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SUPPLEMENTARY INFORMATION

Fungal β -glucan-facilitated cross-feeding activities between Bacteroides and Bifidobacterium species.

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Table S1. *Bacteroides* species tested with all substrates.

	Mycoprotein β-glucan	Yeast β-glucan
<i>Ba. thetaiotaomicron</i> VPI-5482		
<i>Ba. cellulosilyticus</i> WH2		
<i>Ba. cellulosilyticus</i> DSM 14838		
<i>Ba. ovatus</i> ATCC 8483		
<i>Ba. intestinalis</i> DSM 17393		
<i>Ba. fragilis</i> ATCC 25285		
<i>Ba. finegoldii</i> DSM 17565		
<i>Ba. vulgatus</i> ATCC 8482		
<i>Ba. caccae</i> ATCC 43185		
<i>Ba. clarus</i> DSM22519		
<i>Ba. dorei</i> DSM17855		
<i>Ba. egerthii</i> DSM 20697		
<i>Ba. fluxus</i> DSM 22534		
<i>Ba. massiliensis</i> DSM 17679		
<i>Ba. oleiciplenus</i> DSM 22535		
<i>Ba. plebeius</i> DSM 17135		
<i>Ba. salyersae</i> DSM 18765		
<i>Ba. stercolis</i> ATCC 43183		
<i>Ba. uniformis</i> ATCC 8492		
<i>Ba. xylanosolvens</i> XB1A		
<i>Ba. intestinihominis</i> DSM 21032		

<i>Dysgonomonas gadei</i> ATCC BAA-286		
<i>Dysgonomonas mosii</i> DSM 22836		

Green= full growth (OD₆₀₀ 0.9-1.5), Yellow= medium growth (OD₆₀₀=0.4-0.8) and Red= no or low growth (OD₆₀₀=0-0.3). The time for all growths was 24h and all experiments were obtained in triplicates.

Table S2. Primers used in this study.

	Sequence
Bacell WH2	
Bccell WH2_01926 (GH3)	Forward: GCGGCGGCTAGCCAGGAAAAGGCAAATACC Reverse: GCGGCGCTCGAGCTATTGTAAGACAGTAAA
Bccell WH2_01931 (GH157)	Forward: GCGGCGGCTAGCCAATTCAGTTCTTCTCCG Reverse: GCGGCGCTCGAGTCATGTCAGCAACATCTT
Bccell WH2_02537 (GH30_3)	Forward: GCGGCGGCTAGCTGCGGATCGGACCACAAT Reverse: GCGGCGCTCGAGTTATTTCCACTTGAAAGA
Bccell WH2_02541 (GH2)	Forward: GCGGCGGAATCCAACATGAGAGCAAAACG Reverse: GCGGCGCTCGAGTTAATAAAGTTTCCTGAG
Primers for qPCR cross feeding	Forward: 5' -AGCAGGCGGAATTCGATAAG-3' Reverse: 5' -GTGTACAGTGCCAGGCATAA-3'
BT	
BT3312 (GH30_3)	Forward: GCGGCGGCTAGCAATAGTGATGATGCGGAA Reverse: GCGGCGCTCGAGTTACTTTGACTTTGCCCAACG
BT3314 (GH3)	Forward: GCGGCGGCTAGCCAGACACCAGTATATCTA Reverse: GCGGCGCTCGAGCTATCTCAACTCAAATGG
Primers for qPCR cross feeding	Forward: 5' -AGGTGCAGGCAACCT-3' Reverse: 5' -AATTCCCCTTCTCCATGTCC-3'
<i>Bi. breve</i> UCC2003	
Bbr_0109 (GH1)	Forward: GCGGCGGCTAGC ATGACATTGTTTTTCCG Reverse: GCGGCGCTCGAG TCA GAT TCC TTC CTG GAT
Primers for qPCR cross feeding	Forward: AGAGTTTGATCCTGGCTCAG Reverse: CTACGGCTACCTTGTTACGA
<i>Bi. longum</i> subsp. <i>longum</i>	

