Supplemental Data

Unravelling glucan recognition systems by glycome microarrays using the designer approach and mass spectrometry

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Supplemental Results

Linkage analysis of gluco-heptasaccharides with linear sequences and homo-linkages by negative-ion ESI-CID-MS/MS

For ESI-CID-MS/MS analysis of the linear longer chain gluco-oligosaccharides, heptasaccharides (supplemental Table S3) were used as representatives. In the spectrum of α1,2-linked Cyano-7 (Fig. 2*E*), the fragments observed were those with neutral losses of 18/78/120 Da arising from $[M-H]^-$ and the respective C-ions, identifiable for three consecutive residues up to C_5. These are the same fragmentation as those for the corresponding 1,2-linked disaccharide kojibiose (Fig. 2*A*), although the ^{0,4}A–h ions (*e.g.* m/z 1073, 911 and 749) were very weak. In the spectrum of the β1,3-linked Lam-7 (Fig. 2*F*) only a full set C-ions, C_6 to C_1, were observed, with neutral losses of 162 Da, defining the linear sequence. The absence of A-type ions indicated 1,3-linkages as in nigerose (Fig. 2*B*). For the α1,4-linked Malto-7, the spectrum (Fig. 2*G*) had features very similar to those of the 1,4-linked disaccharide maltobiose (Fig. 2*C*). The major ions were those with neutral losses of 60/78/120 Da from the $[M-H]^-$ and the C-ions. These fragments are identifiable for four consecutive residues. Further product-ion scanning, using C_4 (m/z 665) as the precursor, revealed the fragmentation in the lower mass region (supplemental Fig. 2*A*). Similarly, the α1,6-linked Dext-7 showed fragmentation features (Fig. 2*H*) identical to that of the α1,6-linked disaccharide isomaltose (Fig. 2*D*). The Dext-7 showed A-type fragmentation with neutral losses of 60/90/120 Da, apparent only for the first three consecutive residues. Further quasi-MS^3 using fragment ions C_4 (m/z 665) and C_3 (m/z 503) as precursors produced similar A-type fragment ions in the lower mass region, indicating 1,6-linkages (supplemental Fig. 2*B*).

Linkage analysis of a gluco-pentasaccharide isolated from the hydrolysate of barley β-glucan by negative-ion ESI-CID-MS/MS.

A pentasaccharide, Barley-5a, was isolated from barley β-glucan hydrolysate obtained by digestion with a novel cellulase with high transglycosylation activity. The sequence was predicted to be Glcβ1,3Glcβ1,4Glcβ1,4Glcβ1,4Glc based on the literature knowledge of barley β-glucan and the specificity of the enzyme used for cleavage. The MS/MS spectrum (Fig. 4*A*) obtained agreed with the reducing and non-reducing terminal 1,4- and 1,3-linkages, respectively. However, a 1,6-linkage with A-type ion set –60/90/120 was identified for an internal linkage (Fig. 4*A*), which is clearly different from the 1,4-linkage with A-type ion set –60/78/120. Therefore the sequence for this pentasaccharide was assigned as Glcβ1,3Glcβ1,4Glcβ1,6Glcβ1,4Glc, different from the predicted three consecutive 4-linkages at the reducing side. The 2D TOCSY NMR spectrum (supplemental Table S4) of this oligosaccharide is consistent with the above mass spectrometric assignment and the expected β-configuration was corroborated. Six differentiated spin systems were clearly identified and attributable to the glucose residues; one α- and one β-reducing residue, a non-reducing terminal β-residue, and three internal residues characteristic of 3-linked-Glcβ, 4-linked-Glcβ, and 6-linked-Glcβ. The chemical shifts for H6 of the 6-linked glucose (in bold, supplemental Table S4) showed particularly clear glycosylation shifts.

Mixture analysis of *Poria cocos* gluco-oligosaccharide fractions by ESI-CID-MS/MS and of derived NGLs by microarray

Poria cocos polysaccharide was selected as a source of α1,3-linear gluco-oligosaccharides(1). However, the ESI-CID-MS/MS product-ion spectrum of its heptasaccharide fraction Poria-7, obtained by gel filtration chromatography from the acid hydrolysate (BioGel P4 fraction), indicated the presence of 1,4-linked glucose as a minor (20-30 %) contaminant (Fig. 4*B*). This was revealed by the weak A-type ion set –60/78/120 (*e.g.* m/z 1091/1073/1031) unique for 4-linkage (Table 1*B*),

in addition to the major C-ions at m/z 989, 827, 665, 503, 341, consistent with dominant 1,3-linkage. These observations were in accord with our initial microarray binding data with NGLs of *Poria cocos* BioGel P4 oligosaccharide fractions with >DP-2 using *Tm*CBM41, which is known to recognize Glcα1,4Glc-linked sequences (supplemental Fig. S6A). ^{1}H- and ^{13}C-NMR corroborated the presence of α1,3-linked in addition to α1,4-linked sequences (supplemental Table S5). Although the ^{1}H-NMR spectrum at 700 MHz was crowded (not shown), the relative peak heights of resolved resonances, such as those from H5 of the 3-linked Glc and H3 of the 4-linked glucose, indicated that the fraction contained at least 20% of α1,4-linked component. Therefore, the Poria fractions DP3 to DP13 were purified by preparative HPTLC to remove the α1,4-linked contaminants (HPTLC fractions). HPTLC analysis of Poria-7, for example, showed that the contaminant was largely removed (supplemental Fig. 6*B*), and MALDI-MS analyses confirmed the heptasaccharide as the major component (supplemental Fig. 6*C*). The purified Poria-7 was analysed by ESI-MS/MS and the fragment ions from the 4-linked contaminant (m/z 1091/1073/1031) were largely removed (Fig. 4*B*). A series of NGL probes of Poria-DP3 to DP13 was generated (supplemental Table 6*A*) and microarray analysis corroborated purity as the binding signals by the 1,4-linkage-specific *Tm*CBM41 were largely disappeared, whereas strong binding signals were detected with the α1,3-linkage-specific MOPC104E (supplemental Fig. 6*A*).

Saturation Transfer Difference (STD)-NMR analyses of CBMs

STD NMR has been used to investigate the geometry and kinetics of protein-glycan complex formation(2). This technique involves NMR spectroscopy of a mixture of a protein with a ligand in solution. NMR spectra of the mixture are obtained with and without radiofrequency irradiation of the protein, and subtraction of one spectrum from the other. The difference spectrum contains only signals from parts of the ligand interacting with the protein, to which saturation has been transferred. It is therefore possible to use this method to determine binding epitopes on glycan ligands(3).

To complement the observations from microarray analyses the interaction of four β1,3-glucan-binding CBMs with β1,3-linked trisaccharide Lam-3 was analyzed in solution by STD-NMR. These were *Tm*CBM4-2, *Bh*CBM6, *Cm*CBM32 and *Cm*CBM6-2. The STD signals were detected with each of the four CBMs (supplemental Fig. 10*A*), indicating their ability to interact with Lam-3 under the solution phase NMR conditions. By comparing the STDs as percentages of the corresponding unperturbed signals, we were able to distinguish, at the atomic level, the modes of recognition by these CBMs in solution.

With *Tm*CBM4-2 (supplemental Fig. 10*B*), STDs were detected arising from all three glucose residues. The interactions with the H2 protons both of the reducing and the internal residue were particularly strong. These observation are in line with crystal structure evidence(4), in which the binding site is a groove that is long enough to accommodate a hexasaccharide. This can explain the lack of binding signals with Lam-2 and Lam-3 given by this CBM in the microarray analysis (Fig. 5*B*). Whereas, in solution, the trisaccharide tested here, Lam-3, may be completely included in the interior of the groove, neither the Lam-2 and Lam-3, gain adequate access to the binding groove when immobilised at their reducing end on the array surface.

With *Cm*CBM32-2, *Bh*CBM6 and *Cm*CBM6-2 (supplemental Fig. 10*C-E*), STDs were observed mainly from the non-reducing end residue of Lam-3, which implies that the non-reducing terminal makes the closest contacts with the three proteins, although with different contact protons. Literature data for *Cm*CBM32-2 are limited, but the data on *Bh*CBM-6 are in agreement with crystal structure, in which the binding site is described as a small, blocked-off groove(5). With *Cm*CBM6-2, STDs were observed strongly at the non-reducing residue and weakly at the internal and reducing residues. This CBM has a broad specificity and two glycan binding sites, one of which (cleft A) interacts with the NR residue, and the second of which (cleft B) accommodates at least a trimer(6); the STDs cannot distinguish between the two sites but the results might be

interpreted in terms of binding to both sites B and A; or possibly at site B alone. By way of validating the glucose linkage specificity in the STD experiments, we analyzed maltotriose (Glcα1,4Glcα1,4Glc) in the presence of *Tm*CBM4-2. In good agreement with the known specificity of the CBM and our observations in microarray analysis, no STD signals were observed (data not shown).

In sum, for the CBMs that bind more strongly to the non-reducing terminal of the trisaccharide ligand (*Cm*CBM32-2, *Bh*CBM6 and *Cm*CBM6-2) binding could be detected to oligosaccharides as short as DP-2; for *Tm*CBM4-2 which interacts with all the residues of the trisaccharide but most strongly with reducing and with internal residues, a chain length of DP-4 and longer was required on the array to detect binding.

Supplemental Discussion

Observations on Dectin-1 interactions with synthetic branched oligosaccharides by other methods using oligosaccharides in solution

Surface plasmon resonance (SPR) is another analysis system that has also been used in Dectin-1 studies with chemically synthesized oligosaccharides in solution as inhibitors of Dectin-1 binding to a glucan-phosphate-coated biosensor(7). We summarize the results in supplemental Table S8. Among the three branched analogs HE-8^{B5}, HE-9^{B6} and HE-10^{B7} and the linear HE-8, HE-9 and HE-10 (DP-8, DP-9 and DP-10, respectively) analyzed in the SPR inhibition system, the decasaccharide He-10^{B6}, with a DP-9 backbone and a single β1,6 branch on the third residue at the non-reducing end was observed to be most potent inhibitor (IC_{50} 0.029 mM). The branched HE-8^{B5}, with a DP-7 backbone (IC_{50} 0.13 mM) was recorded to be ten times more potent than the longer analog, HE-9^{B6}, with an DP-8 backbone (IC_{50} 1.3 mM).

In another Dectin-1 study using chemically synthesized linear and branched gluco-oligosaccharides, ELISA inhibition assays were performed(8). Here the heptadecasaccharide (Structure 1 below), with a DP-16 backbone and a single β1,6 branch on the third residue at the non-reducing end, as in He-10^{B6}, and a linear DP-16 (Structure 2), were observed to be equally active as inhibitors of Dectin-1 binding to immobilized glucan polysaccharide.

Structure 1

Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc
|
Glcβ-6

Structure 2

Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc

It is possible that the SPR inhibition system reveals effects of the β1,6 branch on oligosaccharides in solution with a particular backbone chain length. This phenomenon requires investigation. The HE-8^{B5} and HE-9^{B6} or a synthetic analog with a backbone chain longer than the nonassacharide that is required to detect binding in the microarrays are not currently available for conversion into NGLs for microarray analyses but will be the subject of future investigations.

Supplemental Methods

Proteins investigated. The proteins investigated and their reported oligosaccharide recognition with references are given in supplemental Table S2.

Lectins - Recombinant murine Dectin-1 extracellular carbohydrate recognition domain (CRD) fused to the Fc portion of human IgG at the *N*-terminus(9) was provided by Gordon Brown (University of Aberdeen, UK). Recombinant human Dectin-1 CRD with an *N*-terminal His_{10}-tag was purchased from R&D Systems (Minneapolis, MN); recombinant murine Dectin-1 CRD with an *N*-terminal His_6-tag was a gift from Sino Biologicals (Beijing, China); recombinant human DC-SIGN CRD with the human Fc fused at *C*-terminus(10) was provided by Yvette van Kooyk (VU University Medical Center, Amsterdam) and by Alessandra Cambi (Radboud University Medical Center, The Netherlands); Concanavalin A (ConA) (biotinylated) was purchased from Vector Laboratories.

Monoclonal antibodies - Mouse myeloma antibody MOPC 104E-IgM was purchased from Sigma; the monoclonal anti-dextran antibodies m.3.4.1G6-IgG3 and m16.4.12E-IgA were from the Kabat collection of carbohydrate antigens and antibodies (currently housed at SRI International) and were prepared as biotinylated antibodies as described(11); vaccine induced anti-β-glucan antibodies 2G8-IgG and 1E12-IgM were prepared as described(12); 1H8-IgG was similarly produced (unpublished).

