

**Supplemental Table S1 | Sequences of sgRNA and primers.**

Code of the specific primers (LL0xx or RBxx), target genes, purpose and sequence (in 5' - 3' direction) with forward oligo (F) and reverse oligo (R).

Code	Gene	Purpose	Sequence (5'→3')
LL030	-	Constant R oligo	AAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC
LL031	<i>Gba1</i>	Target 1 sgRNA F	GCGTAATACGACTCACTATAGGAATAATCACCACAGCAAGGTTTTAGAGCTAGAAATAGC
LL039	<i>Gba2</i>	Target 2 sgRNA F	GCGTAATACGACTCACTATAGGCGGAGGGAGCATCACTCGGTTTTAGAGCTAGAAATAGC
LL042	<i>Gba2</i>	HRM F	GTATGTGTTGTTTTTTTCAGGC
LL043	<i>Gba2</i>	HRM R	GCAATAACGGTTTTGTAGTGG
LL044	<i>Gba1</i>	HRM F	AGTCTCATCGGCAGGATGAG
LL045	<i>Gba1</i>	HRM R	CACTTGGACAGAAAGGTAAATC
LL023	<i>Gba1</i>	Sequencing F	CATTGCCATTTTCGTTTTTAGG
LL007	<i>Gba1</i>	Sequencing R	GGAAGTGTCTTGGACTCTCCAT
LL036	<i>Gba2</i>	Sequencing F	AATGGTGGTACCGAAAGACC
LL037	<i>Gba2</i>	Sequencing R	AGTACTACAGACTTCATCTGC
RB485	<i>hGBA1</i>	<i>hGBA</i> F (Gateway cloning)	GGGGACAAGTTTGTACAAAAAAGCAGGCTccACCACCATGGAGTTTTCAAGTCCTTCC
RB487	<i>hGBA1</i>	<i>hGBA</i> R (Gateway cloning)	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCATCACTGGCGACGCCACAGGTA

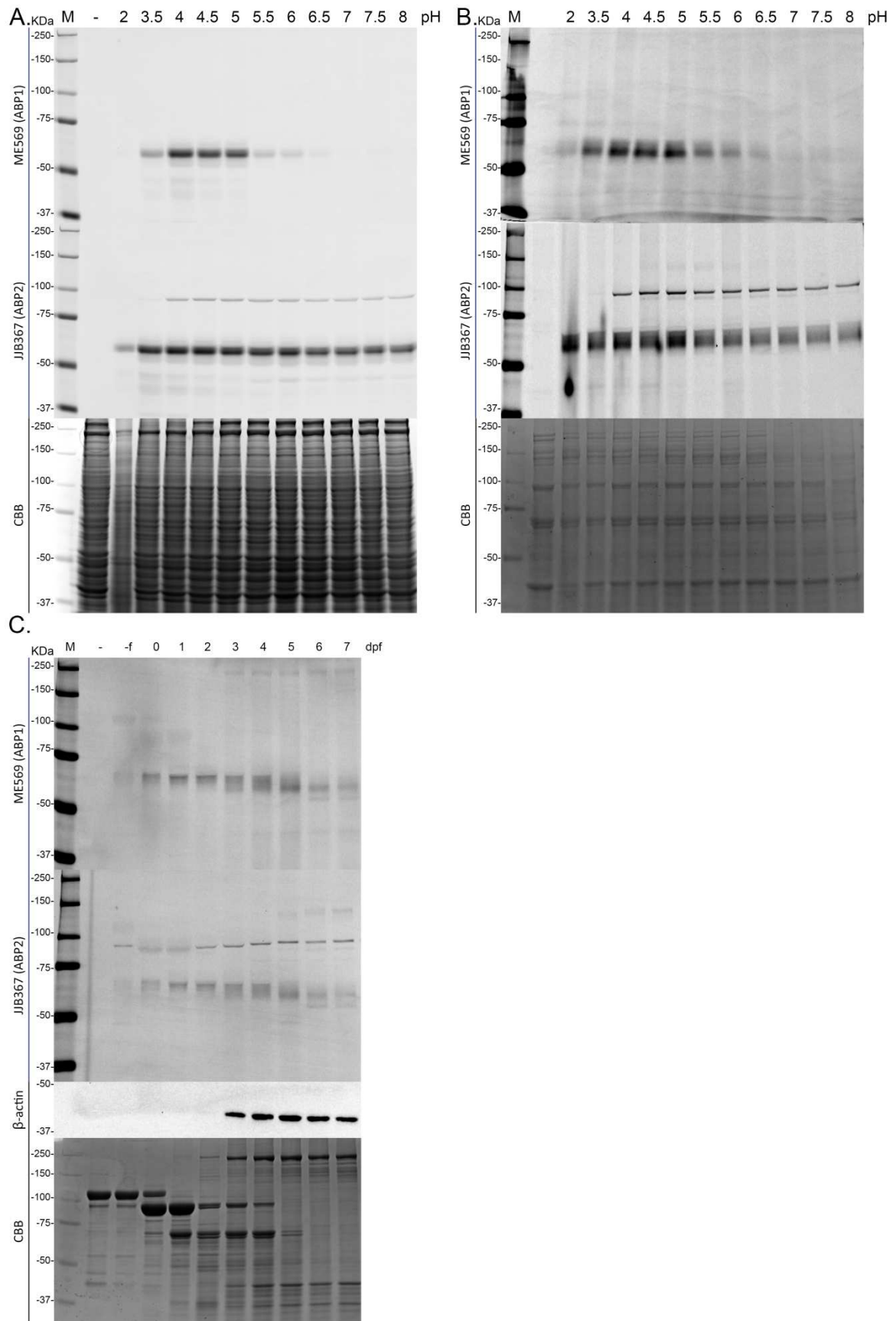
**Supplemental Table S2 | Overview of glycosphingolipid abnormalities in different Gba1-, Gba2- and Gba1:Gba2 deficient animal models.**

Differences in GlcSph, GlcCer and GlcChol in tissues from human patients (Hu) and published GD models including zebrafish (Zf) and mouse (Ms). *Mx1-Cre<sup>+</sup>*: Gba1 deficiency in the white blood cell lineage, *Limp2*: transporter of GBA1 to lysosomes, *Npc1*: exporter of cholesterol from lysosomes; a defect leads to accumulation of cholesterol and glycosphingolipids.