Primers for qPCR cross feeding	Forward: AGAGTTTGATCCTGGCTCAG Reverse: CTACGGCTACCTTGTTACGA
<i>Bi. longum</i> subsp. <i>infantis</i>	
Primers for qPCR cross feeding	Forward: AGAGTTTGATCCTGGCTCAG Reverse: CTACGGCTACCTTGTTACGA
<i>Lb. plantarum</i>	
Primers for qPCR cross feeding	Forward: CAC CGC TAC ACA TGG AG Reverse: CCA CCG CTA CAC ATG GAG TTC CAC T

Figures

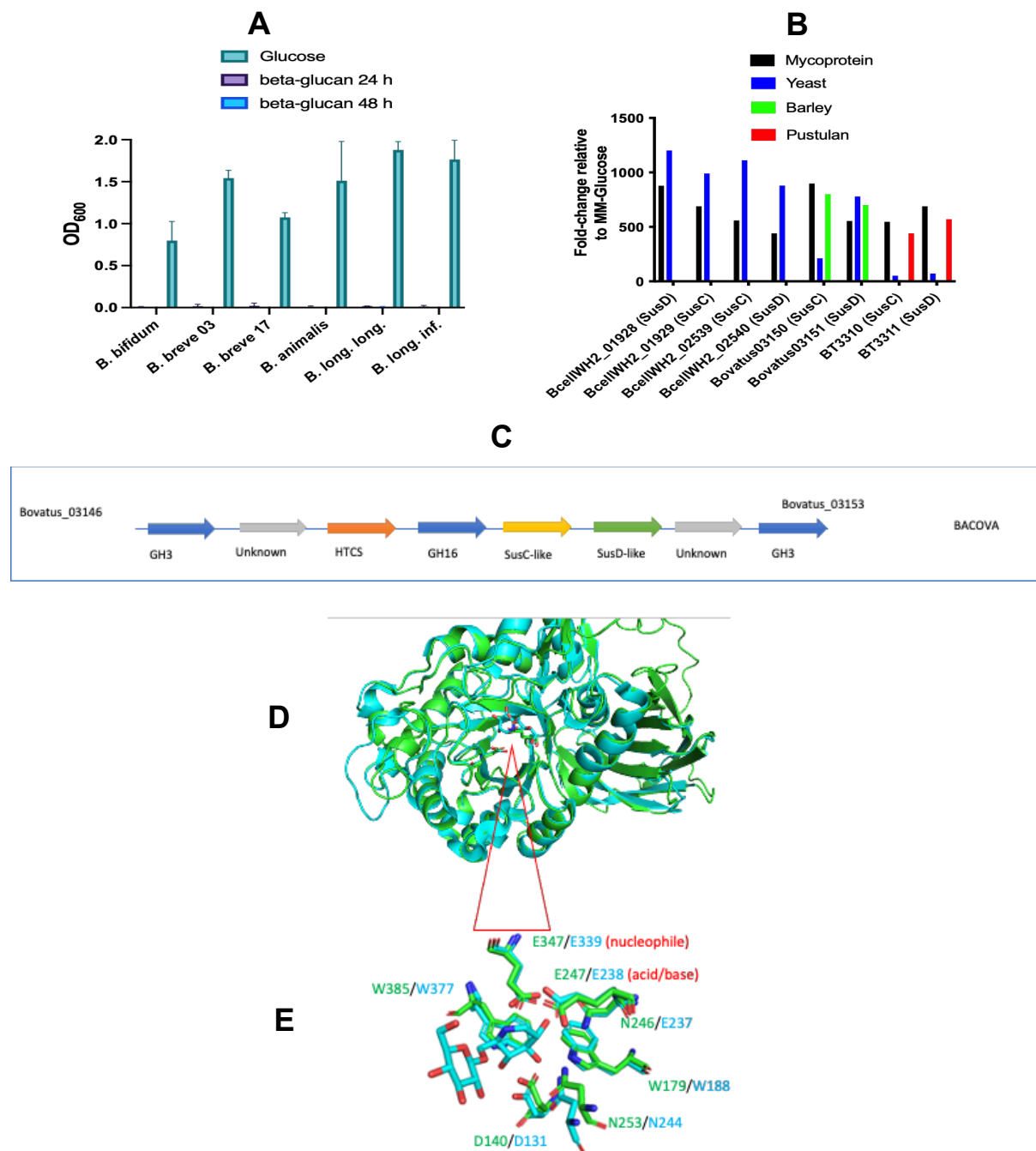


Fig. S1. Growth of *Bifidobacterium* sp. on intact polysaccharides and structural analysis of the different enzymes involved in different GULs. All growth and qPCR experiments have been produced in 3 different independent replicates (n=3). **A.** Ability of *Bifidobacterium* to use glucose, mycoprotein and yeast β -glucan as carbon source. **B.** qPCR analysis of SusC/D pairs in Baccell WH2, Bacova and BT when grown on mycoprotein, yeast, barley and pustulan β -

glucan as carbon source comparing with glucose as monosaccharide. We selected the relevant pairs according to proteomic and bioinformatics analysis, showing the upregulation of the SusC/D pairs in the GULs. **C.** GUL structure in *Bacova* acting on barley β -glucan. **D.** Structural alignment of GH30_3 active on β -glucan. BT3312 (Cyan) and BcellWH2_02537 (green). **E.** Conservation of key residues in the active site; BT3312 (Cyan) and BcellWH2_02537 (green).