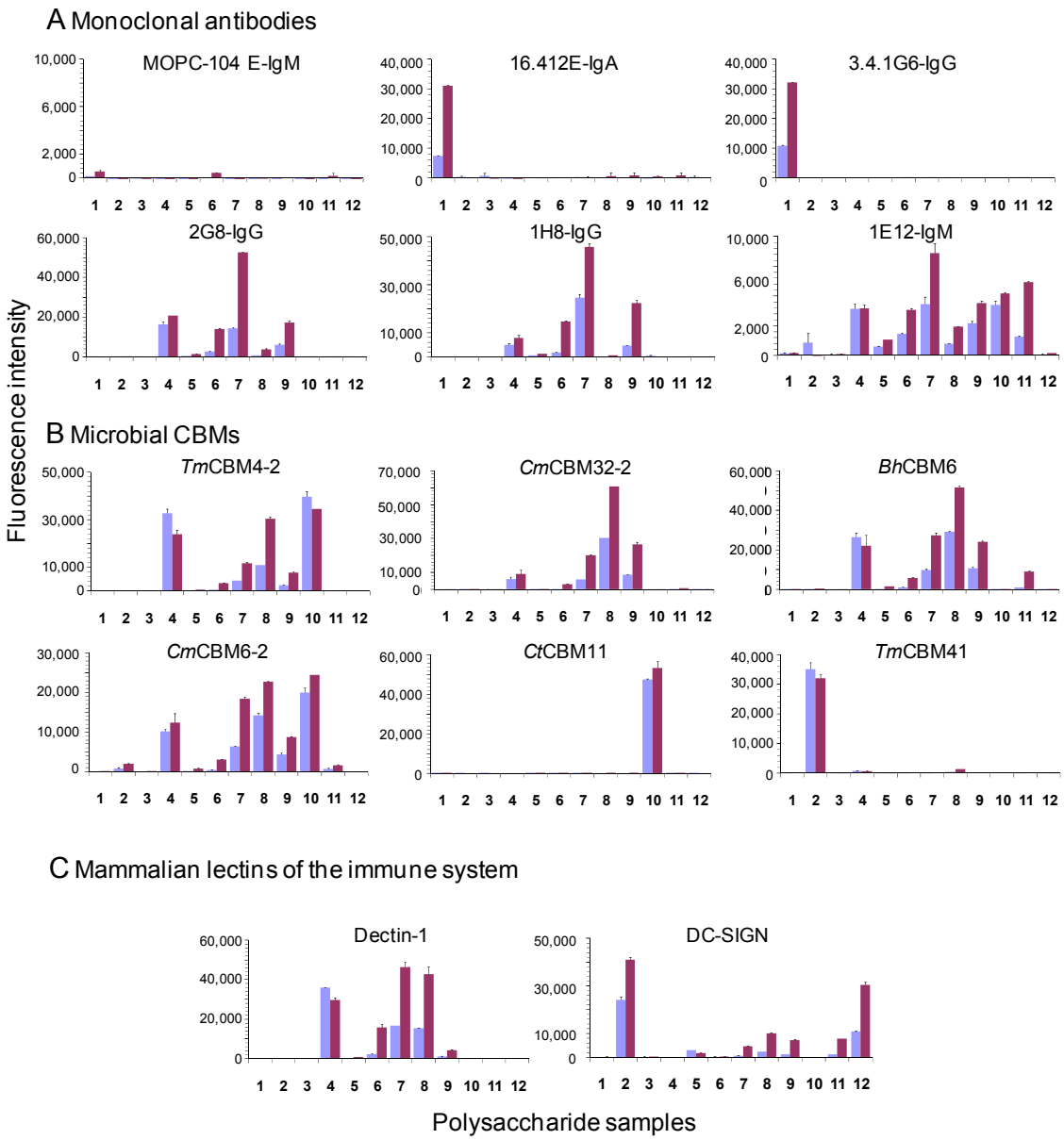
CBMs - The CBMs were produced as recombinant proteins in *Escherichia coli*. Family 6 CBM from *Bacillus halodurans* (*Bh*CBM6), family 4 and family 41 CBMs from the marine hyperthermophile *Thermotoga maritima* (*Tm*CBM4-2 and *Tm*CBM41, respectively), were provided by Alisdair Boraston (University of Victoria, Canada); family 6 and family 32 CBM from the aerobic soil bacterium *Cellvibrio mixtus* (*Cm*CBM6-2 and *Cm*CBM32-2, respectively) were provided by Harry Gilbert (University of Newcastle, UK). Family 11 CBM from *Clostridium thermocellum* (*Ct*CBM11) was prepared as described previously. The recombinant *Bh*CBM6, *Tm*CBM4-2, *Tm*CBM41 and *Cm*CBM6-2 contain *N*- terminal His_6-tags, and C*m*CBM32-2 and *Ct*CBM11 a *C*-terminal His_6-tag (references are in supplemental Table S2).

Oligogluco-fructosides and glucan polysaccharides. *Cyanobacterium* gluco-oligosaccharide fructosides(13) were provided by Eckhard Loos (Regensburg, Germany). The polysaccharides used in this study are listed in supplemental Table S1. A glucan polysaccharide containing α1,3-linked sequence was isolated from *Poria cocos* mycelia(1); Dextran polysaccharide was from *Leuconostoc mesenteroides* (Sigma, Dorset, England); the cyclic β-glucan (CβG) was isolated from *Brucella* spp. as described(14); and pustulan was from *Umbilicaria papullosa* (Calbiochem, Nottingham, UK). Two glucan polysaccharides containing branched β1,3- and β1,6-linkages, grifolan and lentinan, were isolated from the barmy mycelium of *Grifola frondosa*(15) and from *Lentinus edodes*(16), respectively, as described.

Other gluco-oligosaccharides The disaccharides kojibiose (α1,2), isomaltobiose (α1,6), cellobiose (β1,4) and gentiobiose (β1,6) were purchased from Sigma, sophorose (β1,2) from Dextra Laboratories (Reading, England) and nigerose (α1,3) from Wako Chemicals (Neuss, Germany). The α1,4-linked malto-di- to heptasaccharides (Malto-2 to -7) were from Sigma. The β1,3-linked laminaribiose, triose and tetraose (Lam-2 to 4) were from Dextra Laboratories, laminaripentaose and -hexaose (Lam-5 and -6) were from Megazyme, and the laminariheptaose (Lam-7) was from Seikagaku (AMS Biotechnology, Abingdon, England). Gluco-oligosaccharides with mixed linkages were from the following sources: panose (Pano-3) and the gluco-tetraose with the sequence of Glcα1,6Glcα1,4Glcα1,4Glc (Pullu-4) were from Sigma; isopanose (i-Pano-3) was provided by Takashi Tonozuka (Tokyo University of Agriculture and Technology). The following oligosaccharides were from Megazyme: barley glucotrioses (A) and (B), glucotetraoses (A), (B)

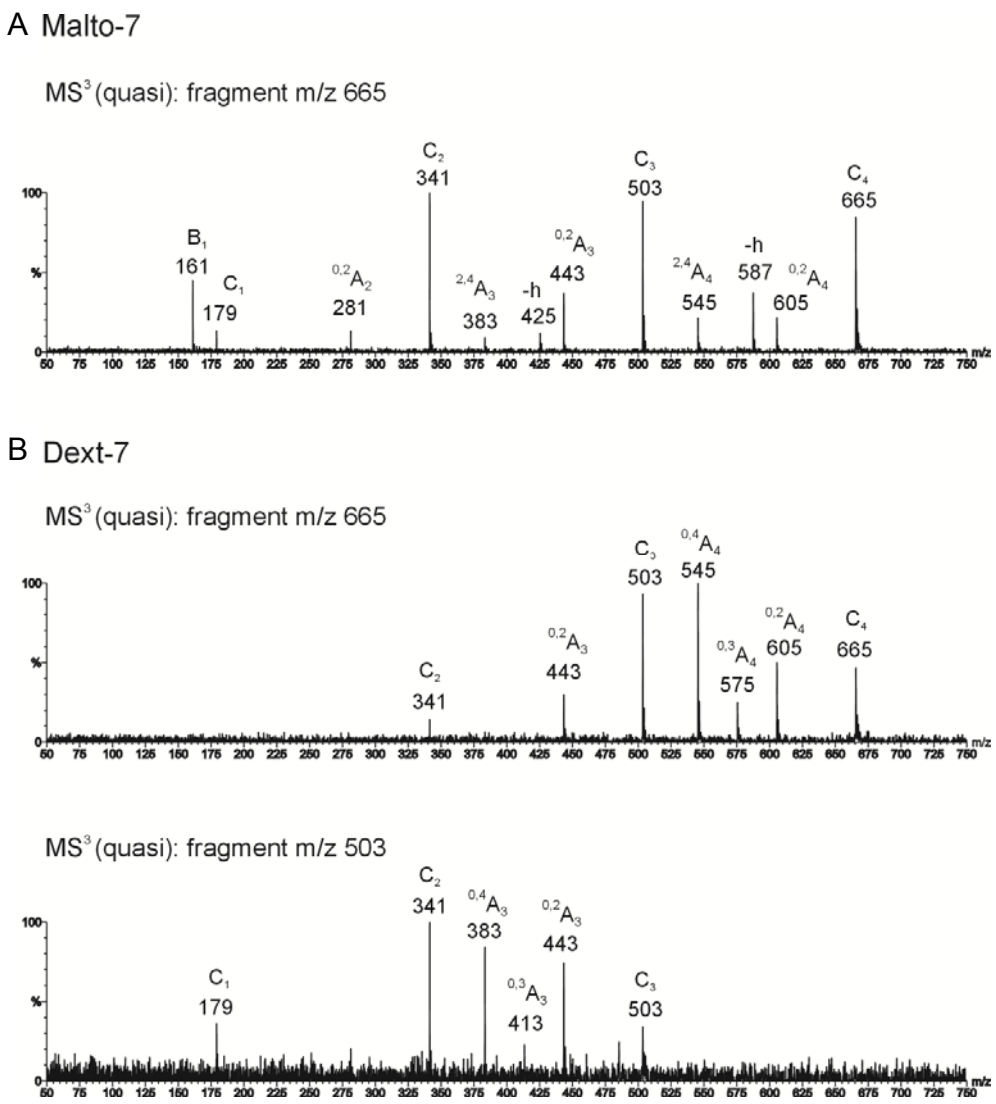
and (C) (Barley-3a and –b, Barley-4a, -b and -c, respectively), Barley penta- and hexasaccharides (Barley-5a and Barley-6a) and pullulan glucotetraose with the sequence of Glcα1,6Glcα1,4Glcα1,4Glc and heptaose with the sequence of Glcα1,6Glcα1,4Glcα1,4Glcα1,6Glcα1,4Glcα1,4Glc (Pullu-4 and –7, respectively). Linear β1,3-linked glucan octa-, nona, and decasaccharides HE-8, HE-9 and HE-10, and branched glucan nona-, deca- and undecasaccharides, HE-9^{B7} -10^{B2}, -10^{B3}, -10^{B5}, -10^{B7} and -$11^{B2,6}$ were synthesized chemically, as described(17). The chemically synthesized hexasaccharide(18), JG-$6^{B1,}$ was a generous gift from Jianxin Gu (Fudan University, Shanghai).

Fig. S1. Microarray analyses using microarrays of 12 soluble glucan polysaccharides to reveal their expression of ligands and antigens for the proteins investigated.



The water-soluble polysaccharide samples (ID 1-12) are listed in supplemental Table S1. (*A*) murine monoclonal antibodies to α- or β-glucans; (*B*) microbial CBMs; (*C*) receptors of the innate immune system: murine Dectin-1 (His-tagged) and DC-SIGN (human Fc chimera). The binding scores are depicted as fluorescence intensities elicited with 30 and 150 pg polysaccharide per spot (blue and purple bar, respectively). The polysaccharides cyclic-β-glucan (ID 3) and laminarin (ID 5) are probably not efficiently retained on the nitrocellulose surface to elicit binding after a binding event due to their low molecular weights. The α1,3-glucose-containing *Poria cocos* polysaccharide (ID 13) is not water-soluble, therefore not included in the microarrays. This explains the lack of binding signals with the α1,3-glucan-specific MOPC-104E-IgM.

Fig. S2. Quasi-MS^3 spectra of gluco-heptasaccharides with homo-linkages Malto-7 (α1,4-linked) and Dext-7 (α1,6-linked).



The fragment ions produced by cone voltage fragmentation were used as the precursors: (*A*) m/z 665 of Malto-7, (*B*) m/z 665 and m/z 503 of Dext-7.

Fig. S3. Negative-ion ES-CID-MS/MS product-ion spectrum of Pullu-7 with hetero-linkages.

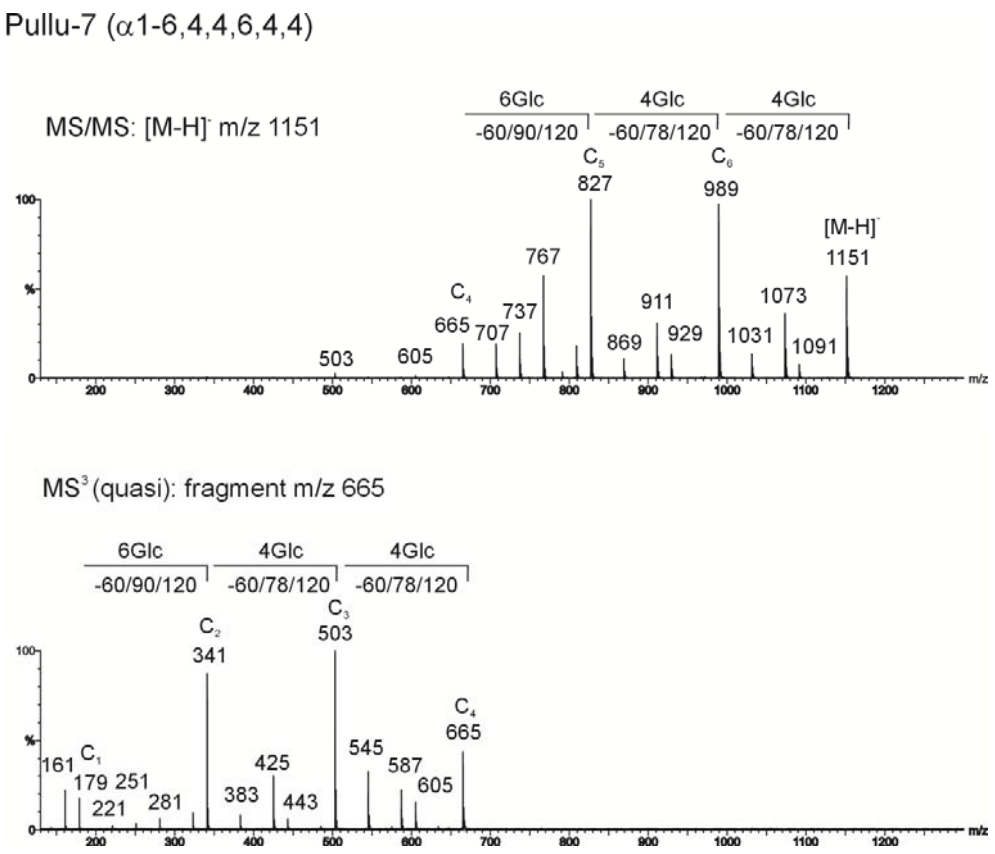


Fig. S4. Negative-ion ES-CID-MS/MS product-ion spectra of gluco-trisaccharides with mixed 1,4- and 1,6-linkages