-: no significant increase or reduction, nd: not determined

		Animal, organ	GlcSph	GlcCer	GlcChol	Reference
Gba1						
	Gaucher disease	Hu: plasma	↑↑↑	↑	↑	(27, 58)
	Gba1 deficient (inhibitor 3)	Zf: larvae	↑↑	↑	↑	This publication
	<i>Gba1<sup>-/-</sup></i> (Full KO)	Zf: larvae Zf: brain	↑↑ ↑↑↑	↑ ↑↑	nd nd	(38)
	<i>Mx1-Cre<sup>+</sup></i> :GD1	Ms: spleen, Ms: liver	↑↑↑ ↑↑	↑ ↑	↑ ↑	(18, 27, 71)
	<i>Limp2<sup>-/-</sup></i>	Ms: spleen, Ms: liver	↑ ↑	- -	nd ↑	(27, 71)
	<i>Npc1<sup>-/-</sup></i>	Ms: spleen, Ms: liver	↑↑ ↑↑	↑ ↑	nd ↑	(71)
Gba2						
	Gba2 KO	Zf: larvae	-	↑	↓	This publication
	<i>Gba2<sup>-/-</sup></i>	Ms: spleen Ms: liver Ms: testis	- - nd	↑ ↑ ↑	↓ ↓ nd	(18, 27, 67, 72)
Gba1:Gba2						
	Gba1:Gba2 KO	Zf: larvae	↑↑	↑	↓	This publication

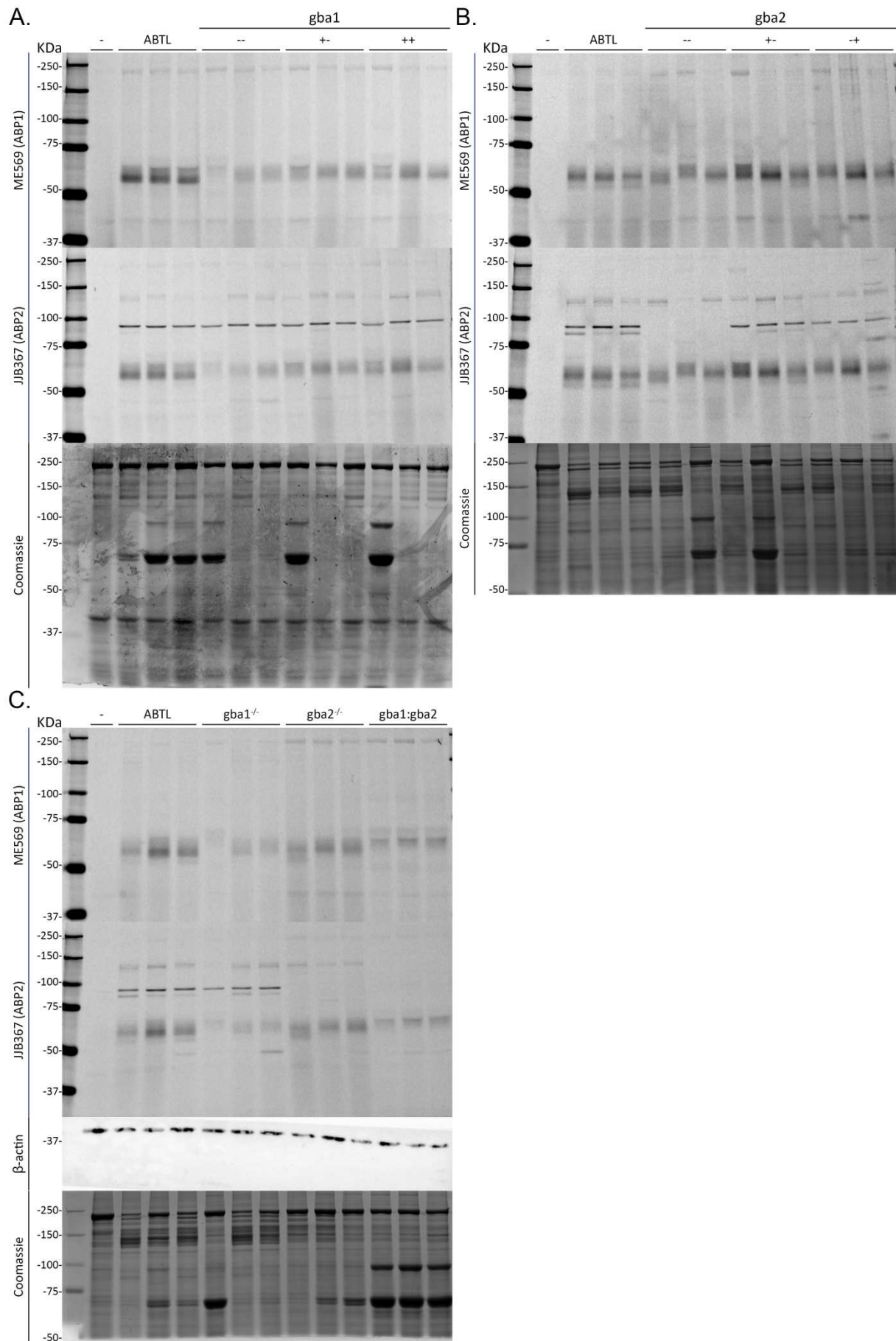
	<i>Mxl</i> -Cre <sup>+</sup> :GD1: <i>Gba2</i> <sup>-/-</sup>	Ms: spleen Ms: liver	↑↑ nd	↑ ↑	nd nd	(18)
	<i>Npc</i> <sup>-/-</sup> : <i>Gba2</i> <sup>-/-</sup>	Ms: brain	↑↑	↑	nd	(28)



