

Psychosine-triggered endomitosis
is modulated by membrane sphingolipids
through regulation of phosphoinositide
4,5-bisphosphate production at the
cleavage furrow

(サイコシンによるエンド

ミトーシスは、分裂溝における
ホスファチジルイノシトール 4,5-ビス
リン酸の生合成を制御する膜の
スフィンゴ脂質類によって調節される)

渡邊 寛

Title

Psychosine-triggered endomitosis is modulated by membrane sphingolipids through regulation of phosphoinositide 4,5 biphosphate production at the cleavage furrow^{*}

Authors

Hiroshi Watanabe ¶, Kyohei Okahara †, Yuko Naito-Matsui †, ||, Mitsuhiro Abe §, Shinji Go††, Jinichi Inokuchi††, Toshiro Okazaki ‡, Toshihide Kobayashi §, Yasunori Kozutsumi †, Shogo Oka ¶ and Hiromu Takematsu ¶, **

¶From the Laboratory of Biological Chemistry, Human Health Sciences, Graduate School of Medicine, Kyoto University, † Laboratory of Membrane Biochemistry and Biophysics, Graduate School of Biostudies, Kyoto University, § RIKEN Frontier Research System and RIKEN, Advanced Science Institute, ‡ Department of Hematology and Immunology, Kanazawa Medical University, †† Division of glycopathology, Institute of Molecular Biomembranes and Glycobiology, Tohoku Pharmaceutical University || Current address: Department of Biochemistry, Kobe Pharmaceutical University

Running title: *Sphingolipids modulate cytokinesis*

**Correspondence should be addressed to Hiromu Takematsu, Laboratory of Biological Chemistry, Human Health Sciences, Graduate School of Medicine, Kyoto University, Room 414, 53 Shogoin-Kawahara, Sakyo, Kyoto, Japan 606-8507 Tel: +81-75-751-3954, E-mail: htakema@pharm.kyoto-u.ac.jp

*** Abbreviations used are as follows: Cer, ceramide; EGFP, enhanced green fluorescent protein; FCM, flow cytometry; Gb3, Gal α 1-4 Gal β 1-4 Glc β 1-1 Cer; GlcCer, Glc β 1-1 Cer; GM1, Gal β 1-3 GalNAc β 1-4 (Sia α 2-3) Gal β 1-4 Glc β 1-1 Cer; GM3, Sia α 2-3 Gal β 1-4 Glc β 1-1 Cer; GSL, glycosphingolipid; IRES, internal ribosomal entry site; LacCer, Gal β 1-4 Glc β 1-1 Cer; MSCV, murine stem cell virus; MS, mass spectrometry; PI, phosphatidylinositol; PIP₂, phosphatidylinositol (4,5) bisphosphate; PDMP, (D-threo)-1-phenyl-2-decanoylamino-3-morpholino-1-propanol; SM, sphingomyelin; SMS, SM synthase; TLC, thin layer chromatography.

ABSTRACT

Endomitosis is a special type of mitosis, in which only cytokinesis, a final step of the cell division cycle is defective, thus resulting in the polyploid cells. Although endomitosis is biologically important process, its regulatory aspects remain elusive. Psychosine, a lyso-galactosylceramide, prevents proper cytokinesis when supplemented to proliferating cells. Cytokinetic inhibition by psychosine does not inhibit genome duplication. Consequently, cells undergo multiple rounds of endomitotic cell cycles, resulting in the formation of giant multiploid cells. Here, psychosine-triggered multiploid cell formation was successfully quantified, enabling demonstration that membrane sphingolipids ratios modulate psychosine-triggered polyploidy in Namalwa cells. Among enzymes that experimentally remodel cellular sphingolipids, overexpression of glucosylceramide synthase to biosynthesize glycosylsphingolipids (GSLs) and neutral sphingomyelinase 2 to hydrolyze sphingomyelin (SM) additively enhanced psychosine-triggered multiploidy; notably, almost all of the cells became polyploid. In the presence of psychosine, Namalwa cells attenuated cell surface SM clustering and suppressed phosphatidylinositol-4,5-bisphosphate (PIP₂) production at the cleavage furrow, both important processes for cytokinesis. Depending on the sphingolipid balance between GSLs and SM, Namalwa cells could be effectively converted to viable multiploid cells with psychosine.

INTRODUCTION

During somatic cell division, the mother cell replicates chromosomes and redistributes the intracellular contents to ensure the functional properties of the two daughter cells. Cytokinesis is the final step of mitosis that divides daughter cells after appropriate segregation of the duplicated cellular contents (Carmena, 2008). In cytokinetic cells, the cleavage furrow, an indentation of the plasma membrane between two nascent daughter cells, further matures into a microtubule-derived midbody (Steigemann and Gerlich, 2009). Endomitosis is a special kind of cell cycle in which only cytokinesis is defective in the mitotic phase, enabling cells to increase cellular size and ploidy. However, the overall picture of ensuring proper endomitosis has remained elusive to date, particularly regarding the membrane molecules involved and how this important mitotic event is regulated.

The cellular membrane is composed of lipids and embedded proteins, and various cell membrane activities are affected by lipids as constituents and/or signaling molecules. One class of membrane lipid constituents are sphingolipids, biosynthesized from sphingosine and its acylated form, ceramide (Merrill Jr. and Sandhoff, 2002). Glycosphingolipids (GSLs^{***}), a glycosylated class of sphingolipids, comprise one of the major membrane components. GSLs are biosynthesized by glycosylation of ceramide, the lipid component of most GSLs. Psychosine is galactosyl-sphingosine, also called lyso-galactosylceramide, which lacks the fatty acid amide bonded to sphingosine in ceramide. Psychosine exhibits various cellular activities when supplied to cell culture (Hannun and Bell, 1987, 1989; Suzuki, 1998; Lloyd-Evans *et al.*, 2003a). Among them, psychosine induces polyploidization of cultured cells due to inhibition of cytokinesis. When the human monocytic cell line U937 was treated with psychosine, the DNA content of the cells could be elevated to a ploidy of 32N (undergoing four rounds of failed cytokinesis in single

cells) within 72 h of culture (Kanazawa *et al.*, 2000). Time-lapse imaging indicated that polyploidization was achieved due to a combination of cytokinetic prevention, lack of polyploidy-mediated apoptosis and progression to S-phase to duplicate chromosomes. Accordingly, the cell size was enlarged in multiploid cells arising from psychosine-treatment.

