

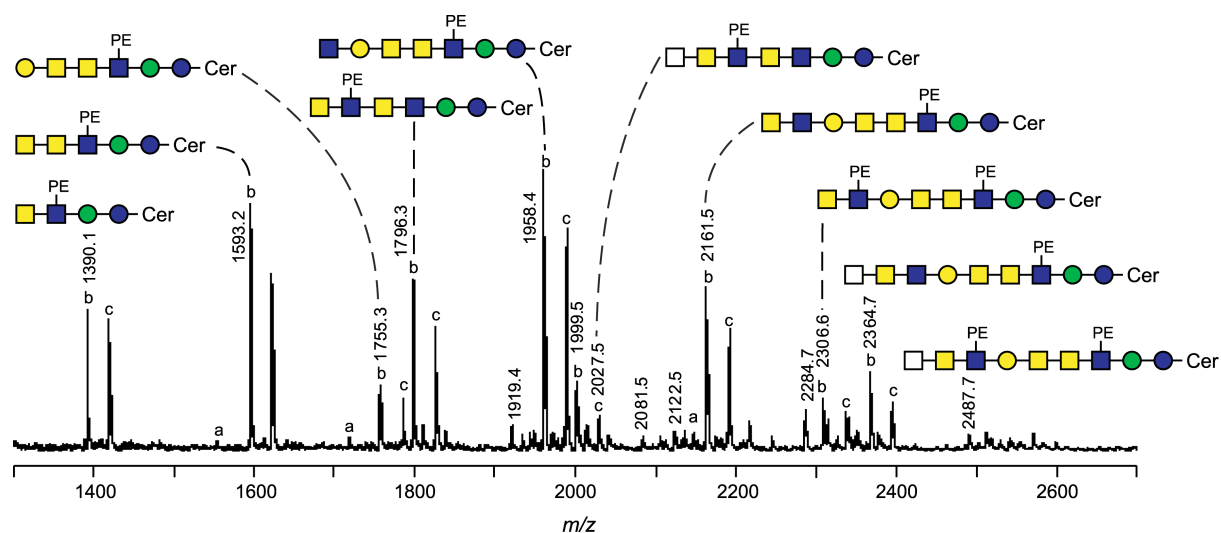
Johswich et al., <http://www.jcb.org/cgi/content/full/jcb.200801071/DC1>

Figure S1. **Negative-mode MALDI-TOF-MS of S2 cell glycosphingolipids.** Glycosphingolipid extracts of nondepleted S2 cells were analyzed by negative-ion mode MALDI-TOF-MS in the reflectron mode using 2,5-dihydroxybenzoic acid as matrix for sample preparation. A list of newly registered masses and assigned glycosphingolipid structures compared with known GSL structures in *Drosophila* is given in Table S1. b, glycosphingolipid with a Cer composition of C14:1 tetradecasphingenine and C20:0 arachidic acid; a and c, glycosphingolipid with Cer masses that are 28 D (two methylene groups,  $C_2H_4$ ) lower (a) or higher (c) than for b. Blue circles, glucose; green circles, mannose; yellow circles, galactose; yellow squares, GalNAc; blue squares, GlcNAc; white square, N-acetylhexosamine; PE, phosphoethanolamine; m/z, mass/charge.