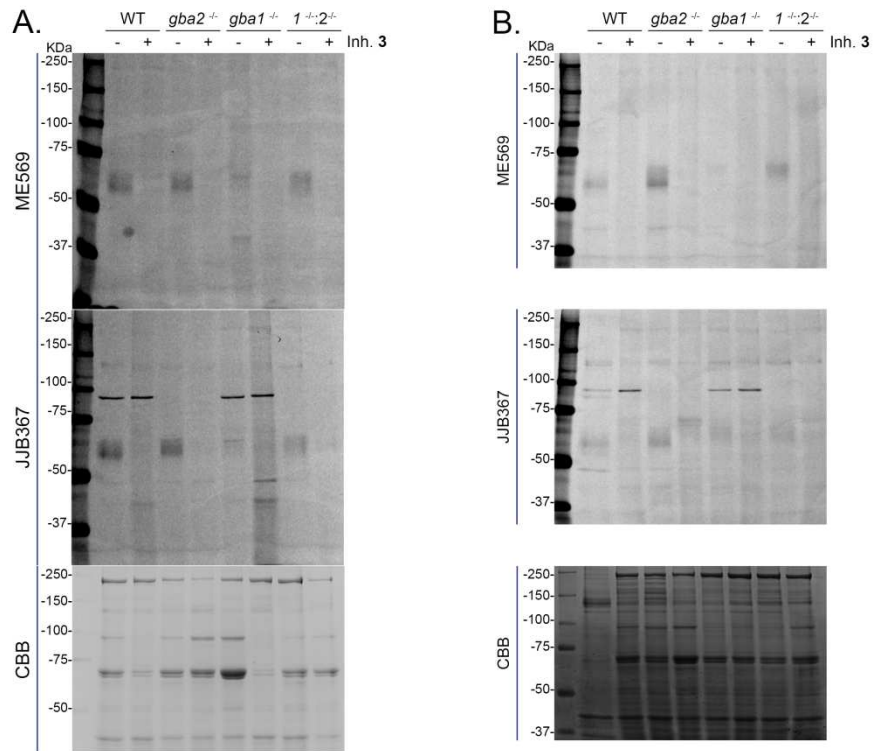
**Supplemental Figure S1** | ABP, Coomassie gels and western blot of figures 1D, 1E and 1F.



**Supplemental Figure S2** | Photos of females (left) and males (right) of WT (A), *gba1*<sup>+/-</sup> (B), *gba2*<sup>-/-</sup> (C) and *gba1*<sup>+/-</sup>;*gba2*<sup>-/-</sup> (D) adult zebrafish (9-12 months of age).

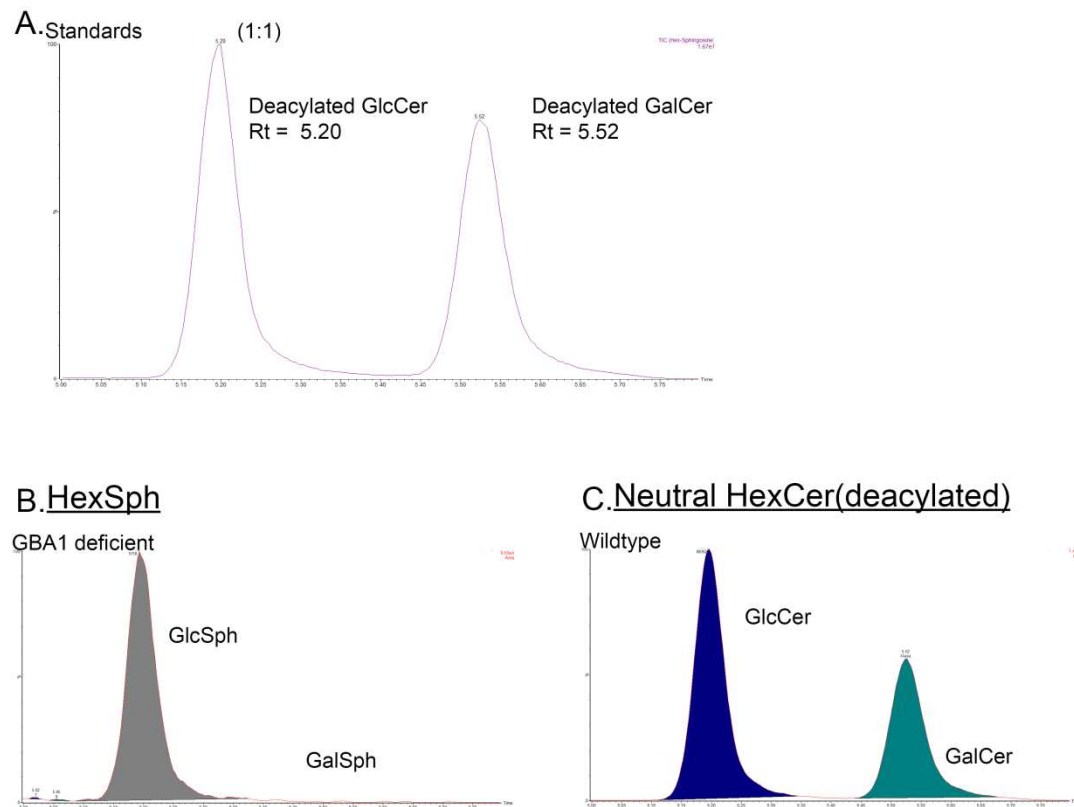


**Supplemental Figure S3** | ABP, Coomassie gels and western blot of figures 2B, 3B and 4A.



Supplemental Figure S4 | ABP, Coomassie gels of figures 5B (a) and duplo (b).

