

- and mass spectrometry-based strategy. *J Mass Spectrom* 2009; 44: 1087–1104.
32. DISWALL M, ANGSTROM J, KARLSSON H et al. Structural characterization of alpha1,3-galactosyltransferase knockout pig heart and kidney glycolipids and their reactivity with human and baboon antibodies. *Xenotransplantation* 2010; 17: 48–60.
 33. DISWALL M, ANGSTROM J, SCHURMAN HJ, DOR FJ, RYDBERG L, BREIMER ME. Studies on glycolipid antigens in small intestine and pancreas from alpha1,3-galactosyltransferase knockout miniature swine. *Transplantation* 2007; 84: 1348–1356.
 34. DISWALL M, ANGSTROM J, SCHURMAN HJ, DOR FJ, RYDBERG L, BREIMER ME. Glycolipid studies in small intestine and pancreas of alpha1,3-galactosyltransferase knockout miniature swine: alpha1,3GALT-KO animals lack alphaGAL antigens and contain novel blood group H compounds. *Transplant Proc* 2008; 40: 543–546.
 35. SLOMIANY BL, SLOMIANY A, HOROWITZ MI. Characterization of blood-group-H-active ceramide tetrasaccharide from hog-stomach mucosa. *Eur J Biochem* 1974; 43: 161–165.
 36. HARA H, LONG C, LIN YJ et al. In vitro investigation of pig cells for resistance to human antibody-mediated rejection. *Transpl Int* 2008; 21: 1163–1174.
 37. SCHEER M, GROTE A, CHANG A et al. BRENDA, the enzyme information system in 2011. *Nucleic Acids Res* 2011; 39(Database issue): D670–D676.
 38. ZHOU D, LEVERY SB. Response to Milland et al.: carbohydrate residues downstream of the terminal Galalpha(1,3)Gal epitope modulate the specificity of xenoreactive antibodies. *Immunol Cell Biol* 2008; 86: 631–632; author reply 633–634.
 39. BAUMANN BC, STUSSI G, HUGGEL K, RIEBEN R, SEEBACH JD. Reactivity of human natural antibodies to endothelial cells from Galalpha(1,3)Gal-deficient pigs. *Transplantation* 2007; 83: 193–201.
 40. BYRNE GW, STALBOERGER PG, DAVILA E et al. Proteomic identification of non-Gal antibody targets after pig-to-primate cardiac xenotransplantation. *Xenotransplantation* 2008; 15: 268–276.
 41. OBUKHOVA P, RIEBEN R, BOVIN N. Normal human serum contains high levels of anti-Gal alpha 1-4GlcNAc antibodies. *Xenotransplantation* 2007; 14: 627–635.
 42. PIERSON RN III, DORLING A, AYARES D et al. Current status of xenotransplantation and prospects for clinical application. *Xenotransplantation* 2009; 16: 263–280.
 43. ROOD PP, TAI HC, HARA H et al. Late onset of development of natural anti-nonGal antibodies in infant humans and baboons: implications for xenotransplantation in infants. *Transpl Int* 2007; 20: 1050–1058.
 44. ZHU A, HURST R. Anti-N-glycolylneuraminic acid antibodies identified in healthy human serum. *Xenotransplantation* 2002; 9: 376–381.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Specificity of 4F10 monoclonal antibody determined by the Glycan-array assay.

Figure S2. Tandem Mass Spectrometry detects iGb3 from isomeric mixtures after multiple rounds of fragmentation that leads to characteristic ions.

Figure S3. Precursor of fucosylated lactosylceramide in pig aortic endothelial cell membranes

Table S1. Structures recognized by the different antibodies and lectins used in the study.

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Tables

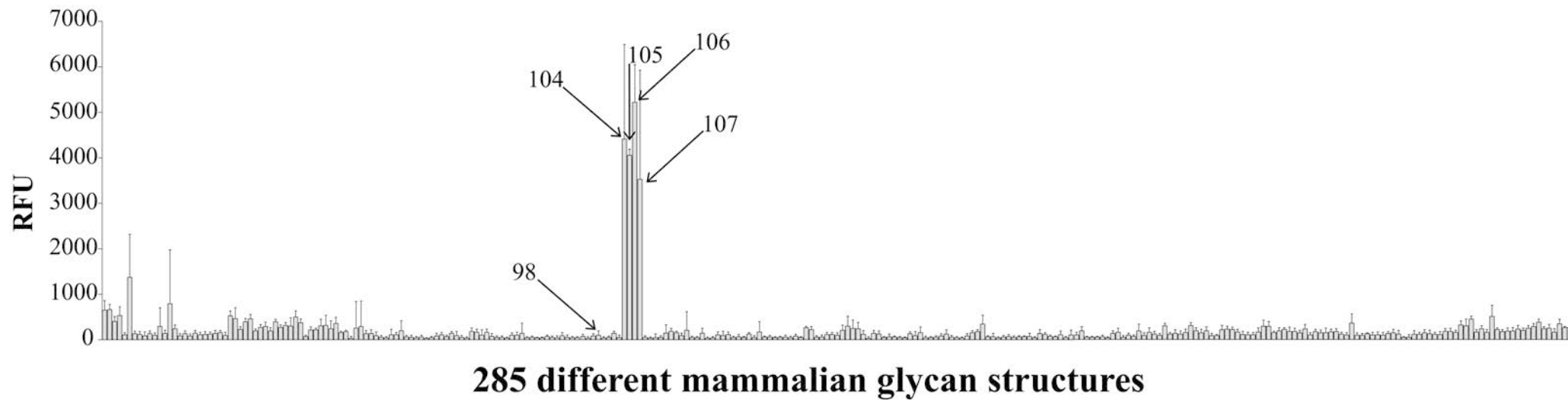
Table S1. Structures recognized by the different antibodies and lectins used in the study.

