Electronic Supplementary Information (ESI)

Recombinant mucin-type proteins carrying LacdiNAc on different *O*-glycan core chains fail to support *H. pylori* binding

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Material and Methods

Verification of the coating efficiency using ELISA and quantification of the fusion protein by mouse IgG2b Fc-specific antibodies

In addition to the glycan quantification, the coating efficiency of our samples in 96-well polysorb plates was checked using an ELISA (Supplementary Fig. 1). Briefly, PSGL-1/mIgG2b carrying LDN on different *O*-glycan core structures was diluted in 4 M GuHCl to 4 µg/mL and coated in 96-well polysorb plates over night at 4 °C. The plates were washed three times with PBS containing 0.05% Tween before blocking for 1 hour with 1% bovine serum albumin (BSA) in PBS containing 0.05% Tween to minimize non-specific binding. The plates were incubated with peroxidase-conjugated anti-mIgG Fc-specific antibody (Sigma-Aldrich) diluted in blocking buffer at 1:2 000 for one hour at room temperature. The plates were washed three times again before incubating with the 3, 3′, 5, 5′-tetramethylbenzidine dihydrochloride substrate (Sigma-Aldrich). The color reaction was stopped by the addition of 2M H_2SO_4 and the optical density (O.D.) of the samples was read at a wavelength of 450 nm in a microplate reader (Synergy 2, Biotek instruments Inc, Winooski, USA). The PSGL-1/mIgG2b concentration was estimated using a dilution series of purified mIgG2b (AbD Serotec, Oxford, UK) in blocking buffer as an internal standard.

Isolation of gastric mucins

The mucin isolation, purification by isopycnic density-gradient centrifugation, and characterization of the samples have been described in detail previously [1]. The human samples were collected after informed written consent and in accordance with approval of the local ethics committee (Lund University Hospital, Lund, and Regionala etikprövningsnämnden i Göteborg, Sweden). The Rhesus monkey sample was collected in accordance with approval of the Uniformed Services University of the Health Sciences animal care and use committee, USA in conjunction with a previous study [2]. The mucin *O*-glycan repertoires were characterized by LC-MS/MS (Supplementary Table 1).

Verification of LDN on the LDN (16R)-BSA conjugate

The LDN-BSA conjugate used in the *H. pylori* binding assay was constructed by Prof. Lothar Elling by preparing LDN in a multi-step chemo-enzymatic synthesis utilizing recombinant glycosyltransferases before conjugating the LDN to lysine groups of bovine serum albumin via squaric acid diethyl ester-linking[3]. In order to verify functional LDN determinants on the LDN(16R)-BSA conjugate, a Galectin-3 ELISA was used. A 0.2 μM concentration of LDN (16R)-BSA in PBS was coated on white opaque 96-well plates in quadruplicates overnight at 4°C. Human serum albumin (HSA; 8μg/ml; Sigma-Aldrich) was coated as a negative control. Subsequent steps were performed at room temperature. PBS (140 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer, pH 7.4) in non-coated wells was used as a negative control. Plates were washed three times with PBS containing 0.05% Tween (PBS-T). Wells were blocked with blocking buffer [0.5% BSA (Sigma-Aldrich) in PBS-T] for 1h. Plates were washed three times with PBS-T. Recombinant human Galectin-3 (Nordic BioSite, Stockholm, Sweden) was diluted 1:500 in blocking buffer and added to the wells for 1h. Plates were washed three times with PBS-T. Streptavidin-Eu (PerkinElmer, Waltham, MA, USA) diluted 1:2,500 in blocking buffer was added to the wells and incubated for 1h. Plates were washed with PBS-T (0.05%) six times. The plates were incubated with Delfia enhancement solution (PerkinElmer; 0.05 M NaOH, 0.1 M phthalate, 0.1% Triton X-100, 50 mM TOPO, 15 mM b-NTA) at 120 rpm (rounds per minute) on an orbital shaker for 5 min. Fluorescence ($\lambda_{excitation}$ = 340 nm and $\lambda_{emission}$ = 615 nm) was measured with the Europium label protocol.

Results

Mucin-type fusion protein coating efficiency.

A secondary peroxidase-conjugated goat anti-mouse IgG Fc antibody was used to verify that the coating efficiency of the different PSGL-1/mIgG2b fusion proteins used in the *H. pylori* binding assay was of the same order of magnitude. There was no statistically significant difference (adjusted $p>0.999$ for all comparisons) between the different fusion proteins in terms of coating efficiency as verified with Kruskal-Wallis with Dunn's multiple comparisons (Supplementary Fig. 1).