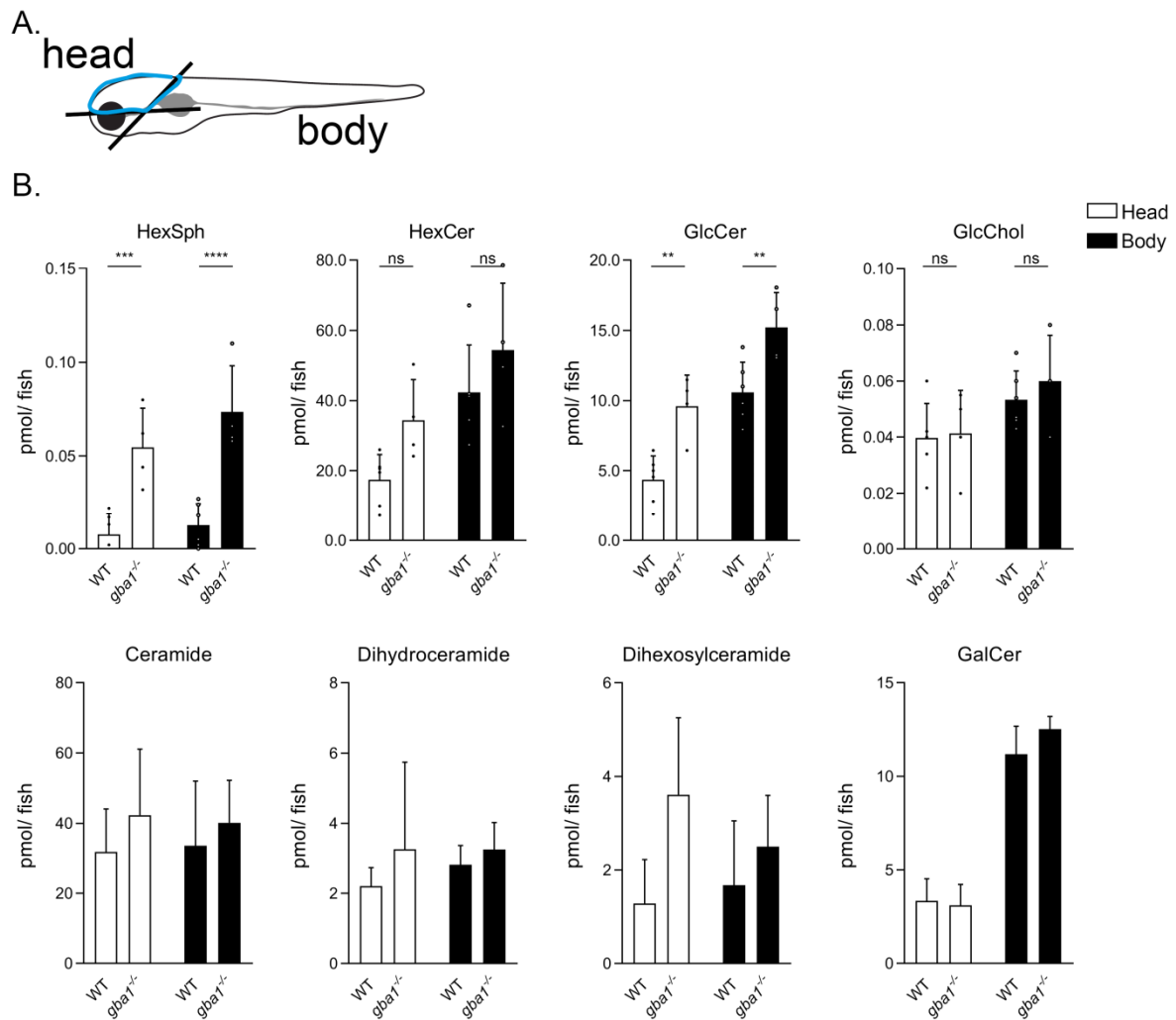
## HILIC separation - HexSph/ HexCer



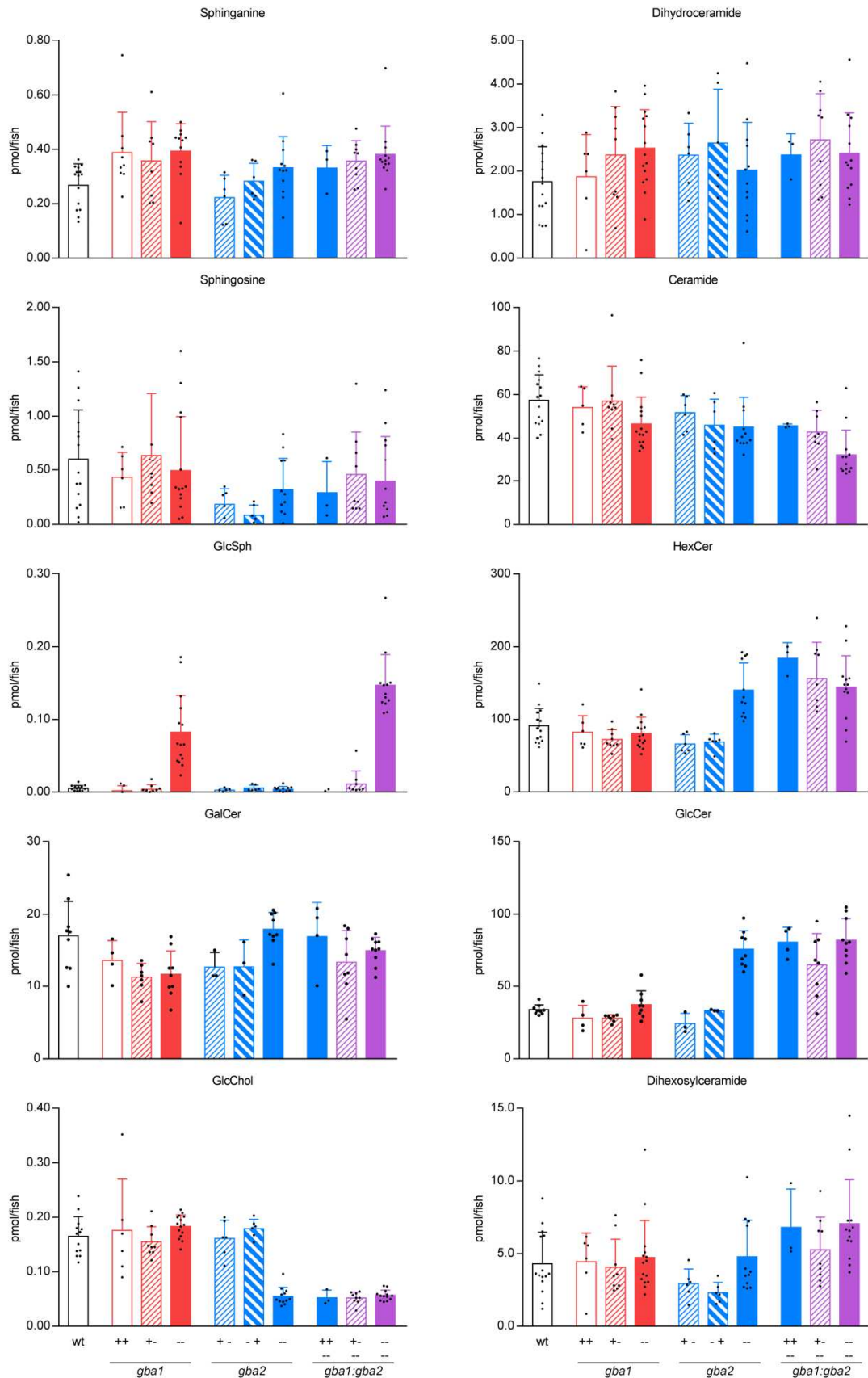
### Supplemental Figure S5 | Elution patterns of HexSph or deacylated HexCer using HILIC separation.