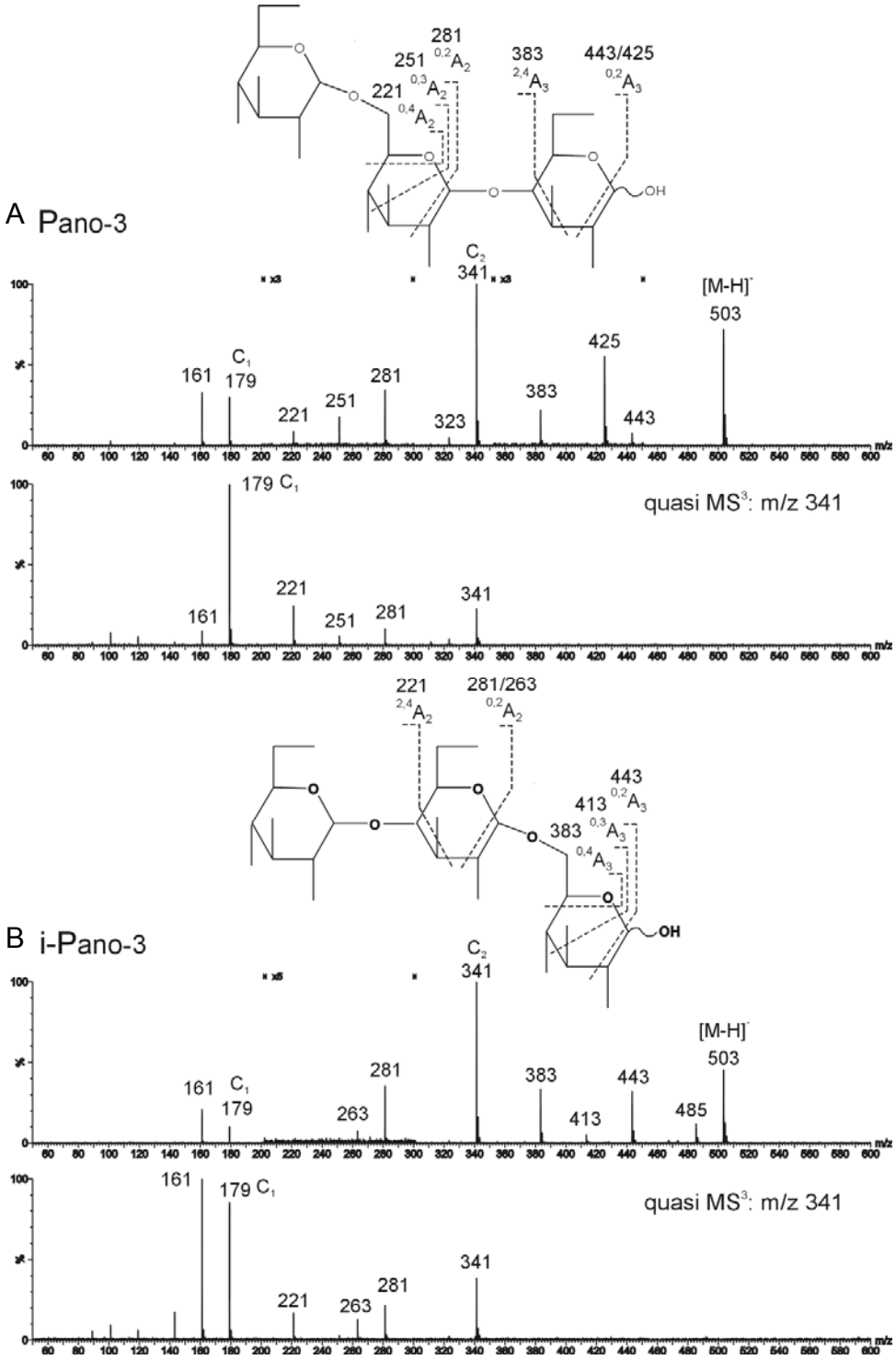
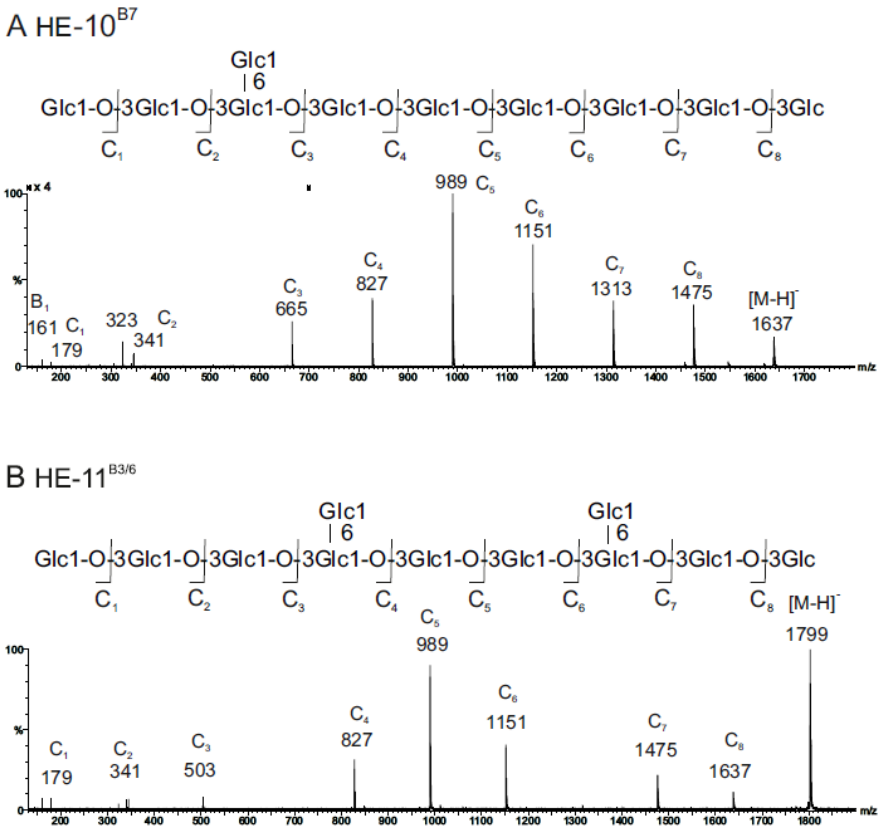
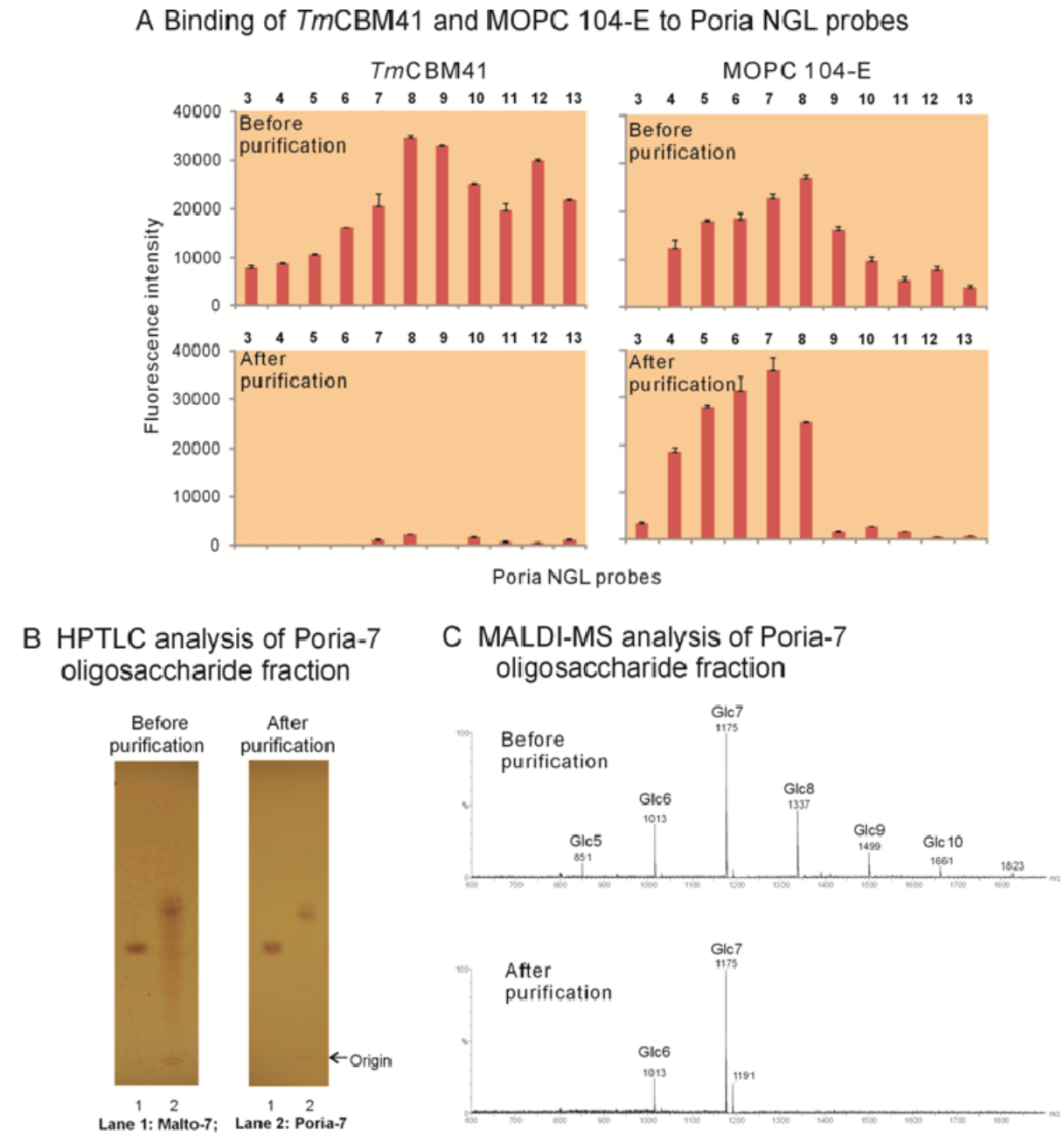


Fig. S5. Negative-ion ES-CID-MS/MS product-ion spectra of 1,3-linked oligosaccharides with 1,6 branching.



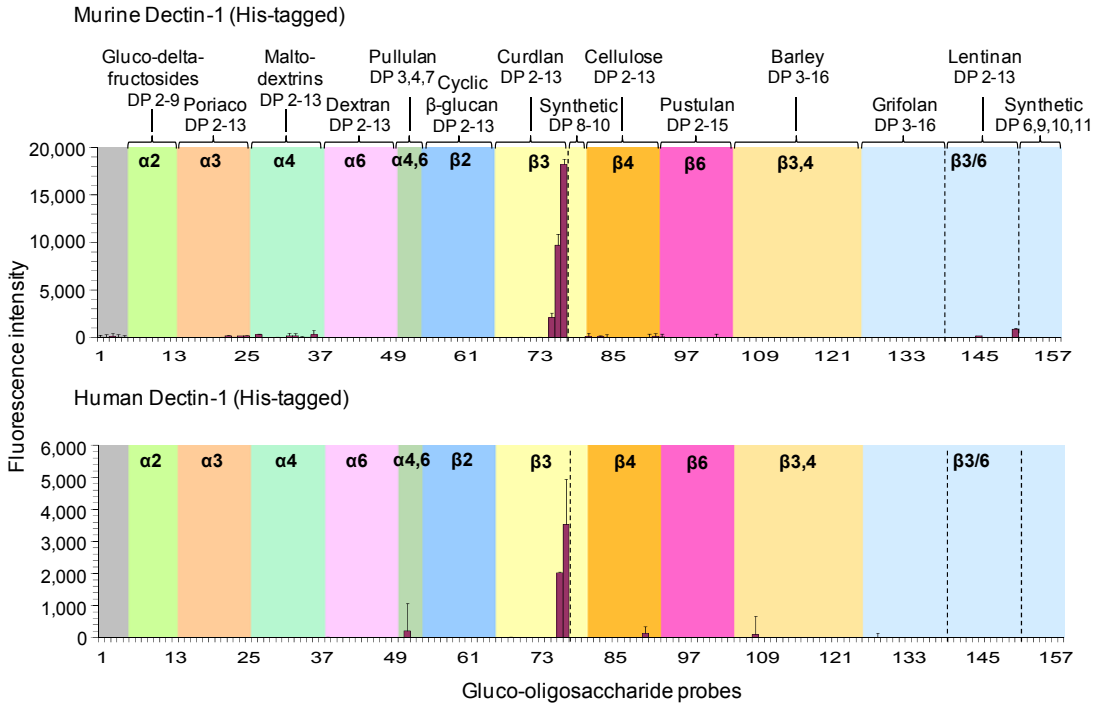
(*A*) Single branched gluco-decasaccharide HE-10^{B7}; (*B*) Double branched gluco-undecasaccharide HE-11^{B3,6}

Fig. S6. Analyses of the α1,3-gluco-oligosaccharide rich Poria fractions, before and after removal of contaminating α1,4-gluco-oligosaccharides.



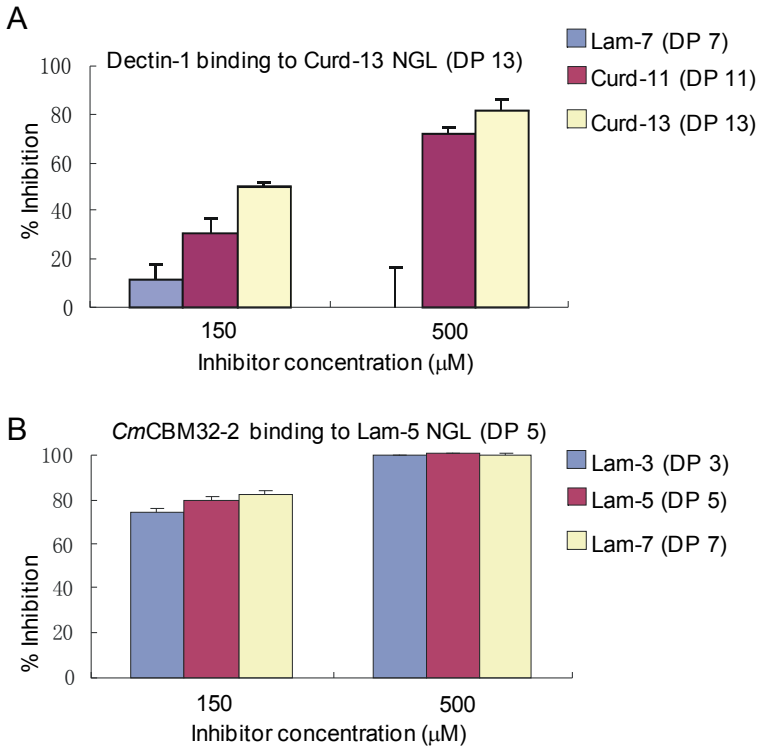
(*A*) Microarray analyses using NGLs derived from Poria oligosaccharide fractions 3-13 (Bio-Gel P4 and HPTLC fractions), tested for binding by an α1,4-glucose specific CBM, *Tm*CBM41, and a α1,3-glucose specific antibody, MOPC 104-E; (*B*) Analytical HPTLC analysis of the Poria-7 oligosaccharide fraction before and after removal of the α1,3-glucose contaminant; Malto-7 was included as a reference; (*C*) MALDI-MS of the Bio-Gel P4 and HPTLC fractions of Poria-7.

