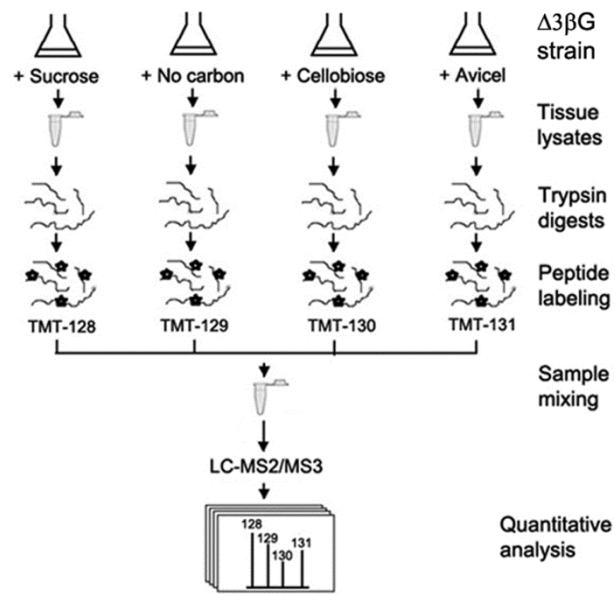


Fig. S2

N MASSRYKDKDAGVVLFSNGQWISWTHTVVAYAFLSALIIGCALHYHKIVKNEWYGYPDE 60  
S -----MARQHSANSVVFNSWVWAHTVCASAAFLCALAVGLSLHYKKIVKNGVAGWPQE 55  
      :.. : \*\*..\*:\*:\* \*\* \* \*\*\*.\*\* :\* :\*\*\*:\*\*\*\*\* \*\*:\*

N WFPSVSATIGDRYPERSIFMIFIAITSGPRFALVGLWYLLTAKPGRVLPKAI AISGVRT 120  
S WWPSVSATIGDWYPERNLFQILIALTSGPRFALVFLWWFVTRVEHPRAAAAIAICGVRT 115  
      \*:\*\*\*\*\* \*\*\*. :\* \*:\*:\*:\*:\*:\* \*\*::\* \*\*\*\*\* \*\*:\*

N LTCGGWYITSTDDHDWHDILMILYIVFTIPWTTGCIALSPPNAKAI-KYRKWIAGSFFG 179  
S VSCGGWYITSSDDHDAHDVLMILYVVCNLPWMLGSI SYTPQGYHASRRQRKIVASLFFG 175  
      :\*\*\*\*.\*\*\*\*\*:\*\*\* \*\*:\*:\*:\*:\* .:\*\* \*.\*: :\* . :\* : \*\* :\*. \*\*\*

N TLVPLVYFFIQHKVHRVAGAYTVYAFFEWALILFDVAFDAVTALDFDTFEVVVKDVKGAS 239  
S ALPPMIYFFIRHKIQRIPGAYTHYSFFEWLIVADVTFDLKSVEFSAIEISVRAVGSSS 235  
      :\* \*:\*:\*:\*:\*:\*:\*: \*\*\*\* \*:\*:\*:\* \*\*:\* \*\*:\*:\*:.. :\*: :\*: \* : \* .:\*

N KGANASSV-----PAAVL-EKEKEKATA-----GVYSAGFNL 270  
S AEIEKPAVSESNPTVTSVQLSTKTAVPKALSVAEAEKINGAKADITFLDRPGLIKSLIF 295  
      : :\* \* \* : . \* \*\* .. : : :

N GPALDIAADVHGFVFWSTLTSLGLVIWYFPLWHMGISGYEAFVMATISPVLLGIRPLRS 330  
S SPTVTYATDLYLSYCSWSVYTSLAITLFYFNIWELGVTGSELAVISTLTPFWLGIRSFRE 355  
      .\*:\* \*:\*:\* .: \*\*. \*\*\*. :. :\*\* \*: \*:\*:\* \* \*:\*:\*:\* \*\*\*\*\* :\*

N LIVNNQ--RLFHVLSLAGLLAYKVHDPVYRLFTVGFVFMGCLAWAATWSSDSVHPSRLE 388  
S SVASHTGQIVVQACVILGMAAYI IDEPLGRLLVVALANMALGIDWAITWSGLSDRISPQD 415  
      :..: :.. : \*:\* \*\* :. :\*: \*\*:.\*. :. : : \*\* \*\*\*, \* : \* :

N SRVIAWLAGLLMSSIAKFAWYTNPIWPVMAENGGWNGTGLVLA ILAVLRFTRKAPLNS 448  
S N-SFILAAGFILANLSKMANHSSNPVWPIVDSRSGGWNKTGLLLALLALAQFASRPPIA 474  
      . : \*\*:::.. :\*: \* :\*\*\*:\*\*\*:.. .. \*\*\*\*\* \*\*\*:\*\*\*:\*\*\*: :\*: : \* : :