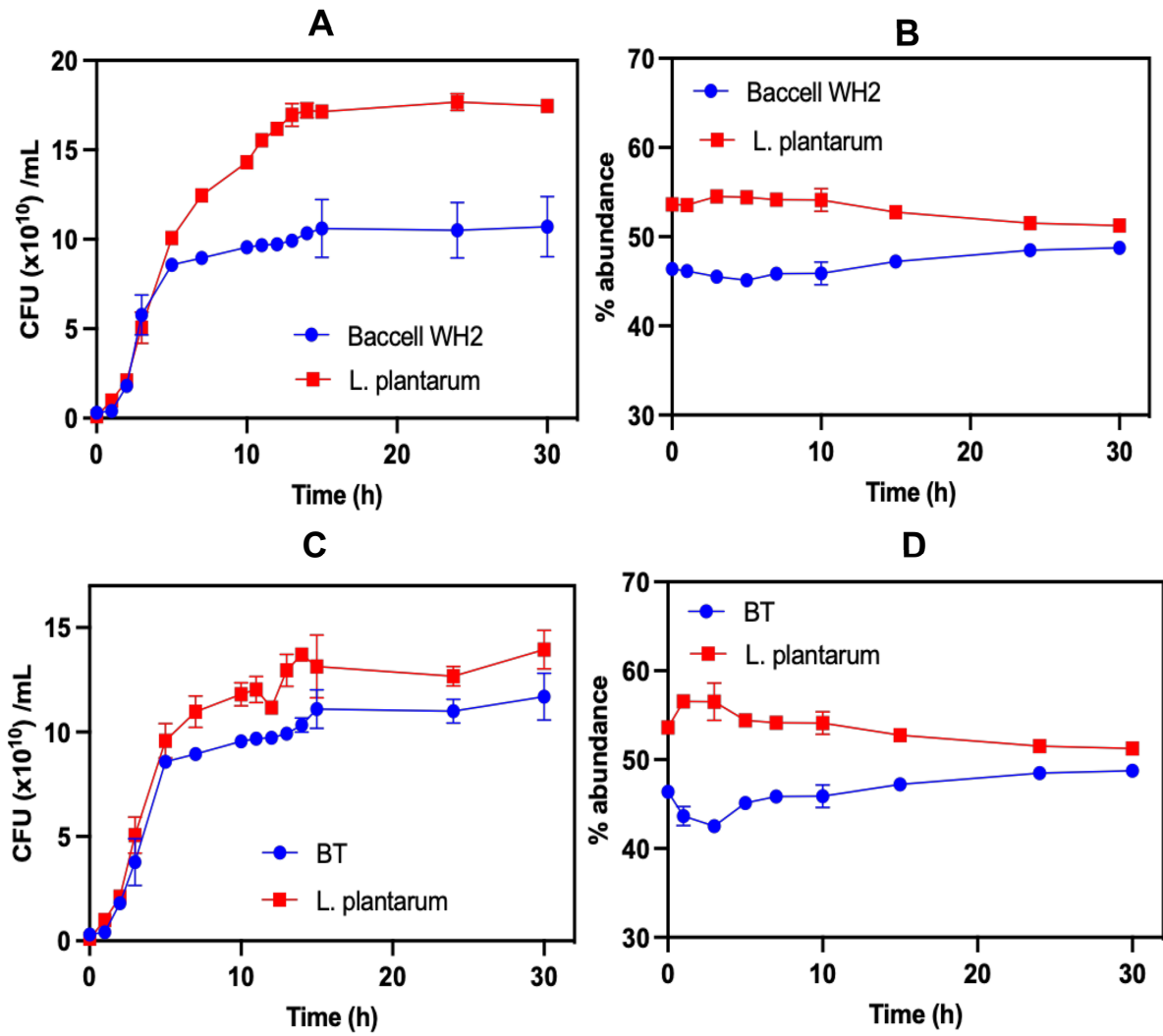


Fig. S2. Cross-feeding experiments between Baccell WH2 and BT and *Lactiplantibacillus spp.* **A.** Colony forming units of Baccell WH2 + *Lactiplantibacillus plantarum*. **B.** Percentage of Baccell WH2 + *Lactiplantibacillus plantarum*. **C.** Colony forming units of BT + *Lactiplantibacillus plantarum*. **D.** Percentage of BT + *Lactiplantibacillus plantarum*. All cross-feeding experiments have been produced in 2 different independent replicates (n=2).