It is noteworthy that, unlike chemical compounds that inhibit cytokinesis, psychosine is a naturally occurring mammalian lipid molecule, although knowledge about its natural expression is very limited (Svennerholm *et al.*, 1980; Suzuki, 1998; Kozutsumi *et al.*, 2002). Psychosine is found in the brains of patients with the lysosomal storage disease globoid cell leukodystrophy (GLD). GLD patients have a genetic mutation of the lysosomal degradation enzyme β -galactosylceramidase, resulting in major accumulation of galactosylceramide (Miyatake and Suzuki, 1972; Wenger *et al.*, 1974). Although its biosynthetic pathway is not established to date, psychosine causes the pathogenesis of GLD due to neuronal toxicity (Svennerholm *et al.*, 1980; Shinoda *et al.*, 1987; Suzuki, 1998). In addition, a giant polyploid cells, called globoid cells, occurs in GLD patients, presence of which is used as a diagnostic marker for the disease. Previous report indicated that similar giant multiploid cell formation is induced by psychosine (Kanazawa *et al.*, 2000). Thus psychosine-mediated multiploid formation is regarded as a key event for globoid cell formation; although molecular basis of formation is still elusive. When the intracellular events were examined, psychosine induced dispersement of TGN46-positive vesicles in COS-7 cells; thus, vesicular intracellular trafficking event(s) downstream of the Golgi apparatus could be affected by psychosine to induce multiploid cells (Kanazawa *et al.*, 2008). Attenuated vesicle transport caused by psychosine could affect delivery of both membrane proteins and lipids to the cleavage furrow.

In general, cellular membranes consist of glycerophospholipids, cholesterol, and

sphingolipids. Sphingolipids consist of sphingosine with various modifications. Ceramides are N-acylated sphingosine molecules that can be further modified with various polar head groups. The hydrophilic head group is provided by glycans or phosphocholine, which produce GSLs and SM in the Golgi apparatus. A cytokinesis study in sea urchin eggs showed that the ganglioside GM1, a GSL species, and cholesterol-enriched lipid microdomains are accumulated during furrow progression (Ng *et al.*, 2005). More recently, it was reported that the midbody of cytokinetic Hela cells accumulates sphingolipid species such as C-24 hexosylceramide (Atilla-Gokcumen *et al.*, 2014). Cellular sphingomyelin (SM) clusters[¶], which were stained using the earthworm toxin lysenin, were also suggested to be involved in phosphoinositide metabolism for cytokinesis completion (Abe *et al.*, 2012). Hence, sphingolipids appeared to play important roles in cytokinesis, it is not clear how GSLs are involved in cytokinesis or related trafficking events.

In the present study, the effects of cellular sphingolipids to produce polyploid cells were examined in the presence of psychosine. Psychosine-mediated polyploidization was quantitatively analyzed in the human B cell line Namalwa, which exhibits robust susceptibility to the effects of psychosine, more responsive than U937 and COS-7 cells, which were shown in previous studies to form multiploid cells upon psychosine treatment (Kanazawa *et al.*, 2000; Kanazawa *et al.*, 2008). Further sphingolipid perturbation studies showed that cellular GSL and SM levels modulated cellular susceptibility to psychosine-induced polyploidization. Evidence was found that membrane SM clustering was disrupted by psychosine. These data suggest that psychosine-triggered polyploidization may be sensitive to ratios of glycosphingolipids and sphingomyelin.

RESULTS

Lack of correlation between TDAG8 expression and psychosine-triggered cytokinetic defects

Psychosine is a lyso-form of galactosylceramide. Currently, the biosynthetic pathway to form lyso-GSL is not known. Psychosine expression is observed in β -galactosylceramidase-deficient animals. Since psychosine elicits various cellular responses (Igisu and Suzuki, 1984; Hannun and Bell, 1987; Okajima and Kondo, 1995; Lloyd-Evans *et al.*, 2003b), the mechanism of how cells respond to psychosine has been debated to date. It was previously suggested that the G protein-coupled receptor TDAG8 is a receptor for psychosine, because TDAG8-expressing cells acquired psychosine sensitivity to induce multiploidy in RH7777 and HEK293 cells (Im *et al.*, 2001). Subsequently, TDAG8 was also proposed as a proton-sensing receptor (Wang *et al.*, 2004). Furthermore, it was shown that macrophages from *Tdag8*-null mice undergo multiploidization upon psychosine treatment (Radu *et al.*, 2006). To date, the receptor responsible for psychosine-mediated polyploidization has yet to be identified. First of all, psychosine-mediated polyploidization was compared among U937, Namalwa and KMS12-PE cell lines to examine the basis for induced cellular ploidy (**Figure 1A**). Among these cells, Namalwa B lymphoma cells were the most sensitive according to detection of 8/16N cells with 2.5 μ M psychosine treatment. U937 cells were less sensitive than Namalwa cells, and myeloma KMS12-PE cells were not polyploidized with 5 μ M psychosine. To determine whether TDAG8 expression correlates with psychosine-mediated multiploid cell nucleation, its expression level was examined in these cell lines (**Figure 1B**). TDAG8 was detected in U937 cells, whereas Namalwa and KMS12-PE cells were negative for staining. The finding that TDAG8-negative Namalwa cells had the highest sensitivity to psychosine is consistent with results in *Tdag8*-null macrophages, where Radu *et al.* showed that TDAG8 does not seem to be involved in

psychosine-induced multiploidy (Radu *et al.*, 2006). Thus, it is unlikely that TDAG8 functions as a specific receptor of psychosine to cause cytokinetic defects.

Quantitative determination and profiling of psychosine-mediated multiploidy

Psychosine susceptibility and resulting ploidy varied among cell types (**Figure 1A**). Therefore, psychosine-induced multiploidy was quantified using six different B cell lines, because quantitative profiling and correlation analyses of cellular phenotypes could be useful to uncover genetic traits (Yamamoto *et al.*, 2007). Lacking a standard procedure to quantitatively evaluate polyploidizing activity among different cell lines, the nuclear status of the cell lines was measured upon treatment with a graded dose of psychosine. Dose-responses of each cell line were different for multiploidization. Therefore, to accurately quantify psychosine-mediated multiploidy, the percentage of >4N cells was determined with incremental doses of psychosine. For normalization, this value was divided by the concentration of psychosine used for each condition. The maximal value was used for each cell line to quantitatively express sensitivity for psychosine-mediated polyploidization. This value was called the “psychosine-mediated ploidy index number” (PPIN). When the six-cell line profile of PPIN was expressed as a web graph (**Figure 1C**), a similarity was found in the pattern with that of cell surface GM1 expression level, measured with the cholera toxin B subunit (CTxB) as in a previous study with the same set of cell lines (Takematsu *et al.*, 2011). The Pearson’s correlation coefficient between these profiles was positive ($r=0.82$). The presence of such a strong positive correlation suggested that cell surface GM1 level could have an effect on psychosine-mediated multiploid cell formation.