A) Elution profile of an equimolar mixture of glucosylceramide and galactosylceramide after microwave-assisted saponification, with deacylated GlcCer (measured as GlcSph) eluting at 5.20 min. and deacylated GalCer (measured as GalSph) eluting at 5.52 min. B) Elution profile of the upper phase separating GlcSph and GalSph of a GBA1 deficient (inhibitor **3** treated) sample. C). Elution profile of microwave-assisted deacylation of the lower phase separating deacylated GlcCer and GalCer (measured as GlcSph and GalSph respectively) of a WT sample.



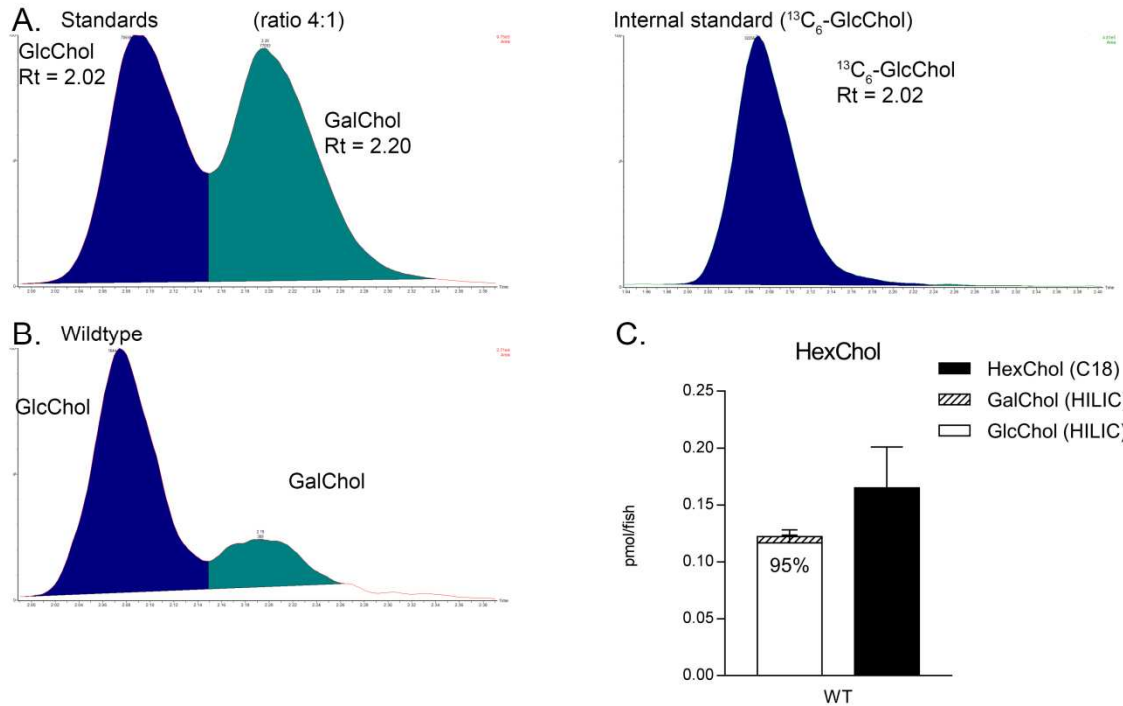


**Supplemental Figure S6** (A) Schematic representation of the dissection of a zebrafish larvae into head and body. (B) Glycosphingolipid levels of dissected head and body regions of WT and *gba1* KO larvae at 5dpf in pmol/ fish. Data is depicted as mean  $\pm$  SD and analysed using One-Way Anova (Dunnett's test) with wt as control group with \*\*\*  $P < 0.001$  and \*\*\*\*  $P < 0.0001$ .