Fig. S7. Carbohydrate microarray analyses of His-tagged murine and human Dectin-1.



Both recombinant proteins showed highly restricted binding to the higher oligomers of linear β1,3-linked glucose generated from curdlan polysaccharide. Please see legend to **Fig. 5** for details.

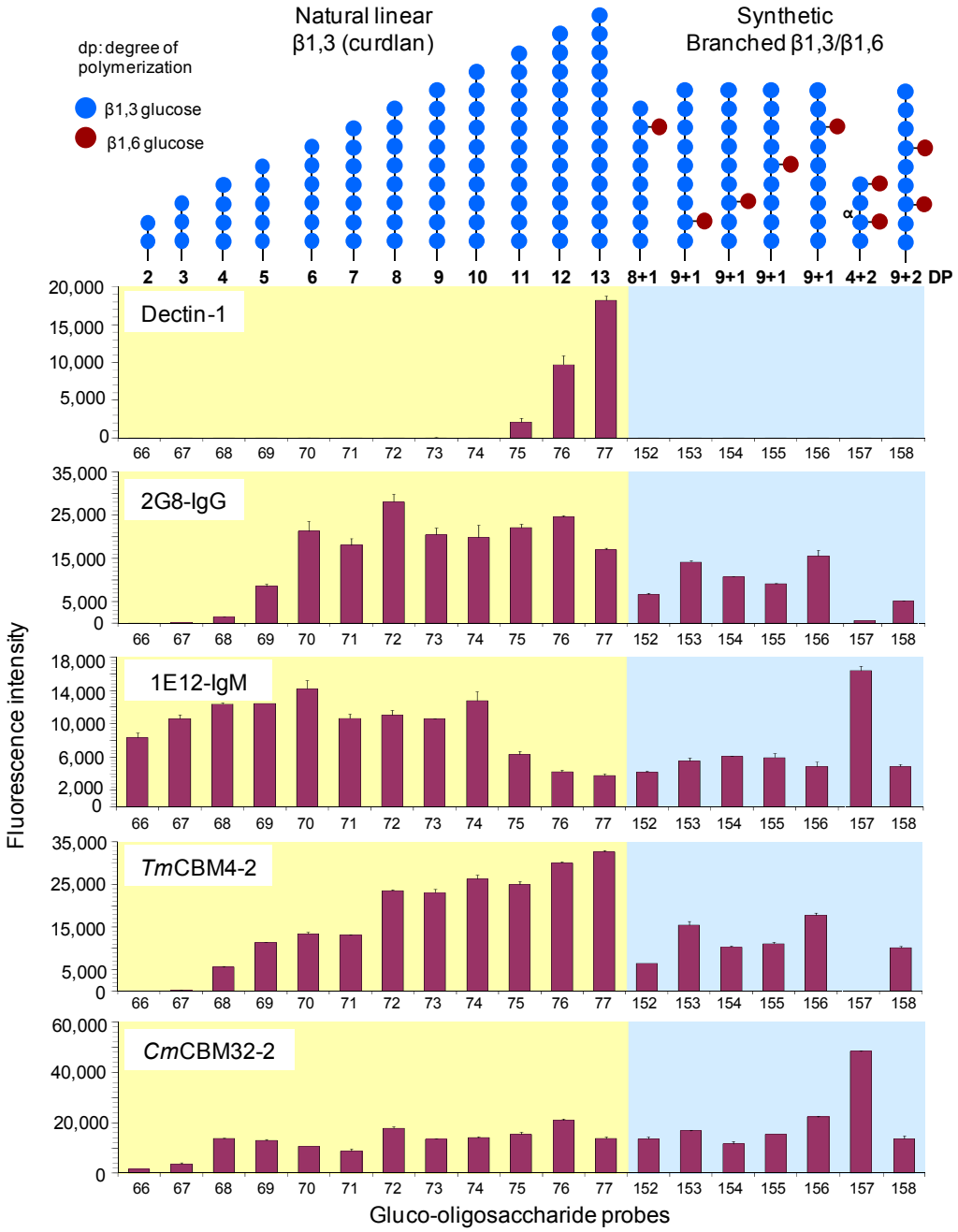
Fig. S8. 'On-array' inhibition of Dectin-1 and *Cm*CBM32-2 binding to immbobilized NGLs with β1,3-linked oligosaccharides



(*A*) Inhibition of the binding of murine Dectin-1 Fc chimera to immobilized β1,3-linked Curd-13 NGL by β1,3-linked oligosaccharides Curd-11 and Curd-13 but not Lam-7; Curd-13 NGL was arrayed at 5 fmol/spot. The 'on-array' inhibition results thus corroborate the chain length requirement for Dectin-1 recognition observed in the microarrary analysis as there was a higher inhibitory activity of the curdlan oligosaccharide fraction with DP-13 over that with DP-11; and a lack of inhibition by the oligosaccharide with DP-7. (*B*) Inhibition of the binding of *Cm*CBM32-2 to immobilized β1,3-linked Lam-5 NGL by β1,3-linked oligosaccharides Lam-3, Lam-5 and Lam-7; Lam-5 was arrayed at 5 fmol/spot. In contrast with Dectin-1, for *Cm*CBM32-2 the short β1,3 oligosaccharides showed high inhibitory activities of binding, thus corroborating the results of the microarray analysis.

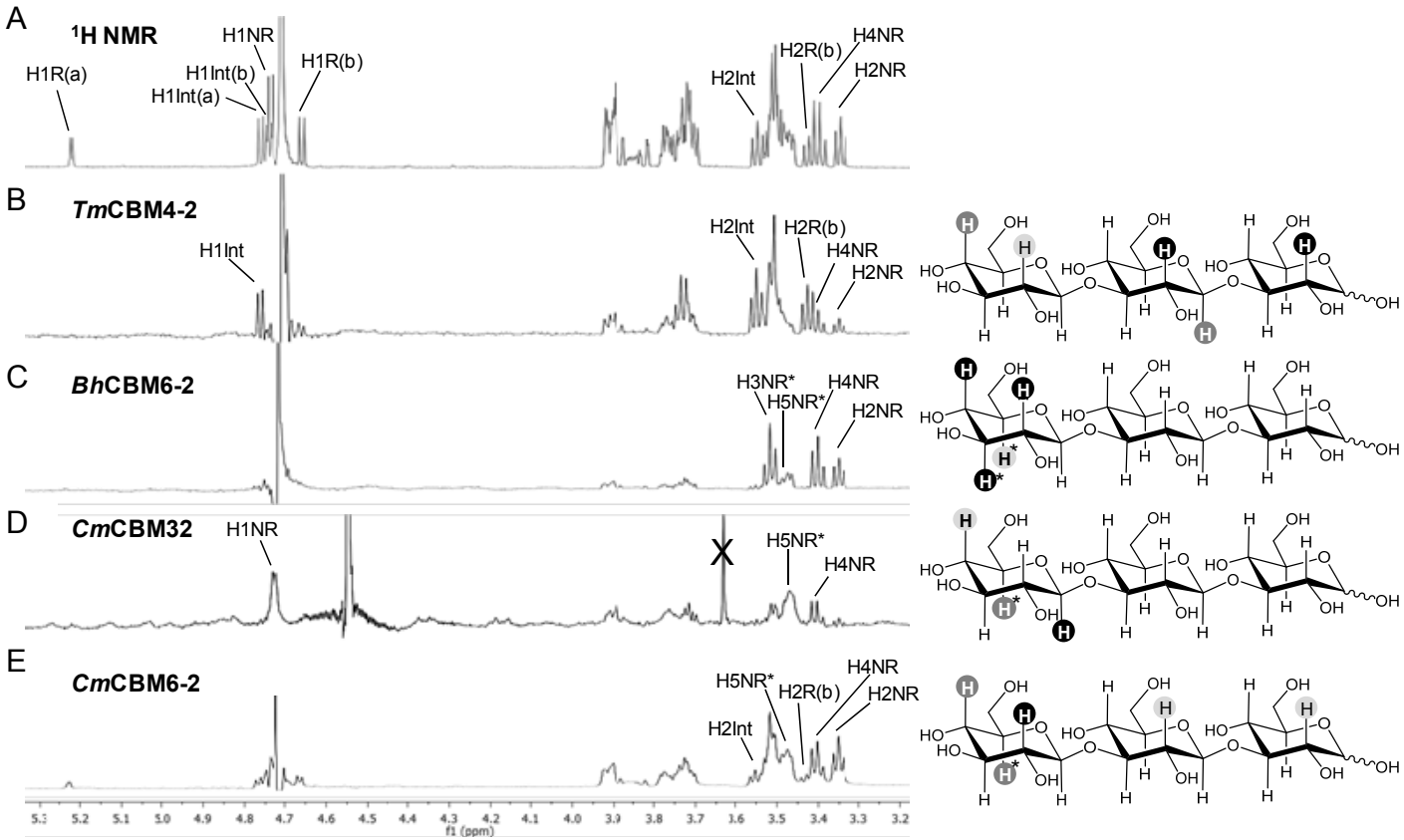
The murine Dectin-1-Fc and *Cm*CBM32-2 were used at non-saturating concentrations of 5μg/ml. The final concentrations of the oligosaccharides used as inhibitors of binding are indicated on the Y axis. The results are expressed as percentage of inhibition of binding as follows: percentage inhibition= [(spot fluorescence intensity no inhibitor - spot fluorescence intensity with inhibitor)/(spot fluorescence intensity no inhibitor- spot fluorescence intensity negative control)] × 100.

Fig. S9. Comparison of the binding of Dectin-1, 2G8-IgG, 1E12-IgM, *Tm*CBM4-2 and *Cm*CBM32-2 to natural linear β1,3-linked and synthetic branched β1,3/β1,6 oligosaccharides.



The results highlight different chain length-dependencies and the abilities of 2G8-IgG and 1E12-IgM antibodies, *Tm*CBM4-2 and *Cm*CBM32-2, but not Dectin-1, to bind branched β1,3/β1,6 oligosaccharides. The glucose linkages are symbolically indicated by diagrams in the top panel; for probe 157 the internal 1,3 linkage has an α-configuration. For probes 6, 8-13 glucose chain lengths of the major components are depicted.

Fig. S10: STD NMR analyses of CBMs with β1,3-linked trisaccharide Lam-3.



(*A*) Reference ^{1}H NMR and STD spectra of Lam-3 in the presence of (*B*) *Tm*CBM4-2, (*C*) *Bh*CBM6-2, (*D*) *Cm*CBM32 and (*E*) *Cm*CBM6-2. Binding epitopes implied by the STD results are shown on the right for each CBM. All the experiments were performed at 30 ºC except for *Cm*CBM32-2 which was measured at 45 ºC. The relative STD effects (highest STD signal normalized to 100%) are illustrated by dark, medium and light gray circles indicating strong (>80 %), medium (40–80%), and weak (<40%) STD effects, respectively. Overlapped STD signals that could not be clearly assigned are not shown on the structures. Asterisks indicate signals for which the STD is an approximate estimate due to partial overlap.

Table S1. Polysaccharides examined. Some of the glucan polysaccharides listed below were selected as sources of gluco-oligosaccharides after their partial depolymerization; reagents used for depolymerization are given. Those that were soluble, polysaccharides ID 1-12, were printed non-covalently onto nitrocellulose-coated slides and analyzed for binding with the proteins investigated as shown in supplemental Fig. S1.

ID	Polysaccharide	Predominant oligosaccharide sequence	Mean molecular mass[1]	Source	Reference	Reagents used for depolymerization
1	Dextran	α1,6-glucose	150 kDa	*Leuconostoc mesenteroides* (Sigma)	(19)	Acid (0.1 M HCl) hydrolysis
2	Pullulan	Mixed α1,4/α1,6-glucose	-	*P. pullulans* (Megazyme)	(20)	
3	Cyclic-β-glucan	β1,2-glucose	3-4 kDa	*Brucella* spp.	(14,21)	Acid (0.01 M HCl) hydrolysis
4	Curdlan[2]	β1,3-glucose	-	*Agrobacterium* sp. ATCC31749	(22)	Enzyme (*endo*-1,3-β-glucanase) digestion
5	Laminarin	Linear β1,3-glucose backbone with occasional monoglucosyl β1,6-glucose branches	7.7 kDa	*Laminaria digitata* (Sigma)	(23)	
6	Neutral soluble β-glucan (NSG)		10-20 kDa	*Saccharomyces cerevisiae* (Biothera)	(24)	
7	Poly-(1,6)-D-glucopyranosyl-(1,3)-D-glucopyranose (PGG)		120-205 kDa	*S. cerevisiae* (Biothera)	(25)	
8	Lentinan		580 kDa	*Lentinus edodes*	(16)	Acid (0.02M TFA) hydrolysis
9	Grifolan	β1,3-glucose backbone with highly ramified oligomeric β1,6-glucose branches	95 kDa	*Grifola frondosa*	(15)	Acid (0.02M TFA) hydrolysis
10	Barley glucan	Mixed β1,3/β1,4-glucose	190 kDa	Barley flour (Megazyme)	(26)	Enzyme (lichenase) digestion
11	Pustulan	β1,6-glucose	20 kDa	*Umbilicaria papullosa* (Calbiochem)	(27)	Acid (0.2 M HCl) hydrolysis
12	Mannan	α1,6-mannose	-	*S. cerevisiae* (Sigma)	(19)	
13	Poria cocos glucan	α1,3-glucose	80-570 kDa	*Poria cocos* mycelia	(1)	Acid (0.01 M TFA) hydrolysis

[1]Where known
[2]Curdlan polysaccharide was solubilized in an alkaline aqueous solution (50mM NaOH) prior printing.

Table S2. Glucan-recognizing proteins investigated and their reported oligosaccharide recognition.