Requirement of glycosphingolipids in efficient psychosine-triggered multiploidization

The GM1 level was hypothesized to be a cellular factor determining psychosine sensitivity. GSL

expression has a propensity to be cell type- and state-specific (Kannagi *et al.*, 1983; Hakomori and Zhang, 1997; Sonnino *et al.*, 2007). To evaluate this positive correlation functionally, Namalwa cells were chosen for the remainder of the present study because they were sensitive to polyploidization. To examine the functional participation of cellular glycosphingolipids (GSLs, biosynthetic pathway is summarized in **Figure 2A**) in psychosine-triggered multiploidization, the effect of GM1 knockdown was examined by means of short hairpin RNA (shRNA)-treated Namalwa cells. Two shRNA species for GM1 synthase (GM1Syn, encoded by *B3GALT4*) exhibited a knockdown effect on cholera toxin B (CTxB) staining, which probes cell surface GM1 expressed on Namalwa cells with high sensitivity (Takematsu *et al.*, 2011). ShRNA corresponds to nucleotide residues 252-272 (Sh252-272) and 311-331 (Sh311-331) which exhibited roughly 70% and 30% reduction in flow cytometric CTxB staining to the control (ShLaminB), respectively (**Figure 2B**). The knockdown was also resulted in loss of GM2 in LC-MS detection whereas GM3 level was not affected (**Supplemental Figure S1**). More prominent suppression in multiploidization was found in Sh252-272 as peak ploidy shifted to 4N in this cells. Although peak ploidy was 8N, similar to control, relative increase in 2N/4N peaks was found in Sh311-331 (**Figure 2C**). These data indicated that cell surface GM1 levels could quantitatively modulate psychosine-triggered multiploidy.

To examine whether or not psychosine-triggered multiploidy could be artificially manipulated by chemical inhibitors, cells were treated with PDMP, a glucosylceramide synthase (GlcCerSyn) inhibitor which reduces cellular GSLs (**Figure 2A**) (Inokuchi *et al.*, 1989). The dosage of PDMP was carefully determined, because its toxicity can cause cell cycle arrest. Addition of PDMP up to 20 μ M did not attenuate proliferation of Namalwa cells (**Supplemental Figure S2**). PDMP treatment dose-dependently reduced cell surface GM1 expression in Namalwa cells (**Figure 2D**).

In the absence of PDMP, psychosine treatment resulted in the induction of 8N and 16N peaks. These multiploid peaks were suppressed with graded dose of PDMP (**Figure 2E**). These data indicated that levels of cell surface GM1, which is reduced by knockdown of *GM1Syn* or PDMP treatment, could somehow affect psychosine-mediated polyploidization.

Evaluation of the involvement of GSL species in polyploidy

Cellular GSLs are present in multiple molecular species, and their balance varies among cells. Moreover, cellular metabolism of various classes of lipids is inter-connected. Thus, in general, caution should be taken to evaluate experimental results obtained from inhibitor treatment, because these chemicals can simultaneously alter other unexpected cellular metabolic pathway(s). To circumvent these potential pitfalls, cells with GSLs altered *via* modulation of glycosyltransferase gene expression were also examined. The major GSL species in Namalwa cells were previously shown to be in the ganglio-series (Takematsu *et al.*, 2011). Human B cells show drastic alteration in GSL species; activated germinal center B cells remodel major GSL species from ganglio-series (such as GM3, GM1) to globo-series (such as Gb3). This was found to be due to the genetic dominance of Gb3 synthase (*Gb3Syn*, lactosylceramide α 1-4 galactosyltransferase, encoded by *A4GALT*) at a pathway branch (Keusch *et al.*, 2000; Kojima *et al.*, 2000; Takematsu *et al.*, 2011) (**Figure 3A**). This dominant effect of *Gb3Syn* was exploited to modulate GSL expression. Importantly, Gb3Syn is a dual-function glycosyltransferase; in addition to α 1-4 galactosyltransferase activity, Gb3Syn can form an intra-Golgi complex with LacCer synthase (encoded by *B4GALT6*). Consequently, mutant Gb3Syn (Gb3Syn-TxT, wherein the DxT motif of the enzyme was converted to TxT) reduced LacCer and ganglio-series GSLs in a dominant negative manner without biosynthesizing the globo-series (**Figure 3B**, **Supplemental Figure S3**) (Takematsu *et al.*, 2011). Therefore, Gb3Syn-TxT cells were useful to

evaluate functional importance of overall GSL expression in psychosine-triggered polyploidy, without using chemical inhibitors. Consistent with PDMP-mediated GSL inhibition (**Figure 2E**), Gb3Syn-TxT cells (exhibiting global GSL reduction downstream of LacCer) were also less susceptible to psychosine-mediated polyploidization showing a prominent decrease in both 8N and 16N peaks and a consequent increase in the 2N peak in the presence of psychosine (**Figure 3C**). Unlike PDMP treatment, Gb3Syn-TxT cells did not alter the GlcCer level (**Figure 3B**, **Supplemental Figure S3**) (Takematsu *et al.*, 2011), yet similar suppression of psychosine-mediated polyploidization was found in these cells (**Figure 3C**). This result was consistent with the suggestion that reduced GM1 could negatively modulate psychosine-triggered multiploidization. To examine the level effects of different glycolipid species, *Gb3Syn* was introduced into Namalwa cells, in which the major GSL species was shifted to Gb3 (**Figure 3B**) (Takematsu *et al.*, 2011). Unlike Gb3Syn-TxT cells, Gb3Syn cells exhibited comparable multiploidization upon psychosine addition. This result indicated that psychosine-mediated multiploidization is not altered by the change from ganglio-series to globo-series GSL(s) (**Figure 3C**). These results suggested that specific GSL species such as GM1 and Gb3 could have similar effects to enhance psychosine-triggered multiploidization.