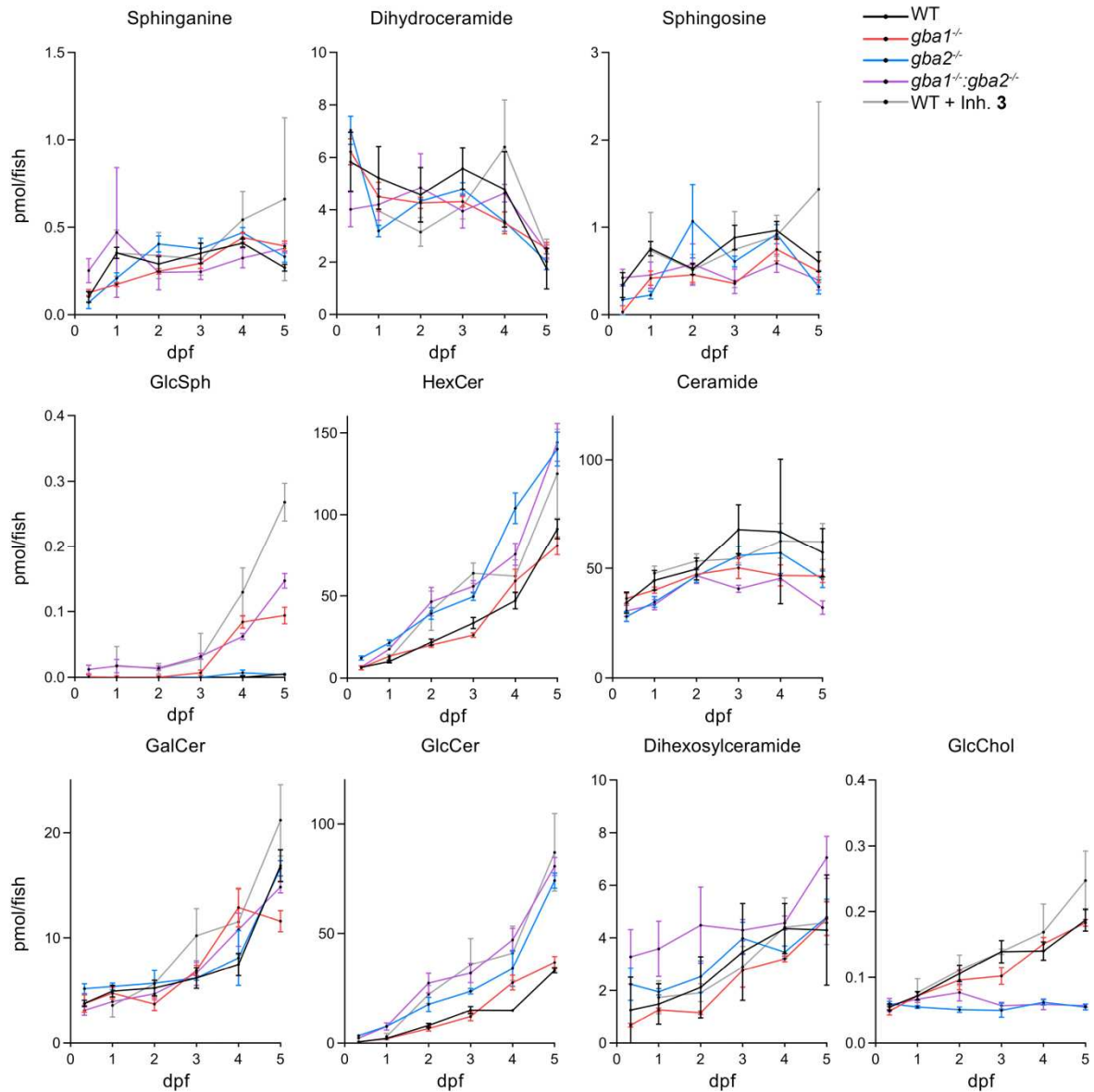


**Supplemental Figure S7** | (Glyco)sphingolipids of *gba1*, *gba2* and *gba1:gba2* genotypes in pmol/fish.

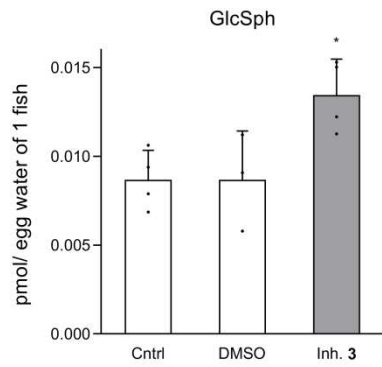
## HILIC separation - HexChol



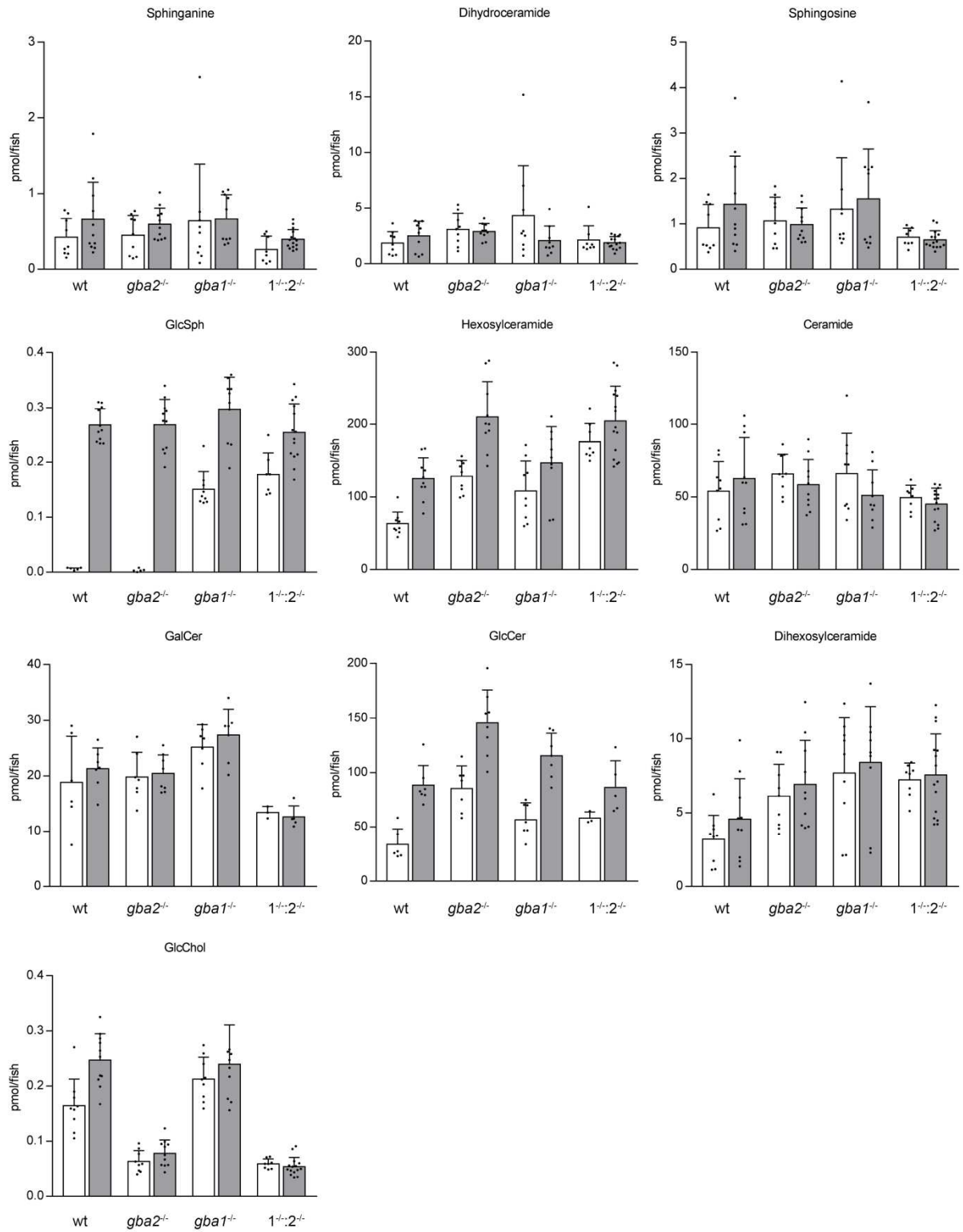
**Supplemental Figure S8** | Elution patterns of HexChol using HILIC separation. A) Elution profile of a mixture of GlcChol and GalChol (ratio of 4:1) and the  $^{13}\text{C}$ -GlcChol internal