N -WGVSQQKNGSSLLSAIGIGGLFFAIHSLSDTSTMILWWGGFPVRGPVSNVHA-YYTI 506  
S PQEIIKTDNSRTWSSSLGLGGTMFALYSLFADSGTIVAWSWTGYPAKGPVPGVMSGAVTI 534  
      : : .\*. : \*:\*:\*:\* \*\*:\*:\*:\*:\*:\*:\*: \* \* \*:\*:\*: .\* : \*\*

N AAMTGGLLIG-VFRPGMI---TNWTAYGVACVGAAMLTYEQWFGYYGALILTYYLMAIA 562  
S SAMCAGLALAGSSSASRVVNHPAWLSIGVGA--CIVMYQCRDWAGYAGGLVYGLFLMSIF 592  
      :\*\* \*\* :. : : \* : \*\*.. : : .:\* \*\* \*:\*: \*\*:\*

N VPMISKAACKNPAVTFGVGFLFYNLLVLFHVWVYAFVPGGPVYREHTDWIMLTTMLTI 622  
S PKMLQESAFRGSALVYYLMWLTITGLILADVWATAYAFVPGGVYMRERTDIVLTAELAI 652  
      \*.:\*: \* . \*.: : :\* \*\*.\*\*\*.\*\*\*\*\*\* \*\*:\*:\* \*\* : : :\*:\*



**Table S1. Overlapped targets between the direct regulons of CLR-1, CLR-2, or XLR-1 and the proteomes up-regulated by cellobiose and/or Avicel**

NCU no.	Locus	Annotation or domain	Up-regulation condition <sup>1</sup>	Correlation between mRNA and protein <sup>2</sup>
<i>Targets of both the CLR-1 and CLR-2 direct regulons</i>				
NCU00801	<i>cdt-1</i>	Cellodextrin transporter	CB vs. NC, AV vs. NC	yes
NCU07340	<i>cbh-1</i>	Exo- $\beta$ -1,4-glucanase or cellobiohydrolase	CB vs. NC, AV vs. NC	yes
NCU09680	<i>gh6-2 or cbh-2</i>	Exo- $\beta$ -1,4-glucanase or cellobiohydrolase	CB vs. NC, AV vs. NC	yes
NCU07190	<i>gh6-3</i>	Exo- $\beta$ -1,4-glucanase cellobiohydrolase	CB vs. NC, AV vs. NC	yes
NCU00206	<i>cdh-1</i>	Cellobiose dehydrogenase	CB vs. NC, AV vs. NC	yes
NCU05864		Signal peptide-containing protein	CB vs. NC, AV vs. NC	yes
<i>Targets of the CLR-2 direct regulon</i>				
NCU08114	<i>cdt-2</i>	Cellodextrin and xylo-dextrin transporter	CB vs. NC, AV vs. NC	yes
NCU08760	<i>NcLPMO9E</i>	Lytic polysaccharide monoxygenase 1	CB vs. NC, AV vs. NC	yes
NCU05956	<i>gh2-2</i>	Intracellular $\beta$ -galactosidase	CB vs. NC	yes
NCU01900	<i>gh43-2</i>	Intracellular $\beta$ -xylosidase	AV vs. NC	yes
NCU00870		SET domain-containing protein	CB vs. NC, AV vs. NC	yes
<i>Targets of the CLR-1 direct regulon</i>				
NCU00326		Calcium homeostasis protein Regucalcin	CB vs. NC, AV vs. NC	yes
NCU05574		Acetyltransferase domain	CB vs. NC, AV vs. NC	yes
NCU07487	<i>bgl-4</i>	Intracellular $\beta$ -glucosidase	CB vs. NC, AV	yes

			vs. NC	
NCU09689		$\alpha/\beta$ -hydrolase domain	CB vs. NC, AV vs. NC	yes
<i>Targets of the XLR-1 direct regulon</i>				
NCU04401		Fructose-bisphosphate aldolase	CB vs. NC, AV vs. NC	yes
NCU09652	<i>gh43-5</i>	Intracellular $\beta$ -xylosidase	CB vs. NC, AV vs. NC	yes
NCU00891		Xylitol dehydrogenase	AV vs. NC	yes
NCU02343	<i>gh51-1</i>	$\alpha$ -arabinofuranosidase	AV vs. NC	yes
NCU08384		Intracellular D-xylose reductase	AV vs. NC	yes
NCU09705		Intracellular aldose 1-epimerase	AV vs. NC	yes

<sup>1</sup> The proteomes up-regulated by cellobiose or Avicel was derived from the comparisons of CB vs. NC or AV vs. NC, respectively (Fold change  $\geq 1.3$ , p-values  $< 0.05$ ).

<sup>2</sup> Correlation analysis between mRNA and protein abundance was performed with the proteome data of this study and the transcriptome data from a previous study (Coradetti ST, Xiong Y, Glass NL. 2013. Analysis of a conserved cellulase transcriptional regulator reveals inducer-independent production of cellulolytic enzymes in *Neurospora crassa*. *MicrobiologyOpen* 2:595-609. <https://doi.org/10.1002/mbo3.94>).