	Antigen	Epitope(s) / structure(s)*	Ref.		Antigen	Epitope(s) / structure(s)*	Ref.
4F10	α Gal	Gal α 1,3Gal-R	(S1)	15.101	iGb3	Gal α 1,3Gal β 1,4Glc β -R	(15)
		Gal α 1,3Gal β 1,3GlcNAc β -R			Gb3	Gal α 1,4Gal β 1,4Glc β -R	(25)
		Gal α 1,3Gal β 1,4GlcNAc β -R					
	iGb3	Gal α 1,3Gal β 1,4Glc β -R	(S1)				
M86	α Gal	Gal α 1,3Gal β 1,4GlcNAc-R	M	Hu anti-αGal	α Gal/iGb3	Gal α 1,3Gal-R	(14)
GT4-31	α Gal	Gal α 1,3Gal-R	(16)	GT6-27	α Gal	Gal α 1,3Gal-R	(16)
25.20	α Gal	Gal α 1,3Gal-R	(15)	24.7	α Gal	ND	(15)
BSI-B4	α Gal	α -D-Gal*	M	UEA-I	H-type	L-Fuc*	M

*In case of lectins mentioned, the reagent recognises terminal sugar structures.

Abbreviations. BSI-B4: *Bandeiraea simplicifolia* lectin; Fuc: fucose; Gal: galactose; Gb3: globotrihexosylceramide 3; GlcNAc: *N*-acetylglucosamine; iGb₃: isoglobotrihexosylceramide 3; M: manufacturer specifications; -R: radical; and UEA-I *Ulex europaeus* lectin.

Figure S1



iGb3

Gb3

 $(\text{Gal}\alpha 1,3\text{Gal}\beta 1,4\text{Glc}\beta 1,1\text{Cer})$ $(\text{Gal}\alpha 1,4\text{Gal}\beta 1,4\text{Glc}\beta 1,1\text{Cer})$