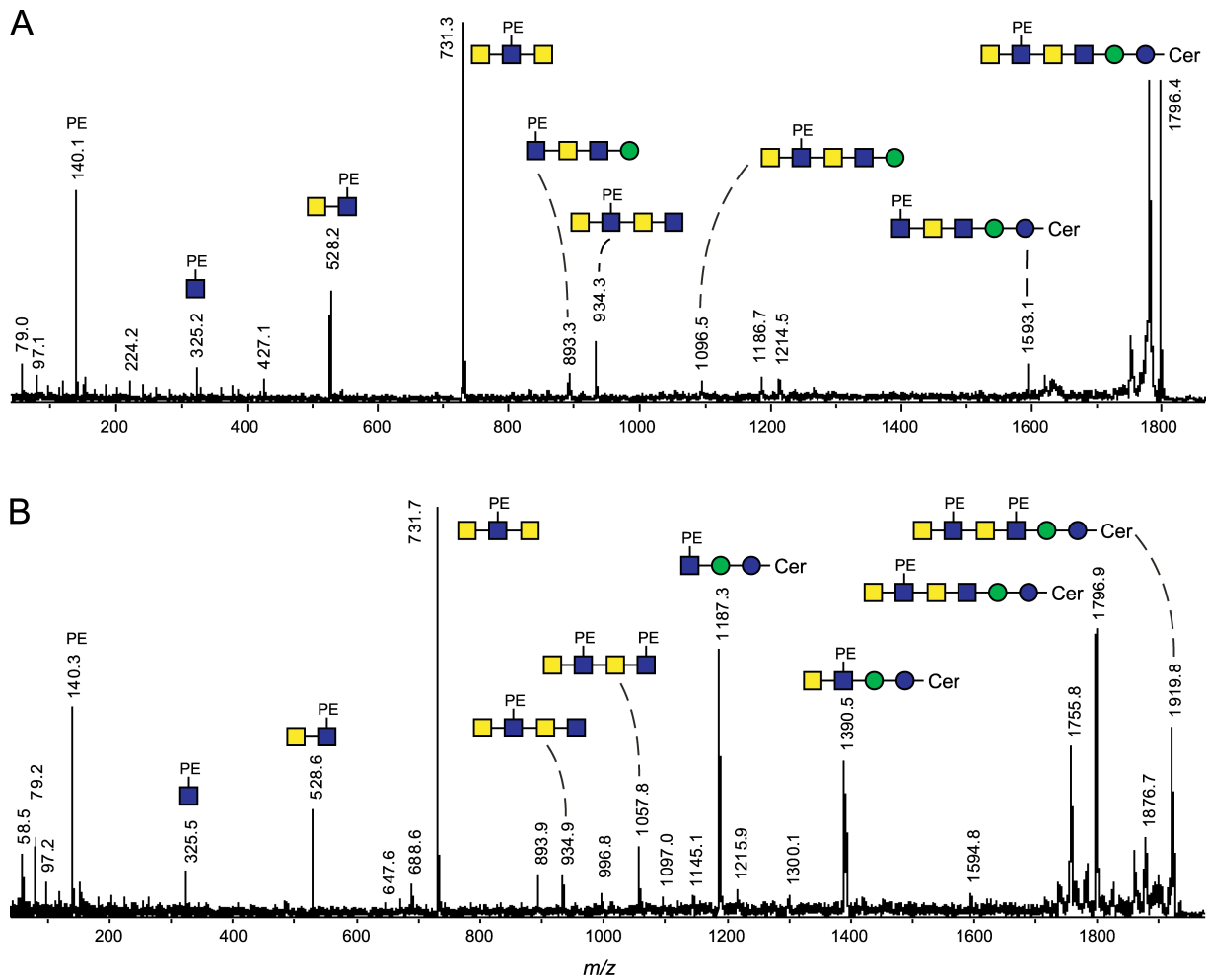


Figure S2. **MALDI-TOF/TOF-MS fragmentation analysis of two zwitterionic glycolipid species containing lacdiNAc tandem repeats.** (A and B) Zwitterionic glycosphingolipid species with a hexasaccharide glycan moiety (mass/charge = 1,796) with one phosphoethanolamine (PE) modification (A) and the hexasaccharide glycan moiety (mass/charge [*m/z*] = 1,919) containing two phosphoethanolamine residues (B) were predicted to contain two hexose and four *N*-acetylhexosamine residues. Fragmentation patterns by MALDI-TOF/TOF-MS (in deprotonated form using 2,5-dihydroxybenzoic acid as matrix) confirmed the presence of a tetra *N*-acetylhexosamine sequence with one or two phosphoethanolamine modifications, which can only be interpreted as a repeat of two lacdiNAc units. Blue circles, glucose; green circles, mannose; blue squares, GlcNAc.