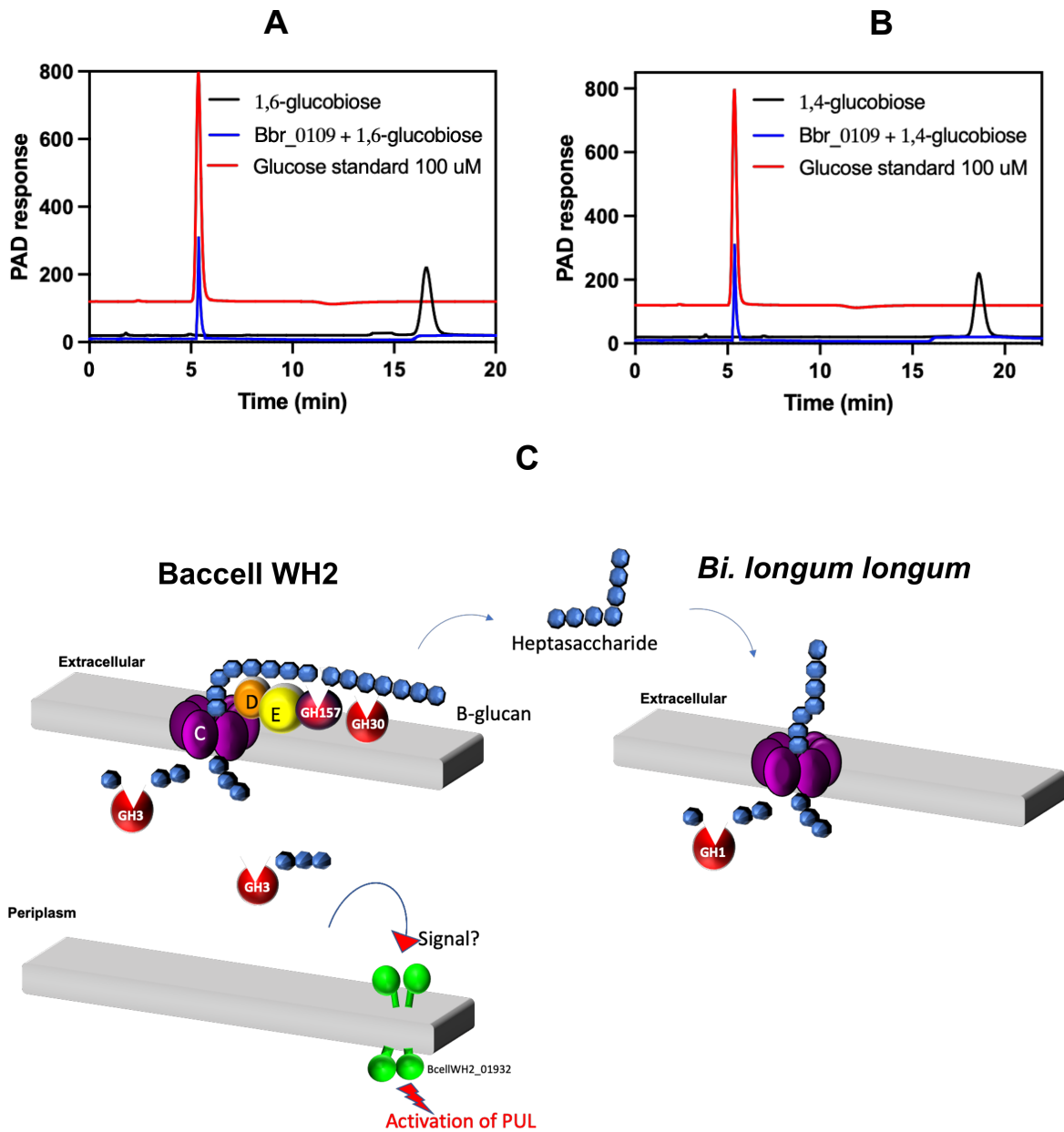