Enhanced psychosine-induced multiploidization upon introduction of GlcCerSyn

Reductions of GSL resulted in resistance to the psychosine-triggered multiploidization (**Figure 2D** and **Figure 3C**). Induction of GSL was useful to examine enhanced cellular response to psychosine. GlcCerSyn (encoded by *UGCG*) responsible for cellular GSL levels was expressed to induce GSL levels. When *GlcCerSyn* was introduced into Namalwa cells, ploidy value was not different from control cells. However, psychosine induced ploidy of the cells (**Figure 4A**). Because the difference in polyploidy was less than two-fold, this experiment was repeated five

times to confirm the reproducibility and statistical significance (**Figure 4B**). These data indicated that levels of GSLs quantitatively affect psychosine-triggered multiploidization.

Additive effect of GlcCerSyn and sphingomyelinase

Modulation of *GlcCerSyn* expression reduces ceramide as well as increases GSLs (**Figure 2A**, **Supplemental Figure S3**). Was the increase in GSLs or reduction in ceramide responsible for this phenotypic change? In addition to the *de novo* pathway, ceramide is also produced by sphingomyelinase (SMase). SM is strongly expressed on the plasma membrane, and thus, could serve as a source of ceramide. Among SMases, neutral SMase2 (nSMase2) functions to scramble ceramide from SM on the cell surface; thus, nSMase2 is a good candidate to alter the SM/ceramide balance (Hofmann *et al.*, 2000; Marchesini *et al.*, 2003; Tani and Hannun, 2007). Unlike *GlcCerSyn* expression, nSMase2 expression was expected to increase ceramide levels, although both of these enzymes favor the biosynthesis of GSLs downstream. When *nSMase2* was expressed, an approximately 10% suppression in SM was detected by densitometry in TLC analyses (**Figure 4C**). By contrast, expression levels of GlcCer, LacCer and GM3 were not affected. To account for the loss of SM, ceramide was increased 3-fold in nSMase2 cells in lipidomic LC-MS analysis (**Supplemental Figure S3**). *GlcCerSyn* expression caused more apparent changes in the GSL levels: an approximate two-fold increase in GlcCer and LacCer and an approximate 25% increase in GM3 were noted on TLC and LC-MS (**Figure 4C**, **Supplemental Figure S3**). In both cases, sensitization of Namalwa cells to psychosine-induced polyploidization was detected (**Figure 4D**). These data indicated that an increase in GSLs rather than a reduction in ceramide sensitizes Namalwa cells to psychosine-mediated inhibition of cytokinesis to produce multiploid cells. The effects of *GlcCerSyn* and *nSMase2* were additive, because simultaneous expression of both enzymes resulted in the most sensitive phenotype to

psychosine (**Figure 4D**). Notably, 2N cells were barely detectable under this condition, which indicated that nearly the entire population of cells in culture was prevented from achieving cytokinesis by psychosine.

Specificity of lyso-sphingolipid species on multiploidy

It was previously reported that both psychosine and gluco-psychosine (GlcPsy) could induce U937 multiploidization (Kanazawa *et al.*, 2000). Since the combination of Gb3Syn-TxT Namalwa cells and GlcCerSyn/nSMase2 Namalwa cells was a useful system to analyze the effect of membrane GSLs, it was used to examine the effect of other lyso-sphingolipid species on multiploidization. Consistently as with U937 cells, GlcPsy induced Namalwa cell multiploidization whereas lyso-LacCer and sphingosylphosphorylcholine (SPC) did not (**Supplemental Figure S4A**). Similar to psychosine treatment (**Figure 3C**), Gb3Syn-TxT cells (reduced GSLs) were less polyploidized by GlcPsy. Unlike psychosine treatment (**Figure 4D**), GlcCerSyn/nSMase2 expression did not enhance GlcPsy-mediated polyploidization at concentration of 5 μ M (**Supplemental Figure S4B**) when compared to control cells. In this condition, however, 2N cells were almost undetectable even in the controls. GlcPsy concentration was titrated to examine GlcCerSyn/nSMase2-mediated enhancement in GlcPsy-triggered multiploidization. At 1.25 μ M, GlcPsy more efficiently induced GlcCerSyn/nSMase2 cell-multiploidy compared to controls (**Supplemental Figure S4C**). This enhancement was not increased at 2.5 μ M GlcPsy probably due to the very efficient multiploidization stimulating activity of GlcPsy.

Cell type-specific effect of GlcCerSyn expression

Given that membrane GSL could modulate psychosine susceptibility, *GlcCerSyn* elevation was

examined to alter the psychosine sensitivity of unresponsive cells. Myeloma KMS12-PE cells were resistant to psychosine-induced multiploidy. (**Figure 1A**). Introduction of *GlcCerSyn* in KMS12-PE cells did not alter this resistance (**Supplemental Figure S5**). Thus, psychosine sensitivity can be modulated by GSL expression levels in susceptible cells (such as Namalwa), but cellular GSL levels alone do not sensitize cells to psychosine; rather, there may be a *bona fide* receptor affected by GSL levels.

Suppression of psychosine susceptibility by sphingomyelin (SM)

Because nSMase2 cells exhibited enhanced psychosine-triggered polyploidization, SM was evaluated as a cellular factor controlling psychosine susceptibility (**Figure 4D**). SM was presumed to be independent of cellular GSLs because single nSMase2 introduction did not alter the GSL level (**Figure 4C**). Namalwa cells were pre-treated with incremented doses of SM. Unlike GSL treatment of Namalwa cells, which had no effect (**Supplemental Figure S6**), SM treatment attenuated psychosine-mediated multiploidization dose-dependently (**Figure 5A**). When cell surface SM was digested by bacterial SMase (bSMase) treatment, which reduced SM levels and increased GSL level (**Supplemental Figure S7**), enhanced psychosine-triggered multiploidy was detected (**Figure 5A**). Therefore, SM levels could negatively modulate the psychosine effect. To perturb cellular GSL/SM biosynthesis with enzyme gene expression, SM synthase (SMS) cDNAs were introduced into Namalwa cells. SMS occurs in two isoforms: Golgi-specific SMS1 (Yamaoka *et al.*, 2004) and plasma membrane/Golgi-specific SMS2 (Tafesse *et al.*, 2007). In Namalwa cells, SMS1 was strongly expressed, and SMS2 expression was weak, according to Western blotting of FLAG-tagged SMS proteins (**Figure 5B**). The apparent presence of the SM cycling pathway (Hannun, 1994) prohibited the cells from elevating SM levels higher than ~20% in the SMS2 cells by TLC (**Figure 5C**). Lipidomic LC-MS showed

a more prominent (~30%) induction of SM in both SMS1 and SMS2 cells (**Supplemental Figure S3**). Therefore, these cells were useful for functional analyses of SM levels for psychosine-triggered multiploidization.