Tentative O-glycan repertoires on Mucin 1 and 2.

Tentative *O*-glycan structures identified on Mucin 1 and 2 were deduced by LC-MS/MS following their release by β-elimination (Supplementary Table 1).

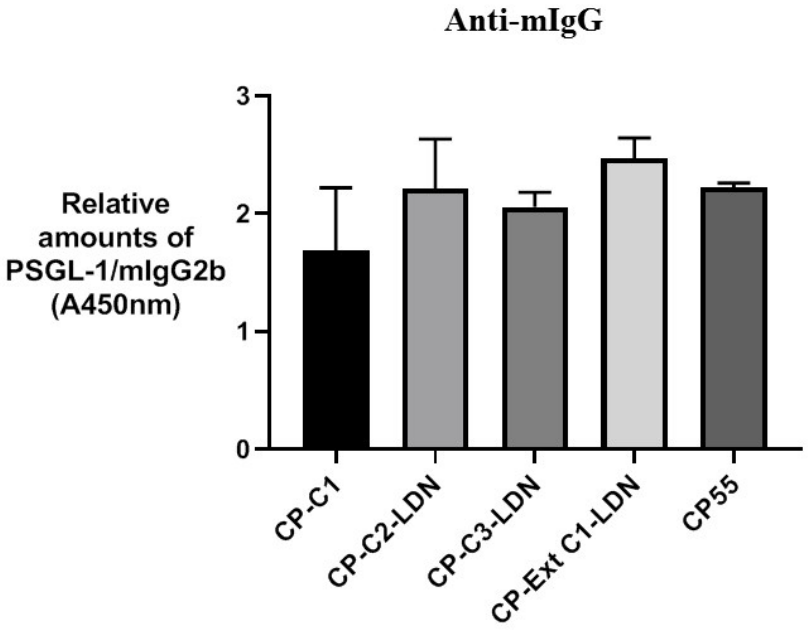
Figure legends

Supplementary Fig. 1.

Binding of the peroxidase-conjugated goat anti-mouse IgG Fc antibody to LDN-carrying PSGL-1/mIgG2b fusion proteins was used to assess their relative coating efficacy in 96-well polysorb plates used for the *H. pylori* binding assay. The absorbance was read at 450 nm and the average optical density values obtained from triplicate wells are shown.

Supplementary Table 1.

Tentative *O*-glycan structures identified by LC-MS/MS on Mucin 1 and Mucin 2. The mucins were purified by isopycnic density-gradient centrifugation and their *O*-glycans released by β-elimination. The percentage of the specific tentative *O*-glycan corresponds to its representation in the total *O*-glycan repertoire. Hex, hexose; HexNAc, *N*-acetylhexosamine; NeuAc, *N*-acetylneuraminic acid; NeuGc, *N*-glycolylneuraminic acid.



Supplementary Fig. 1.

Supplementary Table 1.