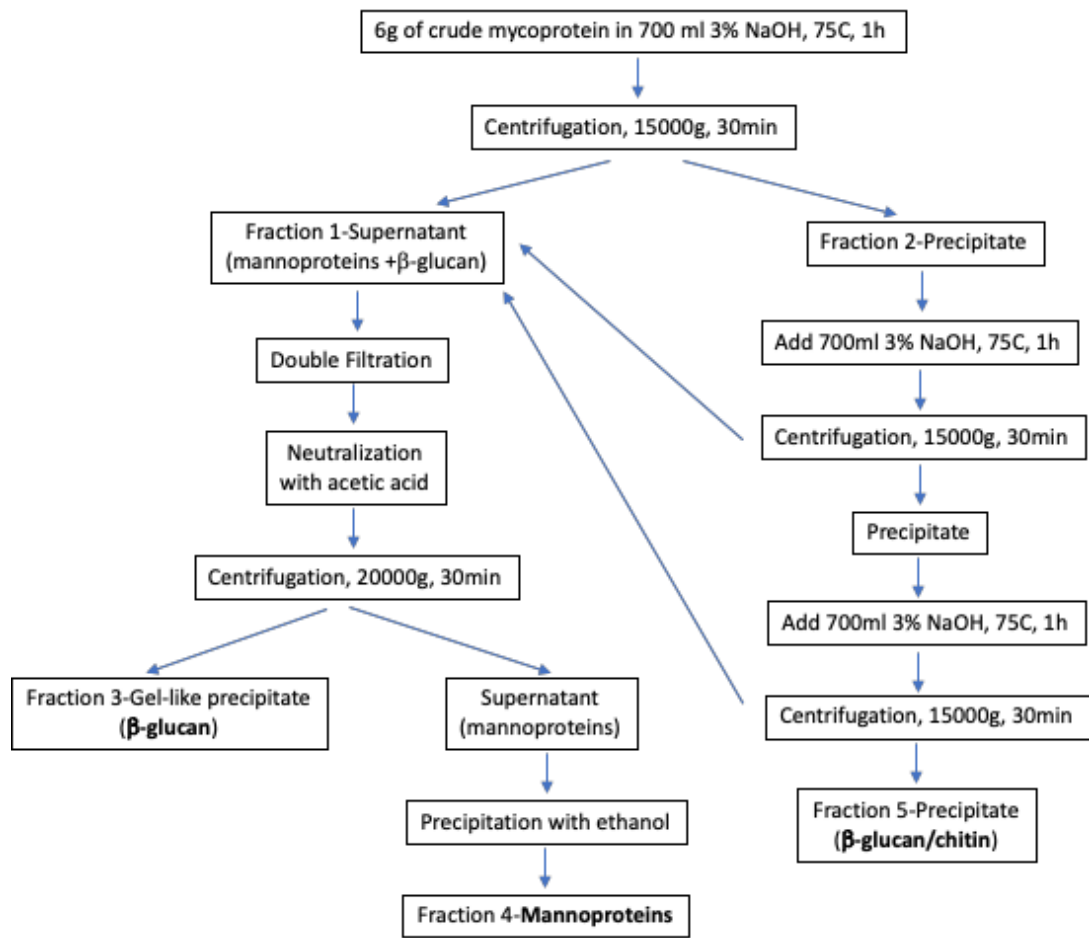


