Supplementary Information

Supplementary Materials and methods

Anti-Galectin Antiserum Generation

Recombinant galectins were purified as described in Materials & Methods. For galectin-9, a construct expressing only the C-terminus of galectin-9 (hGal-9C) was used by cloning the sequence encoding the final 151 amino acids of human galectin-9 into pET32b. Prior to immunization, any fusion tags were removed and separated from the galectin protein by repurification on lactosyl-Sepharose. The purified, tagless galectin proteins were then sent to ProSci for immunization in rabbits. The antiserum was tested by dot blotting and Western blotting to confirm the absence of cross-reactivity with other recombinant galectins.

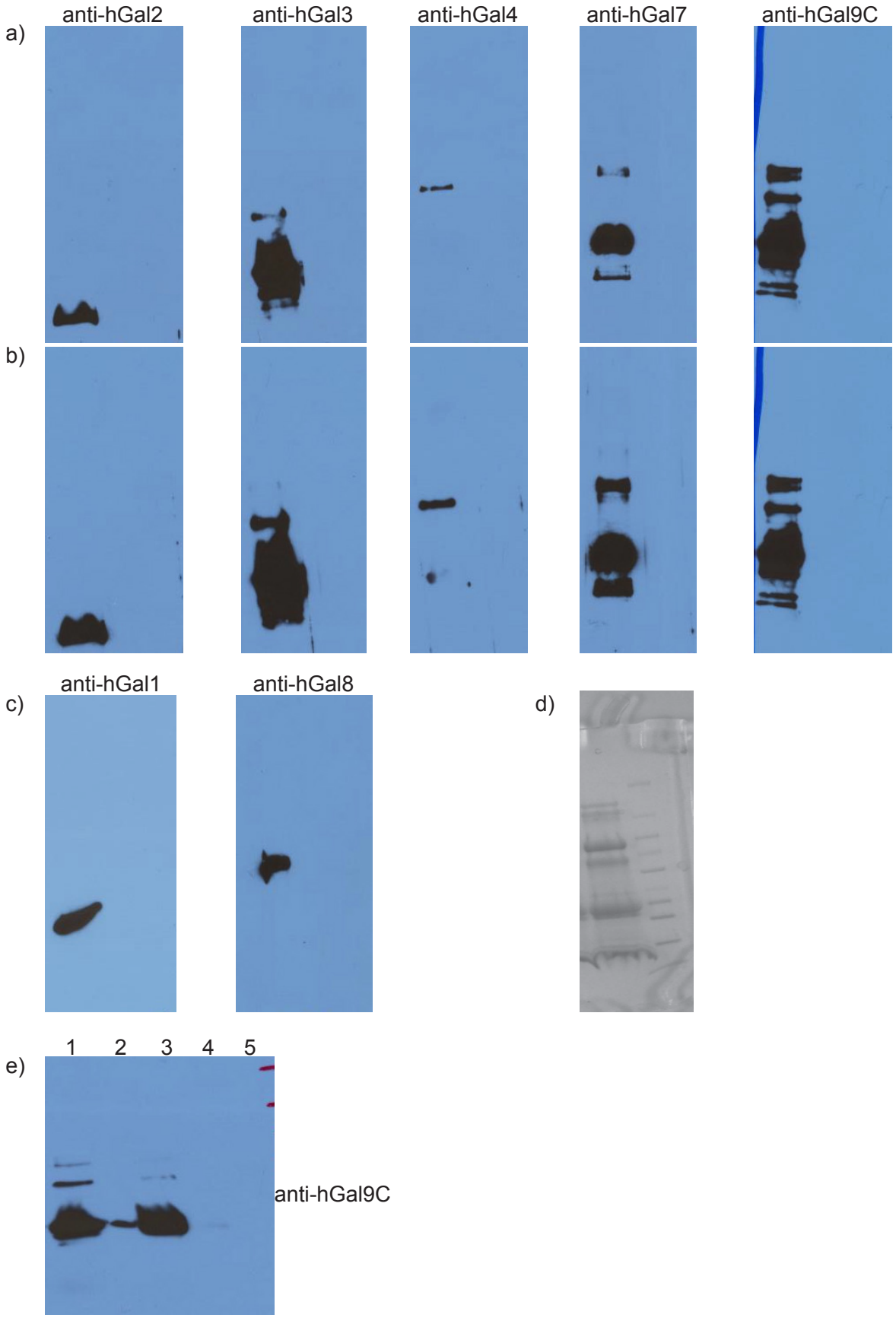
Detection of Galectins in Human Milk

Human milk was obtained from the Mother's Milk Bank (Austin, TX). Whole human milk was first dialyzed into PBS + 14mM β-mercaptoethanol using a 1000Da molecular weight cutoff tubing (Spectrum). Next, the dialyzed sample was centrifuged a low speed and the cream layer removed. The total protein content of the defatted, dialyzed milk was measured by BCA Assay (Pierce). The presence of protein was further assayed by analyzing the defatted, dialyzed milk by SDS-PAGE on a Mini-PROTEAN TGX 4-20% SDS-PAGE gel (Bio-Rad) and Coomassie Brilliant Blue R-250 staining (Bio-Rad).