Proteins	Oligosaccharides
Mammalian lectin receptors of immune system	
Dectin-1 Dendritic cell-associated C-type like lectin-1 (CLEC7A)	Linear β1,3 gluco-oligosaccharides, DP-10 and longer (microarray analysis)(28,29) Synthetic β1,3-linked linear gluco-octasaccharide with a β1,6-linked mono-glucosyl branch (SPR/inhibition of binding to glucanphosphate)(7) Synthetic β1,3-linked linear gluco-hexadecasaccharide with or without a β1,6-linked mono-glucosyl branch (ELISA/inhibition of binding to schizophyllan)(8)
DC-SIGN Dendritic cell-specific ICAM-3-grabbing non-integrin	*N*-linked high-mannose oligosaccharides and fuco-oligosaccharides (Lewis^a, Lewis^x, Lewis^y)(30,31) α1,4-, β1,4- and β1,3-linked gluco-disaccharides (inhibition of binding to gp120 and binding)(32) α1,4-linked gluco-disaccharide and tetrasaccharide (binding to neoglycoconjugates using DC-SIGN expressing cells)(33)
α-glucan antibodies	
MOPC myeloma 104-E-IgM	Linear α1,3 di- to penta gluco-oligosacharides (inhibition of dextran precipitation)(34)
16.412E-IgA Anti-dextran (isomaltosyl hexasaccharide- keyhole limpet hemocyanin) *'Cavity-type'*	Linear α1,6 gluco-oligosaccharides > DP-3 (inhibition of binding to dextran)(35,36) Linear α1,6 gluco-tri- and hexasaccharides (binding to protein-conjugates)(11)
3.4.1G6-IgG3 Anti-dextran (isomaltosyl tetrasaccharide-stearylamine) *'Groove-type'*	Linear α1,6 oligosaccharides (inhibition of binding to dextran); Chain length requirement (>DP-5)(37)
β-glucan antibodies	
2G8-IgG Vaccine-induced with *Candida albicans* *β-glucan* polysaccharide (Protective)	Linear β1,3 gluco-oligosaccharides >DP-4 (microarray analysis)(12,38)
1H8-IgG Vaccine-induced with *C. albicans* *β-glucan* polysaccharide (Potential protective)	Specificity investigated in the present report

1E12-IgM *Vaccine-induced with C. albicans β-glucan polysaccharide* CDR identical to that of 2G8-IgG (Non-protective)	Linear β1,3-, β1,4- and β1,6 gluco-oligosaccharides, DP-2 and longer (microarray analysis)(12)
Microbial CBMs	
***Tm*CBM4-2** *Thermotogoa maritime* laminarinase CAZY family 4	Linear β1,3 gluco-oligosaccharides, >DP- 2, peaks at DP-5 Binding site is a deep groove, requiring the internal oligosaccharide sequence (ITC and X-ray crystallography)(4,39)
***Cm*CBM32-2** *Cellvibrio mixtus* Cellulase CAZY family 32	Specificity investigated in the present report Polysaccharide data available: weak binding to laminarin (linear β1,3 polysaccharide with occasional β1,6 branches) and pustulan (linear β1,6 polysaccharide)(40)
***Bh*CBM6** *Bacillus halodurans* Laminarinase;CAZY family 6	Linear β1,3 gluco-oligosaccharides, >DP- 2, with high affinity at DP-5 and DP-6 Low affinity to β1,2- or β1,6-linked disaccharides Unique binding mode: binding site is small slot that accommodates the non-reducing end glucose residue, and the extended β1,3 oligosaccharide curves around the CBM surface, explaining the increased binding with longer DP-5 and DP-6 chains. (ITC and X-ray crystallography)(5)
***Cm*CBM6-2** *Cellvibrio mixtus* Endoglucanase 5A; CAZY family 6	2 independent glucan binding sites: cleft A and B **Cleft A**: Linear β1,4-gluco-oligosaccharides, DP- 2 and longer; Linear β1,4- xylose oligosaccharides (weak); Linear β1,3-gluco-hexasaccharide **Cleft B**: Linear β1,4-gluco-oligosaccharides, > DP-3; β1,3;β1,4-linked tetrasaccharides Glcβ1,4 Glcβ1,3Glcβ1,4Glc Glcβ1,3 Glcβ1,4Glcβ1,3Glc (ITC and X-ray crystallography) (6,41)
***Ct*CBM11** *Clostridium thermocellum* endoglucanase; CAZY family 11	Strong binding to β1,3;β1,4 gluco-tetrasaccharide Glcβ1,4Glcβ1,4Glcβ1,3Glc but not Glcβ1,3Glcβ1,4Glcβ1,3Glc Relatively weak binding affinity to linear β1,4 gluco-oligosaccharides (ITC and STD-NMR)(42-44)

***Tm*CBM41** *Thermotogoa maritime* Pullulanase; CAZY family 41	Linear α1,4 gluco-oligosaccharides, >DP-2; oligomers DP-5 to DP-7 are bound by 2 CBM molecules Enzymatically released pullulan tetra- and heptasaccharide Glcα1,6Glcα1,4Glcα1,4Glc Glcα1,6Glcα1,4Glcα1,4Glcα1,6Glcα1,4Glcα1,4Glc Non-reducing end Glcα1,6 and internal Glcα1,6-linkage are tolerated, Glcα1,4Glcα1,6Glc not tested (ITC and X-ray crystallography)(45,46)

Table S3. Gluco-oligosaccharides used for development of ESI-CID-MS/MS method.

Designations[1]	Sequences
Linear heptasaccharides with homo-linkages	
Cyano-7	Glcα1,2Glcα1,2Glcα1,2Glcα1,2Glcα1,2Glcα1,2Glc
CβG-7	Glcβ1,2Glcβ1,2Glcβ1,2Glcβ1,2Glcβ1,2Glcβ1,2Glc
Poria-7	Glcα1,3Glcα1,3Glcα1,3Glcα1,3Glcα1,3Glcα1,3Glc
Lam-7	Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glc
Malto-7	Glcα1,4Glcα1,4Glcα1,4Glcα1,4Glcα1,4Glcα1,4Glc
Cello-7	Glcβ1,4Glcβ1,4Glcβ1,4Glcβ1,4Glcβ1,4Glcβ1,4Glc
Dext-7	Glcα1,6Glcα1,6Glcα1,6Glcα1,6Glcα1,6Glcα1,6Glc
Pust-7	Glcβ1,6Glcβ1,6Glcβ1,6Glcβ1,6Glcβ1,6Glcβ1,6Glc
Linear oligosaccharides with hetero-linkages	
Pano-3	Glcα1,6Glcα1,4Glc
i-Pano-3	Glcα1,4Glcα1,6Glc
Pullu-4	Glcα1,6Glcα1,4Glcα1,4Glc
Pullu-7	Glcα1,6Glcα1,4Glcα1,4Glcα1,6Glcα1,4Glcα1,4Glc
Barley-4a	Glcβ1,3Glcβ1,4Glcβ1,4Glc
Barley-4b	Glcβ1,4Glcβ1,4Glcβ1,3Glc
Barley-4c	Glcβ1,4Glcβ1,3Glcβ1,4Glc
Branched decasaccharides	
HE10^{B2}	Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glc Glcβ1,6
HE-10^{B3}	Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glc Glcβ1,6
HE-10^{B5}	Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glc Glcβ1,6
HE-10^{B7}	Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glc Glcβ1,6
Double-branched oligosaccharides	
HE-11$^{B2/6}$	Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glcβ1,3Glc Glcβ1,6 Glcβ1,6

[1]Monosaccharide contents of oligosaccharides are given as Arab numerals.

Table S4. ^{1}H NMR assignments for Barley-5a (Glcβ1,3Glcβ1,4Glcβ1,6Glcβ1,4Glc) in D_2O at 30 ºC.

	Chemical shifts in ppm					
	Glcβ1	3Glcβ1	4Glcβ1	6Glcβ	4Glcα	4Glcβ
H1	4.73	4.53	4.53	4.51	5.21	4.65
H2	3.33	3.50	3.34	3.32	3.56	3.28
H3	3.51	3.75	3.61	3.49	3.82	3.59
H4	3.39	3.51	3.65	3.49	3.63	3.62
H5	3.47	3.51	3.58	3.65	3.85	3.58
H6	3.91	3.91	3.97	**4.20**	3.94	3.95
H6’	3.71	3.75	3.81	**3.88**	3.87	3.80

Table S5. ^{1}H and ^{13}C assignments for the Poriaco-7 fraction containing α1,3-linked Glc with a minor component of α1,4-linked glucan, in D_2O at 30 ºC.

	Chemical shifts in ppm			
	α1→3 linked		α1→4 linked	
	^{13}C	^{1}H	^{13}C	^{1}H
C/H1	102.1	5.36	102.4	5.38
C/H2	73.0	3.67	74.4	3.63
C/H3	83.0	3.89	76.1	3.96
C/H4	72.4	3.42	79.7	3.66
C/H5	74.4	4.03	74.0	3.83
C/H6	63.1	3.83	63.3	3.87
		3.77		3.71

Table S6A. MALDI-MS analysis of NGLs derived from gluco-oligosaccharides or fractions with homo-linkages.

Fraction	Glucose units[1]	Calculated mass[2]		Molecular ions Detected[3,4]							
		M+Na$^+$	[M-H]$^-$	Cyano- (α1,2)[5]	Poriaco- (α1,3)[6]	Malto- (α1,4)[6]	Dext- (α1,6)	CβG- (β1,2)	Curd- (β1,3)[6]	Cello- (β1,4)	Pust- (β1,6)
2	Glc2	1083.67	1059.67	1083.8 (Glc2)	1083.6 (Glc2)*	1059.8 (Glc2)*	1059.9 (Glc2)	1083.7 (Glc2)	1059.7 (Glc2)*	1083.9 (Glc2)	1083.9 (Glc2)
3	Glc3	1245.72	1221.72	1245.6 (Glc3)	1245.9 (Glc3)	1221.9 (Glc3)*	1221.4 (Glc3)	1245.9 (Glc3)	1221.6 (Glc3)*	1246.1 (Glc3)	1246.1 (Glc3)
4	Glc4	1407.77	1383.78	1047.7 (Glc4)	1408.1 (Glc4)	1383.6 (Glc4)*	1383.8 (Glc4)	1407.8 (Glc4)	1383.7 (Glc4)*	1408.2 (Glc4)	1408.1 (Glc4)
5	Glc5	1569.83	1545.83	1569.6 (Glc5)	1570.2 (Glc5)	1545.3 (Glc5)*	1545.7 (100; Glc5) 1383.7 (18; Glc4)	1569.6 (100; Glc5) 1731.7 (11; Glc6)	1545.6 (Glc5)*	1570.1 (100; Glc5) 1408.1 (12; Glc4)	1570.1 (Glc5)
6	Glc6	1731.88	1707.88	1731.7 (100;Glc6) 1569.7 (12;Glc5)	1732.1 (Glc6)	1707.4 (Glc6)*	1707.6 (100; Glc6) 1545.4 (25; Glc5) 1869.4 (25; Glc7)	1732.0 (100; Glc6) 1894.0 (25; Glc7) 1569.9 (23; Glc5)	1707.5 (100; Glc6)* 1869.5 (30; Glc7)	1732.1 (100; Glc6) 1570.1 (35; Glc5)	1732.1 (Glc6)
7	Glc7	1893.93	1869.93	1893.9 (100;Glc7) 1731.8 (12;Glc6)	1894.2 (Glc7)	1869.4 (Glc7)*	1870.0 (Glc7)	1894.1 (100; Glc7) 2056.1 (43; Glc8)	1894.2 (Glc7)*	1894.0 (100; Glc7) 1732.0 (32; Glc6)	1894.1 (Glc7)
8	Glc8	2055.99	2031.99	2055.9 (Glc8)	2055.9 (100; Glc8) 1893.8 (15;Glc7) 2217.9 (10;Glc9)	2031.6 (100; Glc8) 1869.6 (65; Glc7) 2193.8 (32; Glc9)	2031.7 (100; Glc8) 2194.6 (45;Glc9)	2055.9 (100; Glc8) 2217.9 (32;Glc9)	2031.9 (100; Glc8) 1869.9 (90; Glc7) 2194.0 (40; Glc9)	2056.0 (100; Glc8) 1894.0 (35;Glc7) 2217.9 (17;Glc9)	2055.9 (100; Glc8) 1893.8 (40;Glc7)
9	Glc9	2218.04	2194.04	2217.4 (Glc9)	2218.0 (100;Glc9) 2055.9 (20;Glc8) 1893.8 (15;Glc7)	2194.2 (100;Glc9) 2356.3 (47;Glc10) 2031.0 (12;Glc8)	2194.3 (100;Glc9) 2356.3 (37;Glc10) 2032.4 (18;Glc8)	2217.9 (100;Glc9) 2379.9 (29;Glc10)	2193.9 (100; Glc9) 2031.9 (85;Glc8) 2056.0 (30;Glc10)	2217.9 (100; Glc9) 2055.9 (48;Glc8) 2380.0 (17;Glc10)	2218.2 (100;Glc9) 2056.2 (60;Glc8) 2380.3 (20;Glc10)
10	Glc10	2380.09	2356.09	–	2380.1 (100;Glc10) 2218.1 (70;Glc9) 2055.9 (20 Glc8)	2356.0 (100; Glc10) 2518.2 (65;Glc11) 2194.0 (48;Glc9)	2356.3 (100; Glc10) 2518.3 (33;Glc11) 2194.3 (22;Glc9)	2380.0 (100; Glc10) 2542.0 (22;Glc11)	2355.9 (100; Glc10) 2193.9 (85;Glc9) 2517.9 (33;Glc11)	2380.1 (100; Glc10) 2543.0 (78;Glc11) 2218.1 (42;Glc9)	2380.2 (100; Glc10) 2218.2 (85;Glc9) 2542.3 (25;Glc11) 2704.5 (15;Glc12)
11	Glc11	2542.14	2518.15	–	2542.8 (100; Glc11) 2704.4 (50;Glc12) 2866.2 (45;Glc13) 3028.1 (30;Glc14) 2379.7 (13;Glc10)	2518.0 (100;Glc11) 2680.1 (40;Glc12) 2355.8 (30;Glc10)	2518.4 (100; Glc11) 2680.3 (35;Glc12) 2356.5 (25;Glc10)	2541.7 (100; Glc11) 2704.6 (27; Glc12) 2380.7 (13;Glc10)	2517.8 (100; Glc11) 2355.7 (48; Glc10) 2679.9 (28; Glc12)	2542.1 (100; Glc11) 2380.1 (55;Glc10) 2218.1 (20;Glc9) 2704.1 (12;Glc12)	2542.3 (100; Glc11) 2380.2 (85;Glc10) 2651.3 (80;Glc12)
12	Glc12/15[7]	2704.20 3190.36 3166.37	2680.20	–	2704.5 (100;Glc12) 3028.2 (90;Glc14) 2866.4 (53;Glc13) 2542.5 (40;Glc11) 3189.3 (30;Glc15)	2680.1 (100;Glc12) 2518.0 (70;Glc11) 2842.3 (48;Glc13)	2680.5 (100; Glc12) 2518.5 (35; Glc11) 2842.5 (28; Glc13)	2704.7 (100;Glc12) 2866.5 (62; Glc13) 2542.8 (16;Glc11)	2679.6 (100; Glc12) 2517.6 (30;Glc11) 2841.6 (15;Glc13)	2704.2 (100; Glc12) 2542.2 (52;Glc11) 2866.2 (30;Glc13) 2380.5 (20;Glc10)	3191.1 (100; Glc15) 3029.0 (95;Glc14) 2866.1 (60;Glc13)
13	Glc13/15[7]	2866.25 3190.36	2842.25 3166.37	–	2866.1 (100;Glc13) 3189.7 (80;Glc15) 3352.6 (50;Glc16) 3028.6 (33;Glc14) 2703.2 (45;Glc12)	2842.3 (100; Glc13) 2680.3 (47;Glc12) 3004.4 (45;Glc14)	2842.6 (100; Glc13) 3004.6 (28;Glc14)	2866.3 (100; Glc13) 3028.3 (88;Glc14) 2704.4 (15; Glc12)	2842.4 (100;Glc13) 2680.4 (78;Glc12) 3004.4 (25;Glc14)	2866.3 (100; Glc13) 2704.3 (68;Glc12) 2542.3 (28;Glc11) 3028.3 (25;Glc14)	3190.8 (100; Glc15) 3352.7 (80;Glc16) 3028.7 (98;Glc14)