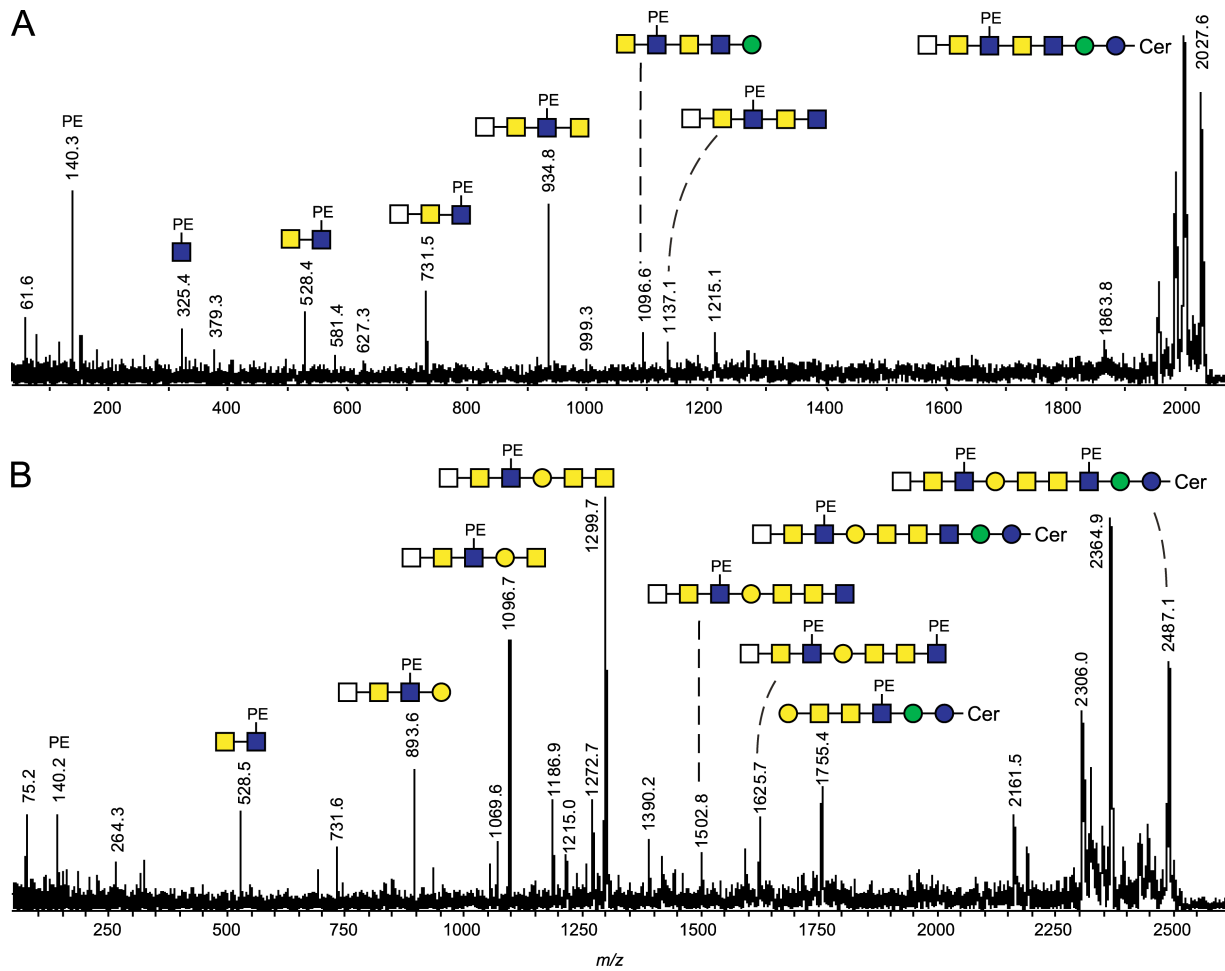


Figure S3. **MALDI-TOF/TOF-MS analysis of two zwitterionic glycolipid species.** (A and B) Zwitterionic glycosphingolipid species with five *N*-acetylhexosamine residues (mass/charge [*m/z*] = 2,027; A) and six *N*-acetylhexosamine sugars (mass/charge = 2,487; B) were analyzed by MALDI-TOF/TOF-MS in a deprotonated form using 2,5-dihydroxybenzoic acid as a matrix. Fragmentation patterns confirmed the presence of an additional terminal *N*-acetylhexosamine residue compared with known structures present in *Drosophila*, probably a GalNAc residue in  $\alpha$ -1,4 linkage in analogy to other *Drosophila* zwitterionic glycosphingolipid structures. Blue circles, glucose; green circles, mannose; yellow circles, galactose; yellow squares, GalNAc; blue squares, GlcNAc; white square, *N*-acetylhexosamine; PE, phosphoethanolamine.

Table S1. **Newly registered zwitterionic glycosphingolipid species**

Zwitterionic GSL	Proposed structure	Registered mass
		<i>D</i>
Nz6*	GalNAc $\beta$ ,4(PE-6)GlcNAc $\beta$ ,3GalNAc $\beta$ ,4GlcNAc $\beta$ ,3Man $\beta$ ,4Glc $\beta$ Cer, GalNAc $\beta$ ,4GlcNAc $\beta$ ,3GalNAc $\beta$ ,4(PE-6)GlcNAc $\beta$ ,3Man $\beta$ ,4Glc $\beta$ Cer	1,796.3/1,824.4 <sup>a</sup>