Fig. S3. Biochemical analysis of Bbr_0109 with different disaccharides and general model for the cross-feeding interactions. **A-B.** HPLC of the Bbr_0109 activity with 1,6-glucobiose (A) and 1,4-glucobiose (B), respectively. All HPLC experiments have been produced in 3 different independent replicates (n=3). **C.** General model for the degradation of fungal β -glucan by Baccell WH2 and cross-feeding with *Bifidobacterium*.

A



B

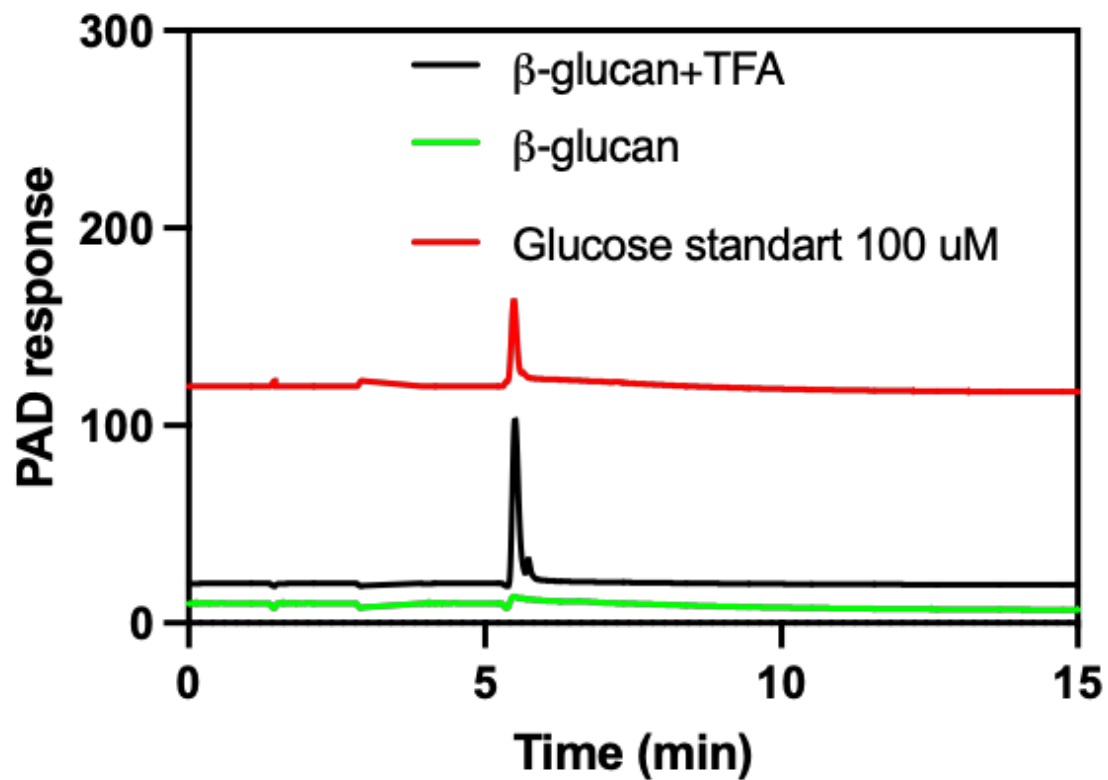


Fig. S4. Analysis of the β -glucan extraction from mycoprotein. **A.** Purification protocol for fungal β -glucan extraction. **B.** HPLC of extracted fungal β -glucan with acid hydrolysis (HCl 2M) to confirm the purity of the sample.