To analyze the milk preparation for galectins, 300μg of human milk protein was then loaded on a Mini-PROTEAN TGX 4-20% SDS-PAGE gel (Bio-Rad) in one lane. A

second lane contained 10μg of galectin as a positive control. Following electrophoresis, the proteins were transferred to a nitrocellulose membrane using an iBlot (Invitrogen). Western Blotting was then performed using the corresponding anti-galectin antiserum at 1:10,000-1:50,000 dilution with 1:5000 goat anti-rabbit IgG-HRP as the detection reagent. Blots were developed with SuperSignal West Pico Reagent (Pierce).

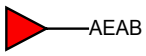
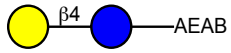
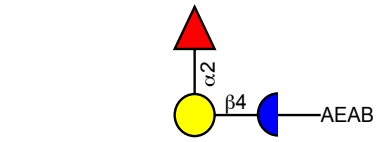
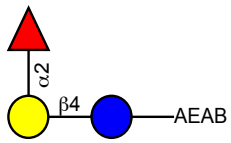
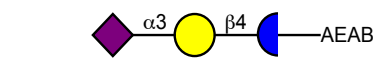
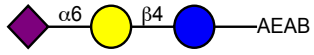
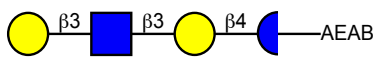
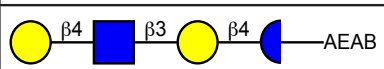
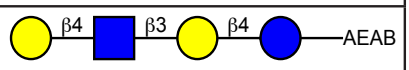
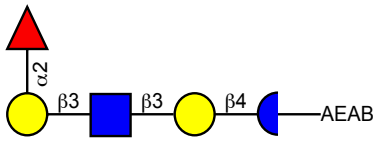
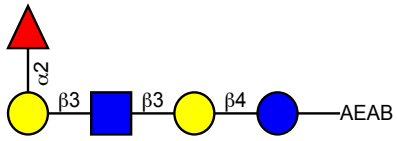
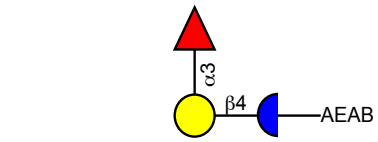
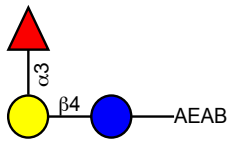
To determine the limit of galectin detection in human milk, 10μg or 5ng of recombinant hGal-9C was run on SDS-PAGE in the presence or absence of 300μg milk protein. 300μg milk protein without added galectin was also run. SDS-PAGE, Western Transfer, and Western Blotting were performed as described above using anti-Gal-9C antiserum.


a)
anti-hGal2
anti-hGal3
anti-hGal4
anti-hGal7
anti-hGal9C
b)
c)
anti-hGal1
anti-hGal8
d)
e)
1 2 3 4 5
anti-hGal9C

Supplementary Figure 1. *Lack of Detection of Galectins in Human Milk*

a-c) 10μg of recombinant galectin (left lane) and 300μg milk protein (right lane) were assayed by Western Blotting with the corresponding anti-galectin antiserum. The anti-hGal-2, -3, -4, -7, and -9C blots were simultaneously developed using either a) 3 minutes or b) 15 minutes of exposure. c) The anti-hGal-1 and -8 blots were simultaneously developed (but separately from the other galectins) using 10 minutes of exposure. d) Coomassie Brilliant Blue R-250 staining of 20μl (~200μg) of defatted, dialyzed milk protein. Left lane: milk, right lane: Spectra Multicolor Broad Range Protein Ladder. e) Limit of detection of galectin-9 in human milk. 10μg or 5ng of recombinant hGal-9C was loaded on an SDS-PAGE gel in the presence or absence of 300μg of milk protein. 20μl milk (~300μg milk protein) was also assayed. The blot was overlaid with anti-hGal-9C antiserum. The lanes contained the following samples: 1: 10μg hGal9C, 2: 5ng hGal-9C, 3: 10μg hGal-9C + 300μg milk protein, 4: 5ng hGal-9C + 300μg milk protein, 5: ~300μg milk protein.

Supplementary Table I. List of HMG structures found on the defined HMG microarray[a, b]

Open-Ring Glycan Structures		Closed-Ring Glycan Structures	
Name (Abbreviation)	Structure	Name (Abbreviation)	Structure
Fucose (Fuc)		Fucose (GG-Fuc)	
Lactose (Lac)		Lactose (GG-Lac)	
2'-fucosyllactose (2'-FL)		2'-fucosyllactose (GG-2'-FL)	
3'-sialyllactose (3'-SL)		3'-sialyllactose (GG-3'-SL)	
6'-sialyllactose (6'-SL)		6'-sialyllactose (GG-6'-SL)	
Lacto-N-Tetraose (LNT)		Lacto-N-Tetraose (GG-LNT)	
Lacto-N-neoTetraose (LNnT)		Lacto-N-neoTetraose (GG-LNnT)	
Lacto-N-Fucopentaose I (LNFPI)		Lacto-N-Fucopentaose I (GG-LNFPI)	
3-fucosyllactose (3-FL)		3-fucosyllactose (GG-3-FL)	

[a]Monosaccharide key: = D-Glucose, = D-galactose, = L-fucose, = D-N-acetylglucosamine, = N-acetylneuraminic acid, = "open-ring" glucose, = "open-ring" fucose

[b]Galactooligosaccharides (GOS) are also found on this microarray but are not depicted here because of the complexity and heterogeneity of the GOS sample used in this study