Nz26*	GalNAc $\beta$ ,4(PE-6)GlcNAc $\beta$ ,3GalNAc $\beta$ ,4(PE-6)GlcNAc $\beta$ ,3Man $\beta$ ,4Glc $\beta$ Cer	1,919.4
Nz7*	(HexNAc1-)GalNAc $\beta$ ,4GlcNAc $\beta$ ,3GalNAc $\beta$ ,4(PE-6)GlcNAc $\beta$ ,3Man $\beta$ ,4Glc $\beta$ Cer	1,999.5/2,027.5 <sup>a</sup>
Nz27*	(HexNAc1-)GalNAc $\beta$ ,4(PE-6)GlcNAc $\beta$ ,3GalNAc $\beta$ ,4(PE-6)GlcNAc $\beta$ ,3Man $\beta$ ,4Glc $\beta$ Cer	2,122.5
Nz9*	(HexNAc1-)GalNAc $\beta$ ,4(PE-6)GlcNAc $\beta$ ,3Gal $\beta$ ,3GalNAc $\alpha$ ,4GalNAc $\beta$ ,4(PE6) GlcNAc $\beta$ ,3Man $\beta$ ,4Glc $\beta$ Cer	2,364.7
Nz29*	(HexNAc1-)GalNAc $\beta$ ,4(PE-6)GlcNAc $\beta$ ,3Gal $\beta$ ,3GalNAc $\alpha$ ,4GalNAc $\beta$ ,4(PE6) GlcNAc $\beta$ ,3Man $\beta$ ,4Glc $\beta$ Cer	2,487.7

GSL, glycosphingolipid. Zwitterionic glycosphingolipids were registered as [M-H]<sup>-</sup> species and are alternative structures to those described by Seppo et al. (2000).  
<sup>a</sup>Glycosphingolipid with a Cer species with a 28-D higher mass, which reflects two additional methylene groups in the Cer moiety.

### References

Seppo, A., M. Moreland, H. Schweingruber, and M. Tiemeyer. 2000. Zwitterionic and acidic glycosphingolipids of the *Drosophila melanogaster* embryo. *Eur. J. Biochem.* 267:3549–3558.