	↓ MS ¹	
1354.8		1354.8
	↓ MS ²	
667.4		667.4
	↓ MS ³	
445.3		445.3
	↓ MS ⁴	
211		257
343		259
371		329
383		413
		415

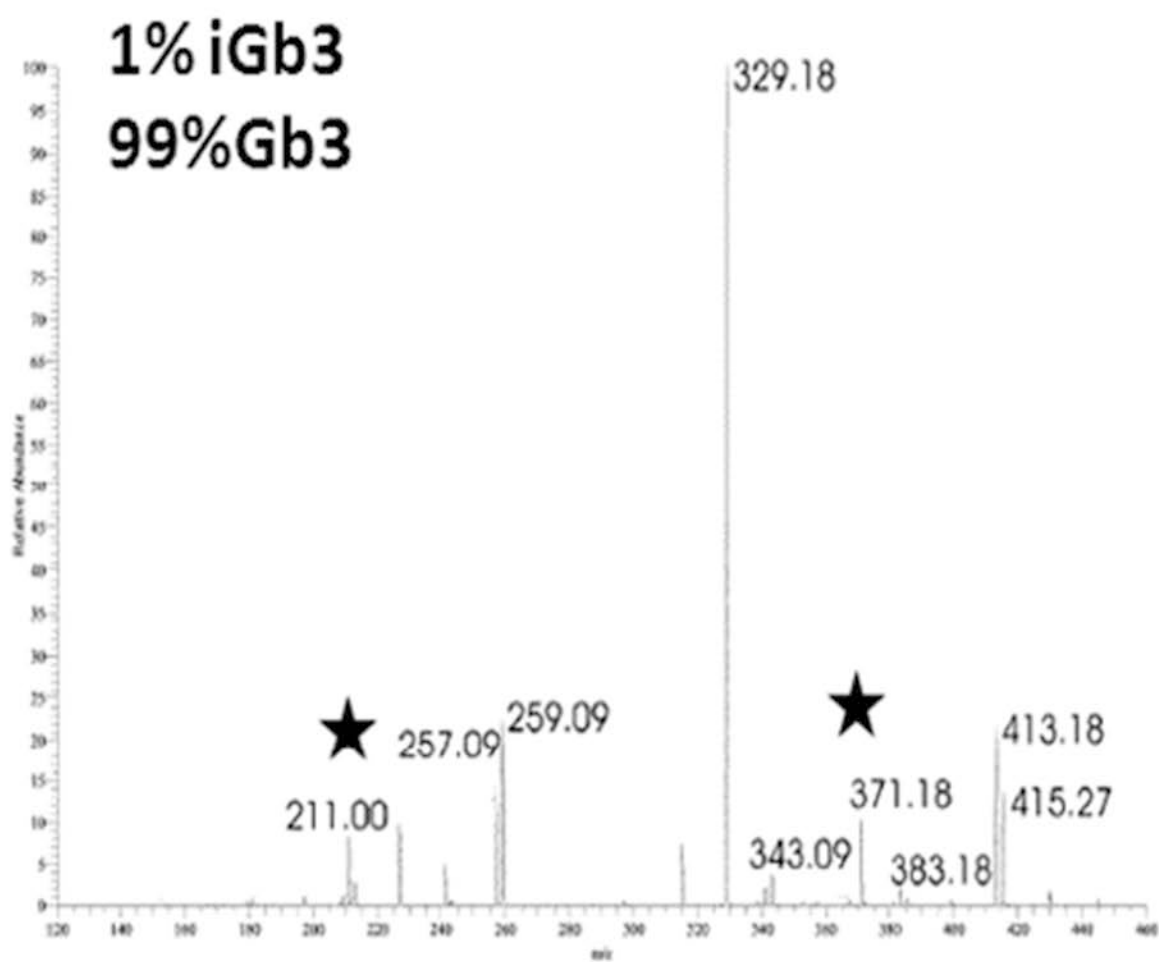
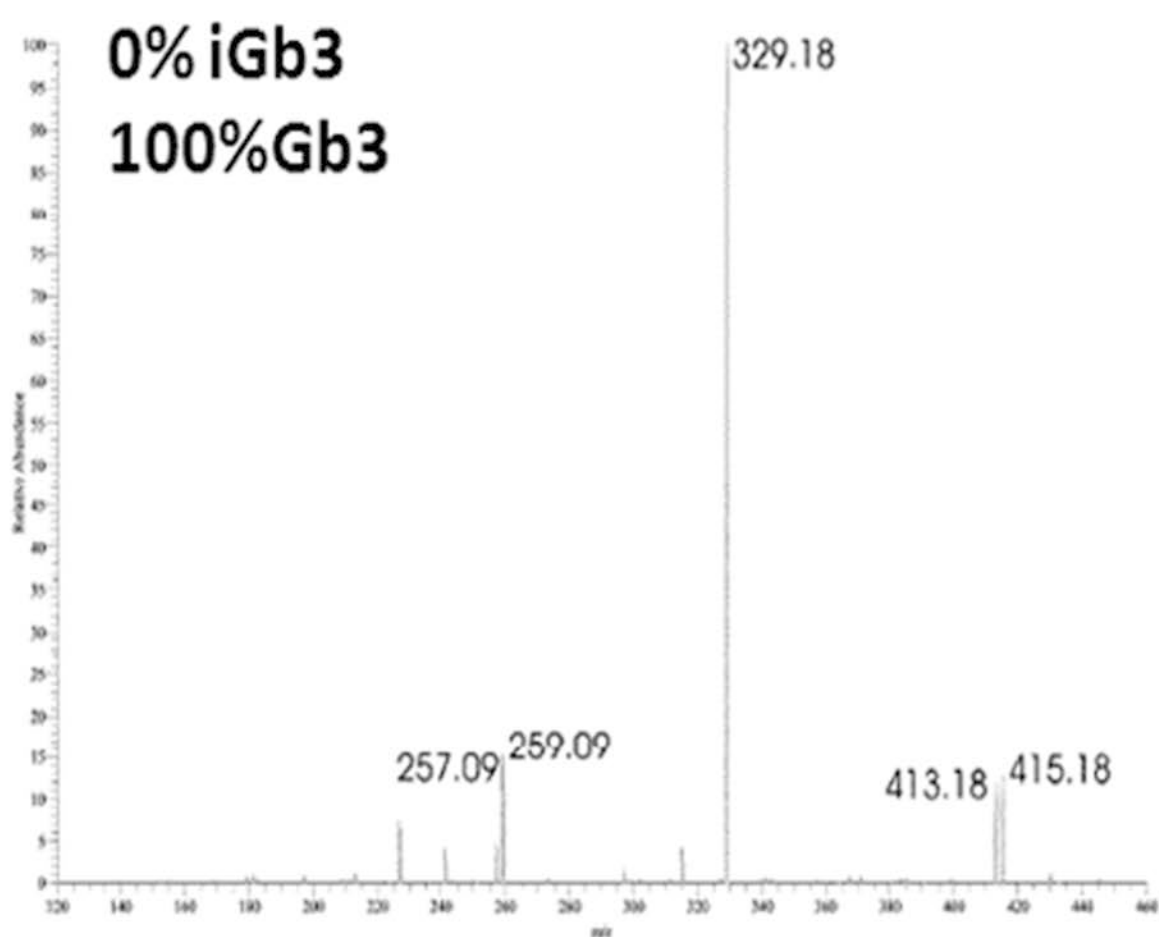


Figure S3

WT