Observed mass [M-nH]n-	Composition	Putative structures	Mucin 1 (%)	Mucin 2 (%)
530	Hex1HexNAc1deHex1	Fucα2Galβ3GalNAcol	10.9	
733-1	Hex1HexNAc2deHex1	Fucα2Galβ4GlcNAcβ3GalNAcol	0.3	
733-2	Hex1HexNAc2deHex1	Fucα2Galβ3(GlcNAcβ6)GalNAcol	5.9	
749	Hex2HexNAc2	Galβ3(Galβ4GlcNAcβ6)GalNAcol		28.0
790	Hex1HexNAc3	Galβ3(GalNAcβ4GlcNAcβ6)GalNAcol		7.2
895-1	Hex2HexNAc2deHex1	Galβ3(Fucα2Galβ4GlcNAcβ6)GalNAcol	0.9	
895-2	Hex2HexNAc2deHex1	Fucα2Galβ3(Galβ4GlcNAcβ6)GalNAcol	5.5	
936	Hex1HexNAc3deHex1	Fucα2Galβ3(GalNAcβ4GlcNAcβ6)GalNAcol	4.8	
1040	NeuAc1Hex2HexNAc2	NeuAcα3Galβ3(Galβ4GlcNAcβ6)GalNAcol		13.1
1041-1	Hex2HexNAc2deHex2	Fucα2Galβ4GlcNAcβ3(Fucα2)Galβ3GalNAcol	0.5	
1041-2	Hex2HexNAc2deHex2	Fucα2Galβ3[Gal(Fuc)GlcNAcβ6]GalNAcol	0.6	
1041-3	Hex2HexNAc2deHex2	Fucα2Galβ3(Fucα2Galβ4GlcNAcβ6)GalNAcol	57.7	
1114-1	Hex3HexNAc3	Galβ4GlcNAcβ3Galβ3(Galβ4GlcNAcβ6)GalNAcol		7.6
1114-2	Hex3HexNAc3	Galβ3(Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcol		24.6
1121	Hex2HexNAc2deHex2Sul1	Fucα2Galβ3[Fucα2(S)Galβ4GlcNAcβ6]GalNAcol	0.3	
1186	NeuAc1Hex2HexNAc2deHex1	NeuAcα3Galβ3(Fucα2Galβ4GlcNAcβ6)GalNAcol	0.8	
1244-1	Hex2HexNAc3deHex2	GalNAcα3(Fucα2)Galβ3[Fucα2Galβ4GlcNAcβ6]GalNAcol	0.4	
1244-2	Hex2HexNAc3deHex2	Fucα2Galβ3[GalNAcα3(Fucα2)Galβ4GlcNAcβ6]GalNAcol	0.2	
1405	NeuAc1Hex3HexNAc3	Galβ3(NeuAcα3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcol		4.8
1406-1	Hex3HexNAc3deHex2	Hex3HexNAc3deHex3	0.4	
1406-2	Hex3HexNAc3deHex2	Hex3HexNAc3deHex3	0.4	
1406-3	Hex3HexNAc3deHex2	Fucα2Galβ4GlcNAcβ3Galβ3[Gal(Fuc)GlcNAcβ6]GalNAcol	0.2	
1406-4	Hex3HexNAc3deHex2	Fucα2Galβ3(Fucα2Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcol	0.7	
1479-1	Hex4HexNAc4	Galβ4GlcNAcβ3Galβ3(Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcol		7.6
1479-2	Hex4HexNAc4	Galβ3(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcol		7.2
1552-1	Hex3HexNAc3deHe	Fucα2Galβ3[Fucα2Galβ4GlcNAcβ3(Fucα2)Galβ4GlcN	0.2	

	x3	**Acβ6]GalNAcol**		
1552-2	**Hex3HexNAc3deHex3**	**Hex3HexNAc3deHex3**	**4.0**	
1552-3	**Hex3HexNAc3deHex3**	**Hex3HexNAc3deHex3**	**0.8**	
1609-1	**Hex3HexNAc4deHex2**	**Fucα2(GalNAcα3)GalGlcNAcβ3Galβ3(Fucα2Galβ4GlcNAcβ6)GalNAcol**	**0.7**	
1609-2	**Hex3HexNAc4deHex2**	**Fucα2Galβ4GlcNAcβ3(GlcNAcβ6)Galβ3(Fucα2Galβ4GlcNAcβ6)GalNAcol**	**0.6**	
1771-1	**Hex4HexNAc4deHex2**	**Fucα2Galβ4GlcNAcβ3(Galβ4GlcNAcβ6)Galβ3[Fuc(Gal)GlcNAcβ6]GalNAcol**	**0.2**	
1771-2	**Hex4HexNAc4deHex2**	**Fucα2Galβ4GlcNAcβ3(Fucα2Galβ4GlcNAcβ6)Galβ3(Galβ4GlcNAcβ6)GalNAcol**	**0.1**	
1771-3	**Hex4HexNAc4deHex2**	**Fucα2Galβ3(Fucα2Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcol**	**0.1**	
1917-1	**Hex4HeNAc4deHex3**	**3Fuc + Galβ4GlcNAcβ3(Galβ4GlcNAcβ6)Galβ3(Galβ4GlcNAcβ6)GalNAcol**	**0.9**	
1917-2	**Hex4HeNAc4deHex3**	**3Fuc + Galβ4GlcNAcβ3(Galβ4GlcNAcβ6)Galβ3(Galβ4GlcNAcβ6)GalNAcol**	**1.6**	
2063	**Hex4HeNAc4deHex4**	**4Fuc + Galβ4GlcNAcβ3(Galβ4GlcNAcβ6)Galβ3(Galβ4GlcNAcβ6)GalNAcol**	**0.5**	

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