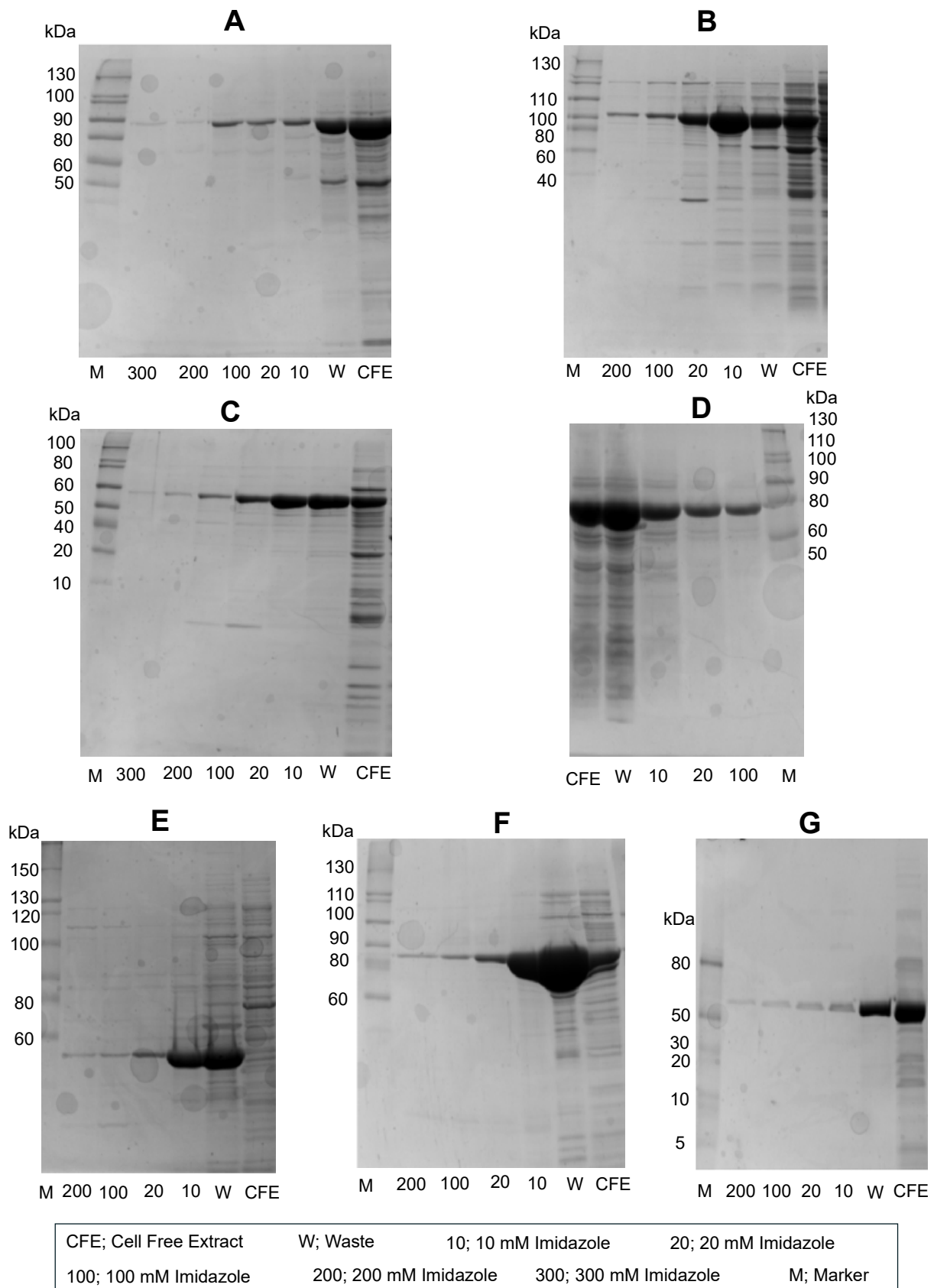


Fig. S5. SDS-gels of the recombinant proteins expressed in this study and the theoretical molecular weight for each of them. **A.** BcellWH2_01926 (GH3, Mw 85423); **B.**

BcellWH2_02541 (GH2, Mw 102478); **C.** BcellWH2_02537 (GH30_3, Mw 57669); **D.** BcellWH2_01931 (GH157, Mw 73658); **E.** BT3312 (GH30_3, Mw 55468); **F.** BT3314 (GH3, Mw 84558) and **G.** Bbr_0109 (GH1, Mw 52130).