[1]Glucose units or degree of polymerization (DP) for the major components in each fraction;[2]Calculated masses for major components are given;[3]Positive-ion MALDI-MS was used for the analysis of AO-NGLS of cyano-, poriaco-, CβG-, cello- and pustulan-series and MNa$^+$ were detected, whereas negative-ion MALDI-MS was used for the analysis of AO-NGLS of malto-, dextran- and curdlan-series and [M-H]$^-$ were detected;[4]Shown in brackets are assigned glucose units; and where multiple components were detected, relative intensities of ions greater 10% are given;[5]Nomenclature of the oligosacchide moieties correspond to the polysaccharide origins;[6]Disaccharides and malto- and curdlan oligosaccharides from commercial sources are asterisked; [7]For the β1,6-linked pustulan series the major components of these fractions are oligomers with DP-15.

Table S6B. MALDI-MS analysis of NGLs derived from gluco-oligosaccharides or fractions with hetero-linkages.

Fractions	Glucose units[1]	Calculated[2] mass MNa^+	Molecular ions Detected[3,4]		
			Barley-(β1-3,1-4)	Grifo-(β1-3/1-6)	Lenti -(β1-3/1-6)
2	Glc2	1083.67	–	–	1083.8 (Glc2)
3	Glc3	1245.72	1241.6 (Glc3)	1245.9 (100; Glc3) 1408.0 (12)	1245.8 (Glc3)
4	Glc4	1407.77	1408.1 (Glc4)	1408.0 (100; Glc4) 1246.0 (18; Glc3) 1570.0 (10; Glc5)	1407.7 (100; Glc4) 1569.7 (12; Glc5)
5	Glc5	1569.83	1569.9 (100; Glc5) 1731.9 (38; Glc6) 1407.9 (12; Glc4)	1570.2 (100; Glc5) 1408.1 (15; Glc4) 1732.2 (11; Glc6)	1570.0 (100; Glc5)
6	Glc6	1731.88	1731.7 (100; Glc6) 1569.7 (19; Glc5)	1703.8 (100; Glc6) 1569.6 (23; Glc5) 1893.0 (12; Glc7)	1732.0 (100; Glc6) 1569.9 (20; Glc5)
7	Glc7	1893.93	1893.6 (100; Glc7) 1731.8 (32; Glc6)	1894.2 (100; Glc7) 1732.2 (22; Glc6) 2056.2 (20; Glc8)	1893.9 (100; Glc7) 1731.9 (32; Glc6)
8	Glc8	2055.99	2055.6 (100; Glc8) 1893.6 (32; Glc7) 2217.6 (23; Glc9)	2056.2 (100; Glc8) 2219.2 (19; Glc9) 1894.2 (17; Glc7)	2055.8 (100; Glc8) 1893.9 (27; Glc7)
9	Glc9	2218.04	2218.3 (100; Glc9) 2055.4 (12; Glc8)	2218.1 (100; Glc9) 2056.1 (32; Glc8) 2380.0 (20; Glc10)	2217.8 (100; Glc9) 2379.7 (85; Glc10) 2055.9 (30; Glc8) 2541.7 (13; Glc11)
10	Glc10	2380.09	2380.7 (100; Glc10) 2218.8 (45; Glc9)	2380.1 (100; Glc10) 2218.2 (33; Glc9) 2542.1 (25; Glc11)	2379.9 (100; Glc10) 2217.8 (75; Glc9) 2056.0 (32; Glc8) 2541.9 (15; Glc11)
11	Glc11	2542.14	2542.6 (100; Glc11) 2704.6 (48; Glc12) 2380.6 (47; Glc10)	2542.0 (100; Glc11) 2380.0 (45; Glc10) 2703.9 (19; Glc12)	2541.8 (100; Glc11) 2379.9 (45; Glc10) 2704.5 (32; Glc12) 2218.8 (12; Glc9)
12	Glc12	2704.20	2705.1 (100; Glc12) 2542.3 (45; Glc11) 2866.9 (28; Glc13)	2703.9 (100; Glc12) 2543.0 (77; Glc11) 2865.7 (52; Glc13)	2703.7 (100; Glc12) 2866.5 (48; Glc13) 2541.8 (45; Glc11) 2379.6 (14; Glc10)
13	Glc13	2866.25	2867.2 (100; Glc13) 2704.2 (45; Glc12) 3028.8 (18; Glc14)	2866.0 (100; Glc13) 2703.9 (62; Glc12) 3027.8 (30; Glc14)	2866.1 (100; Glc13) 3028.0 (80; Glc14) 2704.6 (28; Glc12) 3189.7 (12; Glc15)
14	Glc14	3028.30	3027.9 (100; Glc14) 2866.1 (53; Glc13) 3190.7 (38; Glc15)	3028.3 (100; Glc14) 3190.2 (41; Glc15) 2866.4 (39; Glc13)	–
15	Glc15	3190.36	3189.8 (100; Glc15) 3027.8 (75; Glc14) 3352.7 (40; Glc16) 2866.1 (25; Glc13)	3190.4 (100; Glc15) 3352.9 (50; Glc16) 3028.3 (35; Glc14)	–
16	Glc16	3352.41	3352.8 (100; Glc16) 3190.0 (58; Glc15) 3512.7 (37; Glc17)	3352.0 (100; Glc16) 3514.3 (54; Glc17) 3190.9 (48; Glc15)	–

[1]Glucose units or degree of polymerization (DP) for the major components in each fraction.
[2]Calculated masses for major components are given.
[3]Positive-ion MALDI-MS was used for the analysis and MNa^+ was detected.
[4]Shown in brackets are assigned glucose units; and where multiple components were detected, relative intensities of ions greater 10% are given.

Table S6C. MALDI-MS analysis of NGLs prepared from additional commercial and chemically synthesized gluco-oligosaccharides.

Oligosaccharide designations	Glucose units	Calculated mass $M+Na^+$	Detected Ions[1,2]
Pano-3	Glc3	1245.72	1245.9 (Glc3)
i-Pano-3	Glc3	1245.72	1245.5 (Glc3)
Pullu-4	Glc4	1407.77	1407.9 (Glc4)
Pullu-7	Glc7	1893.93	1893.9 (Glc7)
Barley-3a	Glc3	1245.72	1245.6 (Glc3)
Barley-3b	Glc3	1245.72	1245.7 (Glc3)
Barley-4a	Glc4	1407.77	1407.8 (Glc4)
Barley-4b	Glc4	1407.77	1407.6 (Glc4)
Barley-4c	Glc4	1407.77	1407.8 (Glc4)
Barley-5a	Glc5	1569.83	1570.0 (Glc5)
Barley-6a	Glc6	1731.88	1731.8 (Glc6)
HE-8	Glc8	2055.99	2055.7 (Glc8)
HE-9	Glc9	2218.04	2218.0 (Glc9)
HE-10	Glc10	2380.09	2380.1 (Glc10)
$HE\text{-}9^{B7}$	Glc9	2218.04	2217.9 (Glc9)
$HE\text{-}10^{B2}$	Glc10	2380.09	2380.0 (Glc10)
$HE\text{-}10^{B3}$	Glc10	2380.09	2379.9 (Glc10)
$HE\text{-}10^{B5}$	Glc10	2380.09	2379.7 (Glc10)
$HE\text{-}10^{B7}$	Glc10	2380.09	2380.3 (Glc10)
$HE\text{-}11^{B3/6}$	Glc11	2542.14	2542.0 (Glc11)
$JG\text{-}6^{B1/3}$	Glc6	1731.88	1731.7 (Glc6)

[1]Positive-ion MALDI-MS was used for the analysis and MNa^+ were detected.
[2]Shown in brackets are the assigned glucose compositions.

Table S7A. Oligosaccharide NGL probes included in the gluco-oligosaccharide microarrays, sorted by linkage type and degree of polymerization.

ID[1]	Linkage and sources	Probe designation[3]	Probe Sequence[4,5]
1	Linear Xyl or Manβ4[2] Controls	Xyl5(ß4)	Xylß-4Xylß-4Xylß-4Xylß-4Xyl-DH
2		Xyl6(ß4)	Xylß-4Xylß-4Xylß-4Xylß-4Xylß-4Xyl-DH
3		Man4(ß4)	Manß-4Manß-4Manß-4Man-DH
4		Man5(ß4)	Manß-4Manß-4Manß-4Manß-4Man-DH
5		Man6(ß4)	Manß-4Manß-4Manß-4Manß-4Manß-4Man-DH
6	Linear Glcα2 Cyanobacterium gluco-fructosides	Cyano-2	Glcα-2Glc-AO
7		Cyano-3	Glcα-2Glcα-2Glc-AO
8		Cyano-4	Glcα1-2Glcα1-2Glcα1-2Glc-AO
9		Cyano-5	Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glc-AO
10		Cyano-6	Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glc-AO*
11		Cyano-7	Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glc-AO*
12		Cyano-8	Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glc-AO
13		Cyano-9	Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glcα1-2Glc-AO
14	Linear Glcα3 Poriaco	Nigerose	Glcα-3Glc-AO
15		Poria-3	Glcα-3Glcα-3Glc-AO
16		Poria-4	Glcα1-3Glcα1-3Glcα1-3Glc-AO
17		Poria-5	Glcα-3Glcα-3Glcα-3Glcα-3Glc-AO
18		Poria-6	Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glc-AO
19		Poria-7	Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glc-AO
20		Poria-8	Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glc-AO*
21		Poria-9	Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glc-AO*
22		Poria-10	Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glα-3Glc-AO*
23		Poria-11	Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glα-3Glcα-3Glc-AO*
24		Poria-12	Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glα-3Glcα-3Glcα-3Glc-AO*
25		Poria-13	Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glcα-3Glα-3Glcα-3Glcα-3Glcα-3Glc-AO*
26		Malto-2	Glcα-4Glc-AO
27		Malto-3	Glcα-4Glcα-4Glc-AO
28		Malto-4	Glcα-4Glcα-4Glcα-4Glc-AO
29		Malto-5	Glcα-4Glcα-4Glcα-4Glcα-4Glc-AO

30	Linear Glcα4 Maltodextrins	Malto-6	Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glc-AO
31		Malto-7	Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glc-AO
32		Malto-8	Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glc-AO*
33		Malto-9	Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glc-AO*
34		Malto-10	Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glc-AO*
35		Malto-11	Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glc-AO*
36		Malto-12	Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glc-AO*
37		Malto-13	Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glcα-4Glc-AO*
38	Linear Glcα6 Dextran	Dext-2	Glcα-6Glc-AO
39		Dext-3	Glcα-6Glcα-6Glc-AO
40		Dext-4	Glcα-6Glcα-6Glcα-6Glc-AO
41		Dext-5	Glcα-6Glcα-6Glcα-6Glcα-6Glc-AO*
42		Dext-6	Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glc-AO*
43		Dext-7	Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glc-AO
44		Dext-8	Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glc-AO*
45		Dext-9	Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glc-AO*
46		Dext-10	Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glc-AO*
47		Dext-11	Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glc-AO*
48		Dext-12	Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glc-AO*
49		Dext-13	Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glcα-6Glc-AO*
50	Mixed linked Glcα4,6 Pullulan	Pano-3	Glcα-6Glcα-4Glc-AO
51		i-Pano-3	Glcα-4Glcα-6Glc-AO
52		Pullu-4	Glcα-6Glcα-4Glcα-4Glc-AO
53		Pullu-7	Glcα-6Glcα-4Glcα-4Glcα-6Glcα-4Glcα-4Glc-AO
54	Linear Glcβ2 Cyclic β-glucan	CβG-2	Glcβ1-2Glc-AO
55		CβG-3	Glcβ1-2Glcβ1-2Glc-AO
56		CβG-4	Glcβ1-2Glcβ1-2Glcβ1-2Glc-AO
57		CβG-5	Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glc-AO*
58		CβG-6	Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glc-AO*
59		CβG-7	Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glc-AO*
60		CβG-8	Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glc-AO*
61		CβG-9	Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glc-AO*
62		CβG-10	Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glc-AO*

63		CβG-11	Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glc-AO*
64		CβG-12	Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glc-AO*
65		CβG-13	Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glcβ1-2Glc-AO*
66	Linear Glcβ3 Curdlan	Lam-2	Glcß-3Glc-AO
67		Lam-3	Glcß-3Glcß-3Glc-AO
68		Lam-4	Glcß-3Glcß-3Glcß-3Glc-AO
69		Lam-5	Glcß-3Glcß-3Glcß-3Glcß-3Glc-AO
70		Lam-6	Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glc-AO*
71		Lam-7	Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glc-AO
72		Curd-8	Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glc-AO*
73		Curd-9	Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glc-AO*
74		Curd-10	Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glc-AO*
75		Curd-11	Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glc-AO*
76		Curd-12	Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glc-AO*
77		Curd-13	Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glc-AO*
78	Linear Glcβ3 Synthetic	HE-8	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc-AO
79		HE-9	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc-AO
80		HE-10	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ1-3Glc-AO
81	Linear Glcβ4 Cellulose	Cellobiose	Glcß-4Glc-AO
82		Cello-3	Glcß-4Glcß-4Glc-AO
83		Cello-4	Glcß-4Glcß-4Glcß-4Glc-AO
84		Cello-5	Glcß-4Glcß-4Glcß-4Glcß-4Glc-AO*
85		Cello-6	Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glc-AO*
86		Cello-7	Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glc-AO*
87		Cello-8	Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glc-AO*
88		Cello-9	Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glc-AO*
89		Cello-10	Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glc-AO*
90		Cello-11	Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glc-AO*
91		Cello-12	Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glc-AO*
92		Cello-13	Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glcß-4Glc-AO*
93		Gentiobiose	Glcß-6Glc-AO
94		Pust-3	Glcß-6Glcß-6Glc-AO
95		Pust-4	Glcß-6Glcß-6Glcß-6Glc-AO