standard. B) Elution profile of a WT sample. C) Levels of GlcChol and GalChol as determined by HILIC separation (while bar and striped bar respectively,  $n = 3$ ) in pmol/ fish compared to HexChol as determined using standard methods (black bar; Figure 4B,  $n = 15$ ) in pmol/ fish.



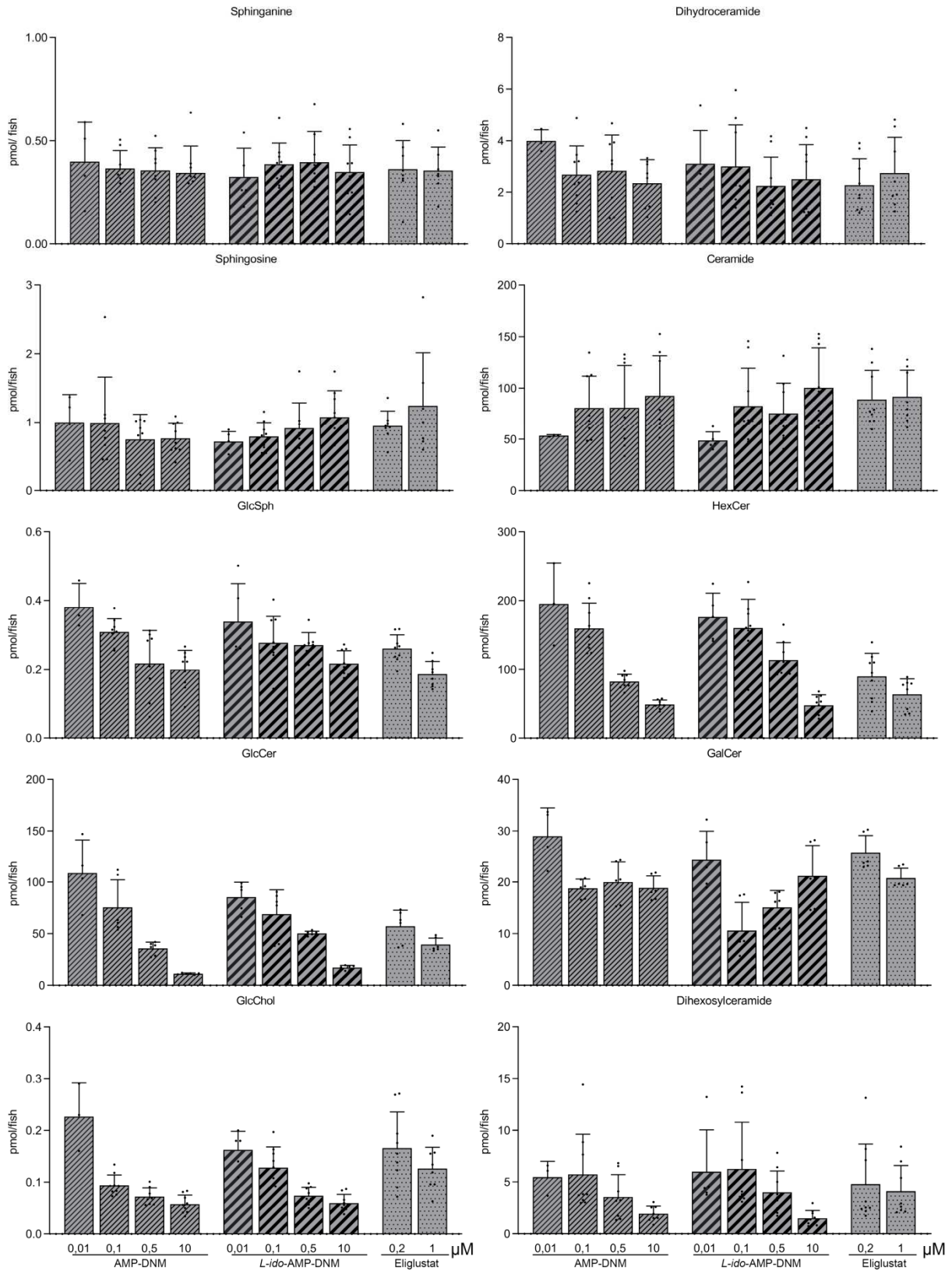
**Supplemental Figure S9** | (Glyco)sphingolipids of *gba1*, *gba2* and *gba1:gba2* embryos at different ages in pmol/fish