Cell surface SM level and organization was monitored using NT-lysenin, an earthworm toxin specifically targeting SM clusters. Monomeric recombinant NT-lysenin was shown to be useful for SM detection (Ishitsuka and Kobayashi, 2004). When SMS1 and SMS2 cells were stained with NT-lysenin, consistent with the data from TLC analyses, a detectable increase in cell surface SM was observed (**Figure 5D**). Comparison of these cells with vector-transformed control cells showed suppressed psychosine-mediated polyploidization in both SMS1 and SMS2 cells (**Figure 5E**). Since the effect was subtle, the mean ploidy was calculated from six independent experiments and results were expressed as the difference from control (**Figure 5F**). These data indicated that increased SM expression relative to GSL/GM1 could be a modulatory factor in psychosine sensitivity. A stronger effect in SMS1 cells than in SMS2 cells could be interpreted as a difference in the intracellular localization of these two isoforms. Collectively, these results indicated that elevation of the SM:GSL ratio reduces susceptibility to psychosine. When other parameters are fixed, SM expression negatively modulates, and GSL expression positively modulates psychosine-triggered multiploidization of Namalwa cells.

Suppressed cell surface SM clustering and PIP₂ production by psychosine

SM could be present on the cell membrane as clusters or domains. In this manuscript, the phrase “cluster of lipids” is used to indicate small aggregates composed of less than 10 lipid molecules, to which lysenin probe binds (Ishitsuka *et al.*, 2004). By contrast, a “domain” is the specific area of the membrane with high labeling density of lipid-binding proteins. Here, the effect of psychosine on SM clusters was examined. Hela cells undergoing cytokinesis showed

lysenin-positive SM cluster enrichment in the outer membrane of cleavage furrows (Abe *et al.*, 2012). Subcellular localization of SM was examined in relation to the cleavage furrow using a monomeric NT-lysenin probe, which avoids formation of probe-mediated SM aggregation (Ishitsuka and Kobayashi, 2004). Psychosine treatment reduced NT-lysenin staining by flow cytometry in Namalwa cells (**Figure 6A**). Equinatoxin II is another SM-binding probe that preferentially binds to SM even when dispersed (Makino *et al.*, 2015). In sharp contrast to lysenin, equinatoxin II stained both control and psychosine-treated cells with roughly equal intensity (**Figure 6A**), indicating that the abundance in cell surface SM per se was not attenuated. Thus, psychosine attenuated SM clustering, which was presumably caused by cholesterol-mediated partitioning of the membrane. When psychosine-treated cells were examined under a fluorescence microscope, consistent overall reduction in lysenin staining was detected in both dividing (**Figure 6B**) and non-dividing cells. Taken together, the data show that psychosine disrupts outer leaflet SM clustering, where phosphatidylinositol-4-phosphate 5-kinase is recruited to biosynthesize PIP₂ at the inner leaflet of the membrane (Abe *et al.*, 2012). To evaluate further, PIP₂ production in the cleavage furrows of dividing cells was detected using the PH-GFP probe, which contains the PH domain from PLC δ (Field *et al.*, 2005). The cleavage furrow PIP₂ level is important for furrow ingression at anaphase (Field *et al.*, 2005). Therefore, psychosine-mediated loss of the cleavage furrow PH-GFP signal was examined in cells during late mitosis. Cleavage furrows of psychosine-treated cells showed a reduced PH-GFP signal more prominently in anaphasic cells (**Figure 6, C and D**), indicating that psychosine disrupted SM clustering which is associated with accumulation of PIP₂ at the cleavage furrow. The number of telophasic cells (with elongated cleavage furrow) was reduced upon psychosine treatment, probably caused by the defect in anaphase of psychosine treated cells. Such results were consistent and more prominent in GlcPsy cells whereas non-polyploidizing lyso-LacCer and SPC

did not exhibit any of these effects, despite similar physical properties shared by all of these lyso-sphingolipids (**Figure 6, C and D**). Therefore, it is proposed here that specific disruption of SM clusters by psychosine at the anaphase cleavage furrow caused compromised PIP₂ production which results in failed cytokinesis at a later stage.

DISCUSSION

In this study, psychosine-triggered multiploid cell formation was first quantified. This led to the realization that this phenomenon could be cell-specific and quantitatively modulated by other cellular factor(s). Cellular sphingolipid species were modifier molecules where GSL could enhance and SM could suppress multiploidy when examined in sphingolipid perturbed-Namalwa cells. Unlike other studies on GSL-perturbation using siRNAs (Atilla-Gokcumen *et al.*, 2014), significant increase in multiploid cells without psychosine was not detected (**Figure 2C**). At least in this system, altered levels of GSLs or SM *per se* did not trigger cytokinetic failure; rather, supplementation of psychosine triggers multiploid cell formation. Therefore, GSLs and SM seemed to modulate a cytokinetic event that is inhibited by psychosine. Here, almost all cells become multiploid when both *GlcCerSyn* and *nSMase2* were overexpressed in Namalwa cells treated with psychosine (**Figure 4D**). In this condition cytokinesis could be effectively disabled without disturbing genome duplication in the cell cycle. In other words, a specific balance among the three different sphingolipid classes seemed to allow cells to undergo successful endomitosis cycles rather than normal mitosis. Although this phenomenon is striking and psychosine may be biosynthesized in certain situations, endogenous psychosine was not detected in Namalwa cells. Present data indicated that lack of psychosine in normal tissue is consistent with normal mitosis.

Relationship of sphingolipid effects on cleavage furrow regression and cytokinesis

Recent developments in the field indicate that sphingolipids are functionally involved in cytokinesis. The present study showed that SM clustering was disturbed both at the cell surface and anaphase cleavage furrow in the presence of psychosine. By contrast, dispersed SM clusters (stained by NT-equinatoxin) were not affected at the cell surface (**Figure 6**). The effect of psychosine clearly differs among these SM-binding probes. Currently, it is not clear how psychosine reduces SM clustering. A recent study showed that the presence of GSL dispersed SMs (Makino *et al.*, 2015). Although psychosine has only one acyl chain, psychosine is a GSL and as such, could physically disrupt SM clustering. In relation to this point, psychosine was shown to disrupt microdomains such as lipid rafts or detergent-insoluble membranes (White *et al.*, 2009). However it is notable that cholesterol depletion by methyl- β -cyclodextrin did not induce multiploidy in Namalwa cells (HW and HT, unpublished observation). Moreover, neither lyso-LacCer (Gal-Glc-sphingosine) nor N-acetyl-psychosine (cell permeable Gal-Cer where the sphingosine 2-amido group is the acetyl form) trigger polyploidization. It is reasonable to suppose that these non-polyploidizing lyso/permeable-lipids would disturb microdomains like psychosine. Therefore, specific structural specificity seems to be responsible for the polyploidizing activity of psychosine (Kanazawa *et al.*, 2000).