96	Linear Glcβ6 Pustulan	Pust-5	Glcß-6Glcß-6Glcß-6Glcß-6Glc-AO
97		Pust-6	Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glc-AO
98		Pust-7	Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glc-AO
99		Pust-8	Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glc-AO*
100		Pust-9	Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glc-AO*
101		Pust-10	Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glc-AO*
102		Pust-11	Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glc-AO*
103		Pust-15	Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glc-AO*
104		Pust-15a	Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glcß-6Glc-AO*
105	Mixed-linked Glcβ3;4 Barley	Barley-3	Glcß-4Glcß-3Glc-AO
106		Barley-3a	Glcβ-3Glcβ-4Glc-AO
107		Barley-3b	Glcβ-4Glcβ-3Glc-AO
108		Barley-4	Glcß-4Glcß-4Glcß-3Glc-AO
109		Barley-4a	Glcβ-3Glcβ-4Glcβ-4Glc-AO
110		Barley-4b	Glcβ-4Glcβ-4Glcβ-3Glc-AO
111		Barley-4c	Glcβ-4Glcβ-3Glcβ-4Glc-AO
112		Barley-5	Glcß-4Glcß-4Glcß-4Glcß-3Glc-AO*
113		Barley-5a	Glcβ-3Glcβ-4Glcβ-6Glcβ-4Glc-AO
114		Barley-6	$(Glcß\text{-}4/3Glcß)_2$-4Glcß-3Glc-AO*
115		Barley-6a	Glcβ-3Glcβ-4Glcβ-6Glcβ-4Glcβ-3Glc-AO; and Glcβ-3Glcβ-4Glcβ-4Glcβ-4Glcβ-3Glc-AO (ratio ~1:1)
116		Barley-7	Glcß-4Glcß-4Glcß-3Glcß-4Glcß-4Glcß-3Glc-AO*
117		Barley-8	$(Glcß\text{-}4/3Glcß)_3$-4Glcß-3Glc-AO*
118		Barley-9	$(Glcß\text{-}4/3Glcß)_4$-3Glc-AO*
119		Barley-10	$(Glcß\text{-}4/3Glcß)_4$-4Glcß-3Glc-AO*
120		Barley-11	$(Glcß\text{-}4/3Glcß)_5$-3Glc-AO*
121		Barley-12	$(Glcß\text{-}4/3Glcß)_5$-4Glcß-3Glc-AO*
122		Barley-13	$(Glcß\text{-}4/3Glcß)_6$-3Glc-AO*
123		Barley-14	$(Glcß\text{-}4/3Glcß)_6$-4Glcß-3Glc-AO*
124		Barley-15	$(Glcß\text{-}4/3Glcß)_7$-3Glc-AO*
125		Barley-16	$(Glcß\text{-}4/3Glcß)_7$-4Glcß-3Glc-AO*
126		Grifo-3	
127		Grifo-4	
128		Grifo-5	

129	Branched Glcβ3/6 Grifolan	Grifo-6	$Glc(ß\text{-}3/ß\text{-}6)_{3\text{-}16}\text{-}AO$*
130		Grifo-7	
131		Grifo-8	
132		Grifo-9	
133		Grifo-10	
134		Grifo-11	
135		Grifo-12	
136		Grifo-13	
137		Grifo-14	
138		Grifo-15	
139		Grifo-16	
140	Branched Glcβ3/6 Lentinan	Lenti-2	$Glc(ß\text{-}3/ß\text{-}6)_{2\text{-}13}\text{-}AO$*
141		Lenti-3	
142		Lenti-4	
143		Lenti-5	
144		Lenti-6	
145		Lenti-7	
146		Lenti-8	
147		Lenti-9	
148		Lenti-10	
149		Lenti-11	
150		Lenti-12	
151		Lenti-13	
152	Branched Glcβ3/6 Synthetic	HE-9B7	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc-AO Glcβ-6
153		HE-10B2	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc-AO Glcβ-6
154		HE-10B3	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc-AO Glcβ-6
155		HE-10B5	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc-AO Glcβ-6
156		HE-10B7	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc-AO Glcβ-6

157		Gu-6B1/3	Glcβ-3Glcα-3Glcβ-3Glc-AO | | Glcβ-6 Glcβ-6
158		HE-11B3/6	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ1-3Glc-AO | | Glcβ-6 Glcβ-6

[1]ID, Probe position in the microarray matching the position in the binding-charts.
[2]Xyl, xylose; Man, mannose;
[3]Abbreviations for oligosaccharide moieties: Cyano-, from cyanobacterium gluco-fructosides; Poria-, from α1,3-linked glucan polysaccharide isolated from *Poria cocos* mycelia; Malto-, from maltodextrins; Dext-, from dextran (MW 200 kDa); Pullu-, from Pullulan; CβG-, from cyclic β1,2-linked glucan isolated from *Brucella* spp; Lam-, laminarioligosaccharides; Curd-, from Curdlan; Cello-, from cellulose; Pust-, from pustulan; Barley-, from barley glucan; Grifo-, from branched glucan polysaccharide grifolan (95 kDa) isolated from the barmy mycelium of *Grifola frondosa*; Lenti- from branched glucan polysaccharide lentinan from *Lentinus edodes*; HE and Gu are synthetic oligosaccharides. The sequences of the gluco-oligosaccharide preparations highlighted in grey, representative of each series, were determined by ESI-CID-MS/MS. In the lentinan derived fractions a minor 1,4-linked glucose contaminant was detected which was corroborated by NMR (not shown).
[4]The oligosaccharide probes are all lipid-linked, neoglycolipids (NGLs); DH, NGLs prepared from reducing oligosaccharides by reductive amination with the amino lipid, 1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine (DHPE)(47); AO, NGLs prepared from reducing oligosaccharides by oxime ligation with an aminooxy (AO) functionalized DHPE(48).
[5]An asterisk indicates the major component when multiple components are present (see supplemental Tables 6*A*-6*C*).

Table S7B. Fluorescence binding intensities elicited with all the proteins investigated. The numerical scores for the fluorescence binding signals are shown as means of duplicate spots at 5 fmol probe per spot (as in Fig.5 and 6 and supplemental Fig. 7) and are representative of at least 2 independent experiments.

ID[1]	Probe[2]	Murine Dectin-1 (Fc)	Murine Dectin-1 (His)	Human Dectin-1 (Fc)	DC-SIGN (Fc)	MOPC-104E (IgM)	16.412E (IgA)	3.4.1G6 (IgG)	2G8 (IgG)	1H8 (IgG)	1E12 (IgM)	*Tm* CBM4-2 (His)	*Cm* CBM32-2 (His)	*Bh* CBM6 (His)	*Cm* CBM6-2 (His)	*Ct* CBM11 (His)	*Tm* CBM41 (His)	ConA
1	Xyl5(ß4)	-	-[3]	-	-	-	-	-	-	-	8,231	-	-	1,600	2,531	-	-	-
2	Xyl6(ß4)	-	-	-	-	-	-	-	-	-	6,095	-	-	2,176	2,872	-	-	-
3	Man4(ß4)	-	-	-	26,527	-	-	-	-	-	-	-	859	-	1,251	-	-	-
4	Man5(ß4)	-	-	-	33,188	-	-	-	-	-	-	-	736	-	3,911	-	-	-
5	Man6(ß4)	-	-	-	8,700	-	-	-	-	-	-	-	-	-	1,937	-	-	-
6	Cyano-2	-	-	-	732	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Cyano-3	-	-	-	7,139	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Cyano-4	-	-	-	3,785	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Cyano-5	-	-	-	1,953	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Cyano-6	-	-	-	1,593	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Cyano-7	-	-	-	3,539	-	-	-	-	-	-	-	-	-	-	-	-	-
12	Cyano-8	-	-	-	3,407	-	-	-	-	-	-	-	-	-	-	-	-	-
13	Cyano-9	-	-	-	3,567	-	-	-	-	-	-	-	-	-	-	-	-	-
14	Glc2(α3)	-	-	-	16,410	800	-	-	-	-	-	-	-	-	561	-	-	-
15	Poria-3	-	-	-	-	4,485	-	-	-	-	-	-	-	-	-	-	-	-
16	Poria-4	-	-	-	-	21,829	-	-	-	-	-	-	-	-	-	-	-	-
17	Poria-5	-	-	-	4,717	37,550	-	-	-	-	-	-	-	-	-	-	-	-
18	Poria-6	-	-	-	2,166	40,972	-	-	-	-	-	-	-	-	-	-	-	-
19	Poria-7	-	-	-	-	47,596	-	-	-	-	-	-	-	-	586	-	1,080	-
20	Poria-8	-	-	-	-	33,957	-	-	-	-	-	-	-	-	664	-	2,114	-
21	Poria-9	-	-	-	-	2,394	-	-	-	-	-	-	-	535	709	-	-	-
22	Poria-10	-	-	-	-	3,064	-	-	-	-	-	-	-	-	612	-	1,528	-
23	Poria-11	-	-	-	-	2,097	-	-	-	-	-	-	-	-	-	-	746	-
24	Poria-12	-	-	-	-	574	-	-	-	-	-	-	-	-	-	-	-	-
25	Poria-13	-	-	-	-	818	-	-	-	-	-	-	-	507	-	-	1,071	-
26	Malto-2	-	-	-	15,895	-	-	-	-	-	-	-	-	-	-	-	1,337	-
27	Malto-3	-	-	-	22,263	-	-	-	-	-	-	-	-	-	-	-	12,382	-
28	Malto-4	-	-	-	8,168	-	-	-	-	-	-	-	-	-	-	-	24,866	-
29	Malto-5	-	-	-	2,717	-	-	-	-	-	-	-	-	-	-	-	25,079	-

30	Malto-6	-	-	-	5,875	-	-	-	-	-	-	-	-	-	-	-	35,382	-
31	Malto-7	-	-	-	5,174	-	-	-	-	-	-	-	-	-	-	-	31,123	-
32	Malto-8	-	-	-	6,250	-	-	-	-	-	-	-	-	-	-	-	39,910	-
33	Malto-9	-	-	-	2,548	-	-	-	-	-	-	-	-	-	-	-	25,146	-
34	Malto-10	-	-	-	4,106	-	-	-	-	-	-	-	-	-	520	-	29,445	-
35	Malto-11	-	-	-	2,928	-	-	-	-	-	-	-	-	-	628	-	28,806	-
36	Malto-12	-	-	-	3,270	-	-	-	-	-	-	-	-	-	502	-	33,815	-
37	Malto-13	-	-	-	2,817	-	-	-	-	-	-	-	-	-	697	-	32,685	-
38	Dext-2	-	-	-	4,239	-	-	-	-	-	-	-	-	-	721	-	-	-
39	Dext-3	-	-	-	2,338	-	1,962	-	-	-	-	-	-	-	-	-	608	-
40	Dext-4	-	-	-	2,251	-	16,255	-	-	-	-	-	-	-	567	-	-	-
41	Dext-5	-	-	-	1,520	-	22,598	-	-	-	-	-	-	-	-	-	-	-
42	Dext-6	-	-	-	1,607	-	36,421	3,472	-	-	-	-	-	-	510	-	-	-
43	Dext-7	-	-	-	-	-	16,038	2,329	-	-	-	-	-	-	603	-	-	-
44	Dext-8	-	-	-	-	-	16,306	5,505	-	-	-	-	-	-	583	-	-	-
45	Dext-9	-	-	-	-	-	25,738	10,288	-	-	-	-	-	-	721	-	-	-
46	Dext-10	-	-	-	-	-	14,628	6,169	-	-	-	-	-	-	559	-	-	-
47	Dext-11	-	-	-	-	-	13,032	6,534	-	-	889	736	-	757	1,162	-	-	-
48	Dext-12	-	-	-	-	-	22,417	10,151	-	-	533	-	-	-	586	-	-	-
49	Dext-13	-	-	-	-	-	12,645	9,545	-	-	-	-	-	-	-	-	-	-
50	Pano-3	-	-	-	1,319	-	1,462	-	-	-	-	-	-	-	-	-	-	-
51	i-Pano-3	-	-	-	2,538	-	-	-	-	-	-	-	-	-	-	-	12,799	-
52	Pullu-4	-	-	-	7,686	-	18,828	-	-	-	-	-	-	-	-	-	582	706
53	Pullu-7	-	-	-	2,411	-	28,691	-	-	-	-	-	-	-	-	-	25,217	869
54	CβG-2	-	-	-	10,660	-	-	-	-	-	3,964	-	3,124	1,458	5,206	-	-	-
55	CβG-3	-	-	-	13,573	-	-	-	-	-	1,908	-	10,005	3,931	9,253	-	-	-
56	CβG-4	-	-	-	16,420	-	-	-	-	-	1,453	-	11,411	7,908	11,729	-	-	-
57	CβG-5	-	-	-	24,457	-	-	-	-	-	2,133	-	28,076	22,382	19,184	-	-	-
58	CβG-6	-	-	-	30,343	-	-	-	-	-	1,463	-	33,048	24,293	20,680	-	-	-
59	CβG-7	-	-	-	17,123	-	-	-	-	-	1,358	-	27,992	20,445	17,484	-	-	-
60	CβG-8	-	-	-	14,431	-	-	-	-	-	1,350	-	22,859	20,293	16,568	-	-	-
61	CβG-9	-	-	-	16,218	-	-	-	-	-	1,510	-	40,293	31,821	23,567	-	-	-
62	CβG-10	-	-	-	8,395	-	-	-	-	-	859	-	26,905	19,052	20,156	-	-	-
63	CβG-11	-	-	-	11,176	-	8,507	1,177	-	-	1,147	-	33,298	25,387	18,986	-	-	-
64	CβG-12	-	-	-	5,417	-	-	-	-	-	-	-	26,480	20,122	15,960	-	-	-