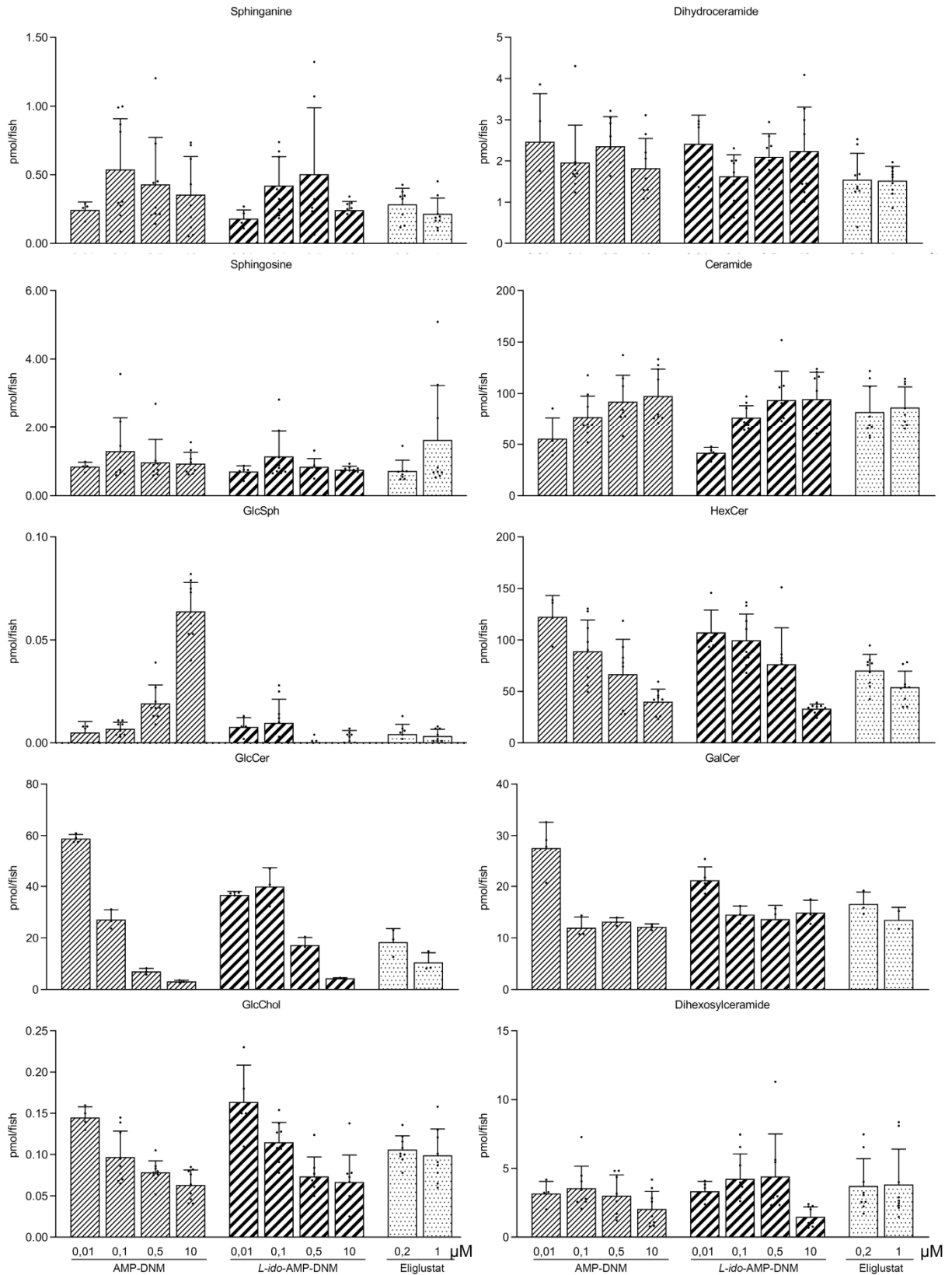
**Supplemental Figure S10** | Excreted GlcSph in egg water: control egg water (no incubation), pooled egg water of DMSO incubated zebrafish individuals (500  $\mu$ L, n = 5 individuals) and pooled egg water of inhibitor **3** incubated zebrafish individuals (500  $\mu$ L, n = 5 individuals). GlcSph is expressed in pmol/ egg water of 1 fish (100  $\mu$ L) and depicted as mean  $\pm$  SD.



**Supplemental Figure S11** | (Glyco)sphingolipids in the different *gba1* and *gba2* genetic backgrounds treated with inhibitor 3 (10  $\mu$ M, grey bars) in pmol/fish.



**Supplemental Figure S12** | (Glyco)sphingolipids of wild-type embryos incubated with 10  $\mu\text{M}$  inhibitor **3** in combination with different concentrations of AMP-DNM (striped bars), L-ido-AMP-DNM (broader striped bars) or Eliglustat (dotted bars) in pmol/fish.



**Supplemental Figure S13** | (Glyco)sphingolipids of wildtype embryos treated with different concentrations of AMP-DNM (striped bars), L-ido-AMP-DNM (broader striped bars) or Eliglustat (dotted bars) in pmol/fish.