Involvement of particular sphingolipid species in cell division

Data revealed that GSL/GM1 enhance and SM suppressed polyploidization triggered by psychosine (**Figure 4 and 5**), most likely due to head group differences. This specificity may be better understood when some specific psychosine target molecules are considered. GSL and SM could modulate putative psychosine target(s). In fact, not only GSLs and/or SM participate in membrane microdomains to regulate cellular signaling (Hakomori and Igarashi, 1993; Iwabuchi

et al., 2000), cellular (glyco)sphingolipids are also involved in membrane-mediated regulation of receptor(s) (Prinetti *et al.*, 2009), cellular signaling components (Hakomori, 2000) and cellular trafficking events (Gkantiragas *et al.*, 2001; Sprong *et al.*, 2001). It has been shown that expression of GSL regulates cell surface receptor behavior in the example of GM3 and insulin receptor signaling (Kabayama *et al.*, 2007), although GM3 also may mediate cellular functions *via* GSL-GSL interactions (Kojima and Hakomori, 1991). More recently, it was shown that specific molecular species of hexosylceramides are enriched in the midbody of dividing cells (Atilla-Gokcumen *et al.*, 2014). It was also shown that a specific molecular species of SM regulates transmembrane proteins through a specific recognition motif (Contreras *et al.*, 2012). Regardless of the mechanisms, the present work could represent a novel/rare cellular system where GSL and SM compete in activity. This system could account for regulation of molecule(s) involved in cytokinesis. Such findings could add to fundamental knowledge for further understanding of cytokinesis, in which the involvement of sphingolipids is emerging (Ng *et al.*, 2005; Abe *et al.*, 2012; Atilla-Gokcumen *et al.*, 2014). This finding may also contribute to understanding molecular mechanisms behind GLD pathogenesis in formation of globoid cells, characteristic cells utilized for disease diagnosis.

Cellular homeostatic regulation of SM levels

Perturbation of cellular SM levels was a rather difficult task. In the steady state Namalwa cells, it is likely that the SM level is homeostatically controlled by the “SM cycle” when SM level is elevated (**Figures 4C and 5C**, unpublished observation H.W. and H.T.) (Hannun, 1994). In addition to accumulation in the form of ceramide detected in lipidomic analyses (**Supplemental Figure S3**), nSMase2-derived ceramide seemed to be efficiently converted to GlcCer when *GlcCerSyn* was introduced. These results suggested that GSL biosynthesis is remotely coupled to

SM metabolism (**Figure 5C, Supplemental Figure S3**) and Namalwa cells may have a transport system that efficiently biosynthesizes GSLs (Tidhar and Futerman, 2013). Thus, psychosine-mediated multiploidy cannot be understood only from SM cellular levels. In any case, it was very consistent that the GSL level positively affected and the SM level negatively affected the cytokinetic defect caused by psychosine. Thus, the sphingolipid environment of the cellular membrane is an important factor controlling cytokinesis, at least in the presence of psychosine. To our knowledge, this is the first report to quantitatively identify cellular factor(s) altering the severity of multiploidization caused by psychosine. This information is useful for further understanding of the mechanisms underlying psychosine-mediated multiploidization and the regulation of the still elusive uncoupling of S-phase and cytokinesis leading to successful endomitosis.

MATERIALS AND METHODS

Reagents and cell culture

Psychosine, GlcPsy, lyso-LacCer and SPC were obtained from Matreya and dissolved in ethanol as a 10 mM stock solution. Sphingomyelin (from bovine brain; Sigma-Aldrich, St. Louis, MO, USA) used for pretreatment was dissolved in ethanol as a 10 mM stock solution. D-threo-1-phenyl-2-decanoylamino-3-morpholino-propanol (PDMP) was dissolved in dimethylsulfoxide as a 10 mM stock solution. Bacterial sphingomyelinase was obtained from SIGMA. KMS-12 PE, Namalwa, U937, KMS-12 BM, Daudi, Raji and Ramos cells were obtained from the Japanese Cell Research Bank and cultured in RPMI 1640 medium supplemented with non-essential amino acids, sodium pyruvate, 2-mercaptoethanol and fetal bovine serum. A total of 1×10^5 cells in 24-well plates were treated with psychosine for 2 days before harvesting for DNA content assessment. The following probe/antibodies were used: biotin-conjugated cholera toxin B subunit (List Biological Laboratories, Campbell, CA, USA); goat anti-human TDAG8 (N-19; Santa Cruz Biotechnology, Santa Cruz, CA, USA); goat polyclonal anti-GST (Amersham Pharmacia Biotech, Uppsala, Sweden); mouse anti-FLAG (M2; Sigma-Aldrich); mouse anti-actin (C4; MP Biomedical, Santa Ana, CA, USA); fluorescein isothiocyanate (FITC)-conjugated anti-goat IgG (Vector Laboratories, Burlingame, CA, USA); and phycoerythrin (PE)-conjugated streptavidin (Caltag, Burlingame, CA, USA); horseradish peroxidase-conjugated anti-mouse IgG (Invitrogen, Carlsbad, CA, USA).

Flow cytometry (FCM)

Cells were stained with antibodies or recombinant toxin subunit in fluorescence activated cell sorting (FACS) buffer [1% bovine serum albumin, 0.1% NaN₃ in phosphate-buffered saline (PBS)]. After washing step(s), stained cells were analyzed by flow cytometry using FACScan or

FACSCalibur (BD Biosciences, Franklin Lakes, NJ, USA), and data were analyzed using FlowJo software (Tree Star Inc, Ashland, OR, USA).