65	CβG-13	-	-	-	8,929	-	-	-	-	-	-	-	30414	25,881	18,989	-	-	-
66	Lam-2	-	-	-	6,100	-	-	-	-	-	8,349	-	1,459	594	4,414	-	-	-
67	Lam-3	-	-	-	4,474	-	-	-	-	-	10,609	-	3,568	1,485	6,577	-	-	-
68	Lam-4	-	-	-	9,079	-	-	-	1,459	-	12,383	5,597	13,762	10,846	17,831	-	-	-
69	Lam-5	-	-	-	5,608	-	-	-	8,565	4,841	12,451	11,304	12960	14,041	17254	-	-	-
70	Lam-6	-	-	-	2,980	-	-	-	21,301	12,936	14,211	13,442	10,577	18,786	15,325	-	-	-
71	Lam-7	-	-	-	1,313	-	-	-	18,059	10,659	10,658	13,077	8,775	17,649	13,182	-	-	-
72	Curd-8	-	-	-	2,200	-	-	-	28,066	18,360	11,046	23,535	17,841	30,340	21,756	-	-	-
73	Curd-9	-	-	-	1,127	-	-	-	20,473	15,466	10,618	23,065	13,468	27,402	17,454	-	-	-
74	Curd-10	-	-	-	657	-	-	-	19,828	13,746	12,767	26,266	14,111	28,568	16,927	-	-	-
75	Curd-11	629	2,111	-	-	-	-	-	22,139	16,898	6,318	24,966	15,359	29,733	20,037	-	-	-
76	Curd-12	3,698	9,698	2,030	-	-	-	-	24,639	16,820	4,244	30,060	20,952	30,026	25,282	-	-	-
77	Curd-13	5,734	18,227	3,529	-	-	-	-	16,971	12,260	3,795	32,683	13,775	30,747	17,497	-	-	-
78	HE-8	-	-	-	2,770	-	-	-	21,359	9,703	6,287	17,714	28,715	29,793	32,676	-	-	-
79	HE-9	-	-	-	1,019	-	-	-	14,179	8,213	4,633	13,629	18,795	21,742	25,574	-	-	-
80	HE-10	-	-	-	644	-	-	-	13,558	7,808	5,630	12,809	16,714	26,513	22,459	-	-	-
81	Cello-2	-	-	-	1,0701	-	-	-	-	-	8,899	-	1,188	1,024	5,376	-	-	-
82	Cello-3	-	-	-	4,881	-	-	-	-	-	10,943	-	2,890	1,128	12,973	-	-	-
83	Cello-4	-	-	-	2,600	-	-	-	-	-	9,840	-	2,775	1,567	12,059	-	-	-
84	Cello-5	-	-	-	1,267	-	-	-	-	-	8,567	-	1,833	1,138	9,883	-	-	-
85	Cello-6	-	-	-	-	-	-	-	-	-	9,603	-	836	-	7,304	-	-	-
86	Cello-7	-	-	-	721	-	-	-	-	-	8,418	-	2,132	1,878	12,699	-	-	-
87	Cello-8	-	-	-	-	-	-	-	-	-	8,137	-	2,625	2,544	14,129	-	-	-
88	Cello-9	-	-	-	1,918	-	-	-	-	-	9,387	-	7,450	8,500	23,494	-	-	-
89	Cello-10	-	-	-	1,060	-	-	-	-	-	11,388	-	4,861	5,975	22,170	-	-	-
90	Cello-11	-	-	-	542	-	-	-	-	-	6,272	-	3,647	4,673	17,387	-	-	-
91	Cello-12	-	-	-	-	-	-	-	-	-	6,736	-	2,463	2,857	13,632	-	-	-
92	Cello-13	-	-	-	-	-	-	-	-	-	6,787	-	2,623	4,699	15,876	-	-	-
93	Pust-2	-	-	-	16,283	-	-	-	-	-	12,094	-	2,628	715	4,226	-	-	-
94	Pust-3	-	-	-	3,471	-	-	-	-	-	5,229	-	4,072	1,479	6,542	-	-	-
95	Pust-4	-	-	-	3,601	-	-	-	-	-	4,129	-	6,818	3,555	8,992	-	-	-
96	Pust-5	-	-	-	4,612	-	-	-	-	-	3,822	-	9,130	5,817	10,958	-	-	-
97	Pust-6	-	-	-	2,723	-	-	-	-	-	4,976	-	6,310	5,132	11,814	-	-	-
98	Pust-7	-	-	-	1,279	-	-	-	-	-	2,802	-	4,500	3,949	9,337	-	-	-
99	Pust-8	-	-	-	1,053	-	-	-	-	-	2,402	-	4,439	4,363	7,920	-	-	-

100	Pust-9	-	-	-	2,560	-	-	-	-	-	1,027	-	5,114	5,197	6,729	-	-	1,363
101	Pust-10	-	-	-	9,969	-	-	-	-	-	1,249	-	7,081	7,636	7,865	-	-	6,261
102	Pust-11	-	-	-	6,530	-	-	-	-	-	995	-	1,731	3,200	3,932	-	-	2,471
103	Pust-15	-	-	-	6,794	-	-	-	-	-	1,141	-	6,999	10,597	9,811	-	-	15,850
104	Pust-15a	-	-	-	3,498	-	-	-	-	-	694	-	2,688	4,761	5,804	-	-	6,397
105	Barley-3	-	-	-	3,439	-	-	-	-	-	12,258	-	5,416	2,068	14,050	-	-	-
106	Barley-3a	-	-	-	2,924	-	-	-	-	-	9,369	-	3,993	3,490	7,660	-	-	-
107	Barley-3b	-	-	-	3,516	-	-	-	-	-	10,305	-	4,121	1,135	11,583	-	-	-
108	Barley-4	-	-	-	685	-	-	-	-	-	6,977	-	4,853	1,756	22,093	-	-	-
109	Barley-4a	-	-	-	1,673	-	-	-	-	-	9,799	-	5,565	4,730	16,313	-	-	-
110	Barley-4b	-	-	-	1,109	-	-	-	-	-	6,261	-	4,381	1,351	20,337	-	-	-
111	Barley-4c	-	-	-	3,914	-	-	-	-	-	8,504	-	6,427	3,181	21,189	-	-	-
112	Barley-5	-	-	-	-	-	-	-	-	-	6,343	-	4,326	3,110	19,285	-	-	-
113	Barley-5a	-	-	-	3,072	-	-	-	773	-	10,831	-	11,006	10,886	21,104	-	-	-
114	Barley-6	-	-	-	-	-	-	-	-	-	7,473	-	4,124	4,306	24,017	-	-	-
115	Barley-6a	-	-	-	4,697	-	-	-	1,412	-	13,401	1,808	22,154	24,640	35,452	-	-	-
116	Barley-7	-	-	-	-	-	-	-	-	-	7,265	-	6,471	5,257	22,968	7,375	-	-
117	Barley-8	-	-	-	-	-	-	-	-	-	4,937	1,309	4,813	5,854	24,158	8,756	-	-
118	Barley-9	-	-	-	-	-	-	-	-	-	3,633	1,552	4,062	5,166	20,539	11,866	-	-
119	Barley-10	-	-	-	-	-	-	-	-	-	2,705	1,004	708	2,007	14,614	8,503	-	-
120	Barley-11	-	-	-	-	-	-	-	-	-	3,749	4,152	4,398	7,049	23,780	25,472	-	-
121	Barley-12	-	-	-	-	-	-	-	-	-	2,123	2,010	723	2,241	15,117	14,574	-	-
122	Barley-13	-	-	-	-	-	-	-	-	-	2,028	2,025	594	2,121	14,750	14,413	-	-
123	Barley-14	-	-	-	-	-	-	-	-	-	1,078	5,381	686	3,710	18,294	23,499	-	-
124	Barley-15	-	-	-	-	-	-	-	-	-	1,917	8,006	1,738	5,538	20,531	37,621	-	-
125	Barley-16	-	-	-	-	-	-	-	-	-	858	3,993	640	3,250	14,286	24,280	-	-
126	Grifo-3	-	-	-	5,861	-	-	-	-	-	8,004	-	10,327	6,490	10,478	-	-	-
127	Grifo-4	-	-	-	4,045	-	-	-	691	-	6,736	-	10,702	9,189	13,168	-	-	-
128	Grifo-5	-	-	-	2,465	-	-	-	1,696	-	5,705	-	12,051	11,336	16,648	-	-	-
129	Grifo-6	-	-	-	1,395	-	-	-	1,231	-	5,161	1,213	11,821	10,556	17,118	-	-	-
130	Grifo-7	-	-	-	1,803	-	-	-	1,934	550	4,319	2,750	12,804	17,580	16,532	-	-	-
131	Grifo-8	-	-	-	1,544	-	-	-	2,562	887	4,184	4,662	13,429	18,250	18,378	-	-	-
132	Grifo-9	-	-	-	-	-	-	-	3,408	960	3,269	3,420	9,784	13,663	13,035	-	-	-
133	Grifo-10	-	-	-	-	-	-	-	4,217	1,741	3,370	5,259	11,249	14,050	14,813	-	-	-
134	Grifo-11	-	-	-	-	-	-	-	4,459	2,577	3,314	5,638	10,594	17,630	14,773	-	-	-

135	Grifo-12	-	-	-	-	-	-	-	3,424	1,681	2,714	5,912	9,730	15,880	14,004	-	-	-
136	Grifo-13	-	-	-	-	-	-	-	3,278	2,014	2,265	7,109	11,035	18,527	14,003	-	-	-
137	Grifo-14	-	-	-	-	-	-	-	2,570	1,050	1,491	3,645	4,577	8,831	7,461	-	-	-
138	Grifo-15	-	-	-	-	-	-	-	2,900	1,271	1,877	4,568	7,499	13,090	10,736	-	-	-
139	Grifo-16	-	-	-	-	-	-	-	2,663	1,632	1,471	6,249	9,740	16,741	11,848	-	-	-
140	Lenti-2	-	-	-	9,906	-	-	-	-	-	6,797	-	5,158	1,767	7,953	-	-	-
141	Lenti-3	-	-	-	9,639	-	-	-	-	-	6,596	-	10,436	4,015	10,790	-	-	-
142	Lenti-4	-	-	-	6,362	-	-	-	846	-	7,591	848	15,111	9,075	14,444	-	1,380	-
143	Lenti-5	-	-	-	18,339	-	-	-	4,553	1,433	9,105	5,552	33,313	27,947	26,199	-	2,095	-
144	Lenti-6	-	-	-	8,559	-	-	-	12,763	4,464	5,549	6,754	22,172	21,553	21,988	-	1,021	-
145	Lenti-7	-	-	-	5,063	-	535	-	14,011	6,393	5,063	9,698	2,1470	23,565	23,225	-	920	-
146	Lenti-8	-	-	-	4,013	-	-	-	14,462	7,256	6,354	11,594	19,612	30,943	23,785	-	1,032	-
147	Lenti-9	-	-	-	1,708	-	-	-	10,875	5,633	5,942	9,156	18,017	21,553	22,493	-	8,086	-
148	Lenti-10	-	-	-	831	-	-	-	6,559	2,415	2,880	5,609	9,681	13,685	15,211	-	3,900	-
149	Lenti-11	-	-	-	-	-	-	-	4,022	1,750	1,968	4,851	5,835	9,859	10,253	-	2,046	-
150	Lenti-12	-	-	-	1,291	-	-	-	12,720	7,826	4,456	12,142	17,599	26,903	22,025	-	3,781	-
151	Lenti-13	-	-	-	1,411	-	-	-	15,353	8,824	4,945	12,489	22,955	27,399	29,975	-	8,971	-
152	HE-9B7	-	-	-	611	-	-	-	6,668	2,894	4,198	6,466	13,597	15,083	14,109	-	-	-
153	HE-10B2	-	-	-	689	-	-	-	14,149	8,164	5,537	15,433	16,772	27,279	26,476	-	-	-
154	HE-10B3	-	-	-	601	-	-	-	10,706	6,677	6,110	10,285	11,626	20,848	22,015	-	-	-
155	HE-10B5	-	-	-	932	-	-	-	9,151	5,268	5,930	11,093	15,411	25,953	21,486	-	-	-
156	HE-10B7	-	-	-	978	-	-	-	15,534	6,760	4,861	17,789	22,306	34,617	28,547	-	-	-
157	Gu-6B1/3	-	-	-	20,839	-	-	-	590	-	16,376	-	48,324	25,675	30,669	-	-	-
158	HE-11B3/6	-	-	-	-	-	-	-	5,151	3,374	4,884	10,154	13,708	19,831	19,449	-	-	-

[1]ID, Probe position in the microarray matching the position in the binding-charts and in supplemental Table 7*A*.

[2]In the β1,6-linked pustulan series, fractions containing oligomers with >DP-8 as major components (probes 100-103) there was evidence of a minor contaminant containing α-linked mannose as detected by ConA binding. The weak binding detected to the NGLs of oligosaccharide fractions derived from branched β1,3/β1,6 lentinan (probes 142-151) was likely to the presence of an α1,4-linked glucose contaminant as corroborated by MS/MS (supplemental Table 7*A*, footnote 3) and NMR (not shown).

[3] '-' refers to a fluorescence intensity < 500.

Table S8: Summary of Dectin-1 interactions with linear β1,3- and selected branched β1,3/β1,6-linked glucose sequences. SPR inhibition data taken from Adams et al 2008(7).

Pos[1]	Probe name	Probe sequence	SPR[2] IC50	Microarray Fluorescence intensity[3]	On array % inhibition
	Linear natural or synthetic				
72	Lam-7	Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glc	>1mM	<100	No inhibition
79	HE-8	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc	1.1 mM	<100	-
80	HE-9	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc	2.6 mM	<100	-
81	HE-10	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ1-3Glc	0.7 mM	<100	-
76	Curd-11	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc[4]	-[5]	2,111	0.15 mM 25% 0.5 mM 75%
77	Curd-12	Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glcß-3Glc[4]	-	9,698	-
78	Curd-13	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc[4]	-	18,227	0.15 mM 50% 0.5 mM 80%
	Branched synthetic				
-	HE-8[B5]	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc | Glcβ-6	0.13 mM	-	-
-	HE-9[B6]	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc | Glcβ-6	1.3 mM	-	-
153	HE-9[B7]	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc | Glcβ-6	-	<100	-
157	HE-10[B7]	Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glc | Glcβ-6	0.029 mM	<100	-

[1]Position in the microarray; [2]Surface plasmon resonance competition experiments using a glucan-phosphate-coated biosensor chip(7); [3]Spot fluorescence intensity on the present study; [4]Major components in factions; [5]Not tested

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