Quantitative determination of cellular ploidy

Multiploidy of the cells was examined as reported previously (Kanazawa *et al.*, 2000). Briefly, cells treated with psychosine for 2 days were fixed in 70% ethanol overnight. After washing with PBS, the cells were incubated with RNase A (DNase-free; Nacalai Tesque, Kyoto, Japan) to remove RNA. The resultant cellular DNA was stained with propidium iodide (Nacalai Tesque) for subsequent FCM analyses using a FACScan or a FACSCalibur (BD Biosciences) flow cytometer for FL-2 channel detection. To exclude doublets, cells were gated using the FL-2 Area and FL-2 Width. Average cell ploidy was calculated from the abundance ratio of each nuclear phase. In the multiploidization experiments, the comparison was made only using control cells cultured side-by-side. The “psychosine-mediated ploidy index number” (PPIN) value was developed to profile psychosine-induced multiploidy in six different B cell lines, in which normalization of the cell-to-cell differences of psychosine sensitivity seemed appropriate. The concentration of psychosine to achieve maximum multiploidy was determined. Values of the maximum percentage of each cell with a ploidy $>4N$ were divided by the concentration of psychosine to calculate the PPIN, for normalization of cell variations in psychosine sensitivity and degree of multiploidy. When PPIN is not needed, average ploidy was calculated and depicted on the upper right of histograms.

Short hairpin RNA-mediated knockdown of GM1 synthase

Target sequences for *GMISyn* (*B3GALT4*) shRNA were chosen by NCBI database (NCBI Reference Sequence : NM_003782.3). Oligonucleotide corresponding to 252 to 272 and 311 to

331 was inserted into pENTR-U6 vector (invitrogen) and transferred to pLenti6 (invitrogen) lentivirus expression vector. Lentivirus plasmid vectors were transiently transfected into 293FT packaging cells and lentivirus produced in the medium was utilized for subsequent infection. Infected cells were polyclonally selected in the culture medium containing 10 µg/ml blasticidin S for at least 3 weeks.

Mass spectrometry analyses of gangliosides

For the extraction of total lipid, Namalwa cells with knockdown *GM1Syn* were suspended in chloroform:methanol (1:1) and the mixture was sonicated, incubated at 40°C for 1 h then centrifuged. The supernatant was retained and the pellet was subjected to the same extraction by same procedure using chloroform:methanol (1:2). The first and second supernatants were combined and evaporated. GM3(d18:1-[¹³C]16:0) was added for internal standards to sample. The extracted lipids were subjected to mild alkaline hydrolysis and were desalted with a Bond-elute C18 (Agilent Technology).

GM3 and GM2 molecular species were quantified using HPLC coupled with electrospray ionization tandem mass spectrometry (MS/MS) in multiple reaction monitoring (MRM) negative ionization mode. The Thermo Fisher triple stage quadrupole (TSQ) Vantage AM (Waltham, MA) instrument was calibrated by directly infusing a mixture of GM3 species extracted from milk, all ion source parameters and ionization conditions were optimized to improve sensitivity. Total lipid extracts from cells were dissolved in methanol and were injected onto a Thermo Fisher Accela 1250 HPLC pump (Waltham, MA) and separated using a Develosil carbon 30 column (C30-UG-3-1 x 50mm, Nomura Co. Ltd, Japan). The gradient program employed started with 100% solvent A (20% H₂O / 50% 2-propanol / 30% methanol containing 0.1% acetic acid and 0.1% ammonia) for 5 min then ramped to 100% solvent B (2% H₂O / 50% 2-propanol / 48%

methanol containing 0.1% acetic acid and 0.1% ammonia) over 30 min. One hundred percent solvent B was maintained for 4 min, then the solvent was returned to 100% solvent A over 1 min and held there for 10 min. The flow rate throughout the duration of the chromatographic run was 50 μ L/min.

The abundance of each molecular species was compared based on its relative percentage of the internal standards GM3(d18:1-[13 C]16:0). Total GM3 and GM2 values were calculated by taking the sum of the each molecular species detected, with the assumption that all species have similar ionization efficiency comparable to the internal standard (**Supplemental Figure S1**). Signal strength for each lipid species were documented with table format (**Supplemental Table S2**).

Lipidomic analyses

Genetically modified Namalwa cells were subjected for lipid isolation and analyzed for mass spectrometry as previously reported (Ogiso *et al.*, 2014; Veillon *et al.*, 2015). Protein amount in the residue after lipid extraction was determined using a BCA Protein Assay Kit (Thermo-Fisher Scientific). Each level of measured lipids was normalized to the protein amount.

Retrovirus-mediated gene transfer

The preparation of Namalwa cells using modified mouse stem cell viral (MSCV) vectors (Clontech) encoding human *GlcCerSyn*, *Gb3Syn* and *Gb3Syn-TxT* combined with the internal ribosomal entry site (IRES)-controlled *EGFP* was reported previously (Takematsu *et al.*, 2011). A similar vector with a blasticidin resistance gene as a selection marker was constructed to replace *EGFP*. The MSCV-IRES system was used throughout this study such that bicistronic expression of the cDNA of interest is warranted with selective marker expression. The full open reading frame of human neutral sphingomyelinase 2 (nSMase2) cDNA was amplified and

subcloned into pDONR221. The LR recombinase (Invitrogen) reaction was utilized to create the MSCV-*nSMase2*-IRES-*Blast* plasmid vector. MSCV plasmid vectors were transfected into Plat-A packaging cells (Morita *et al.*, 2000) to prepare infectious retrovirus. The prepared retrovirus was spin-infected to Namalwa cells as reported previously (Takematsu *et al.*, 2011), and infectants were polyclonally sorted by GFP-positivity or selected using 10 µg/ml blasticidin S for at least 2 or 3 weeks, respectively.

Electroporation-mediated gene delivery

Because the retroviral vector lacks a strong promoter for transgene expression, plasmid vector was utilized to express SM synthase (SMS) 1/2 cDNA (Shakor *et al.*, 2011). FLAG-tagged *SMS1/2* cDNAs were subcloned into an expression vector downstream of the *EF1a* promoter, which bicistronically expresses the blasticidin resistance gene under the IRES. The blasticidin-selected stable polyclonal cells expressed SMS1 or SMS2 according to Western blotting.

Thin-layer chromatography (TLC)

Cellular lipids were extracted as reported previously (Miyake *et al.*, 1995). Briefly, after washing with PBS, 6×10^7 cells were lysed with chloroform-methanol (2:1). The lipid fraction was prepared by Folch's participation and saponificated to remove phospholipids. Sphingolipid fractions were desalted and separated using TLC plates. SM was visualized using primulin under ultraviolet light, and GSLs were visualized using orcinol-sulfate. Data was digitally captured and densities of each band from at least three experimental replicates were analyzed by densitometry.

Cellular surface staining using a non-toxic variant (NT) of lysenin and equinatoxin II probes

The preparation of GST-NT-lysenin and EGFP-NT-lysenin was reported previously (Abe *et al.*, 2012). Briefly, GST-NT-lysenin was expressed in BL21 cells and purified using glutathione-Sepharose 4B resin. GST-NT-lysenin, anti-GST and FITC-conjugated anti-mouse IgG were mixed and incubated overnight at 4°C to form a staining complex. A total of 4×10^5 cells was stained using the “staining pre-complex” for 30 min at room temperature and analyzed by FCM. The non-toxic variant of equinatoxin, NT-equinatoxin II-EGFP-His, was prepared as reported previously (Makino *et al.*, 2015) and utilized for flow cytometric detection.

PH-GFP participation at cleavage furrows

The PH domain of PLC δ 1 was modified from a previously reported plasmid, pEGFP-N1-PH, which was kindly provided by Dr. Tamas Balla (Varnai and Balla, 1998). The PH-GFP fragment was subcloned into the pENTR1A donor vector and subsequently cloned into the pSP72-EF1-DC-IRES-*Blast*-PolyA destination vector by an LR clonase reaction. The pSP72-EF1-*PH-GFP*-IRES-*Blast*-PolyA plasmid was introduced into Namalwa cells by electroporation and selected using 10 μ g/ml blasticidin S. A selected clone (Namalwa-PH-GFP-1) with appropriate fluorescent intensity was used to monitor PIP₂ concentration at the cleavage furrow. Briefly, 4×10^5 Namalwa-PH-GFP-1 cells/ml were cultured overnight with 5 μ M psychosine. After washing, cells were fixed using fixation buffer [4% PFA/0.2 M sucrose in PBS] for 15 min at room temperature. Cells were then suspended in mounting solution with 4',6-diamidino-2-phenylindole (DAPI) for fluorescent microscopic observation as reported previously (Takematsu *et al.*, 2011).

Experimental replication and statistical evaluation

Data were expressed as means $\pm$ standard error of the results of (at least) triplicate experiments.

The statistical significance of the observed differences was evaluated using the Student's *t*-test. All experiments were performed at least twice, and representative results are shown. For profile correlation analyses among multiple cell panels, Pearson's correlation coefficients ($-1 \leq r \leq 1$) were employed, in which the value of 1 indicated a perfect correlation and -1 a perfect negative correlation.

Footnote

[¶]In the present manuscript, we defined the phrase “cluster of SM” as small aggregates composed of less than 10 SM molecules (See text).

The authors declare no financial conflicts related to this manuscript.

*This work was supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Acknowledgements

Authors would like to thank Dr. Makoto Ogiso (Kanazawa Medical Univ) for LC-MS analyses, Dr. Takayuki Kanazawa (Shionogi & Co.) for the evaluation of PPIN values and Dr. Toshio Kitamura (University of Tokyo) for Plat-A cells. We would like to thank Dr. Roger Laine (Louisiana State Univ) for discussion.

References

- Abe, M., Makino, A., Hullin-Matsuda, F., Kamijo, K., Ohno-Iwashita, Y., Hanada, K., Mizuno, H., Miyawaki, A., and Kobayashi, T. (2012). A Role for Sphingomyelin-rich Lipid Domains in the Accumulation of PIP2 to the Cleavage Furrow during Cytokinesis. *Mol Cell Biol* 32, 1396-1407.
- Atilla-Gokcumen, G.E., Muro, E., Relat-Goberna, J., Sasse, S., Bedigian, A., Coughlin, M.L., Garcia-Manyes, S., and Eggert, U.S. (2014). Dividing cells regulate their lipid composition and localization. *Cell* 156, 428-439.
- Carmena, M. (2008). Cytokinesis: the final stop for the chromosomal passengers. *Biochem Soc Trans* 36, 367-370.
- Contreras, F.X., et al. (2012). Molecular recognition of a single sphingolipid species by a protein's transmembrane domain. *Nature* 481, 525-529.
- Field, S.J., Madson, N., Kerr, M.L., Galbraith, K.A., Kennedy, C.E., Tahiliani, M., Wilkins, A., and Cantley, L.C. (2005). PtdIns(4,5)P2 functions at the cleavage furrow during cytokinesis. *Curr Biol* 15, 1407-1412.
- Gkantiragas, I., Brugger, B., Stuken, E., Kaloyanova, D., Li, X.Y., Lohr, K., Lottspeich, F., Wieland, F.T., and Helms, J.B. (2001). Sphingomyelin-enriched microdomains at the Golgi complex. *Mol Biol Cell* 12, 1819-1833.
- Hakomori, S., and Igarashi, Y. (1993). Gangliosides and glycosphingolipids as modulators of cell growth, adhesion, and transmembrane signaling. *Adv Lipid Res* 25, 147-162.
- Hakomori, S., and Zhang, Y. (1997). Glycosphingolipid antigens and cancer therapy. *Chem Biol* 4, 97-104.
- Hakomori, S.I. (2000). Cell adhesion/recognition and signal transduction through glycosphingolipid microdomain. *Glycoconj J* 17, 143-151.
- Hannun, Y.A. (1994). The sphingomyelin cycle and the second messenger function of ceramide. *J Biol Chem* 269, 3125-3128.
- Hannun, Y.A., and Bell, R.M. (1987). Lysosphingolipids inhibit protein kinase C: implications for the sphingolipidoses. *Science* 235, 670-674.
- Hannun, Y.A., and Bell, R.M. (1989). Regulation of protein kinase C by sphingosine and lysosphingolipids. *Clin Chim Acta* 185, 333-345.
- Hofmann, K., Tomiuk, S., Wolff, G., and Stoffel, W. (2000). Cloning and characterization of the mammalian brain-specific, Mg²⁺-dependent neutral sphingomyelinase. *Proc Natl Acad Sci U S A*

97, 5895-5900.

Igisu, H., and Suzuki, K. (1984). Progressive accumulation of toxic metabolite in a genetic leukodystrophy. *Science* 224, 753-755.

Im, D.S., Heise, C.E., Nguyen, T., O'Dowd, B.F., and Lynch, K.R. (2001). Identification of a molecular target of psychosine and its role in globoid cell formation. *J Cell Biol* 153, 429-434.

Inokuchi, J., Momosaki, K., Shimeno, H., Nagamatsu, A., and Radin, N.S. (1989). Effects of D-threo-PDMP, an inhibitor of glucosylceramide synthetase, on expression of cell surface glycolipid antigen and binding to adhesive proteins by B16 melanoma cells. *J Cell Physiol* 141, 573-583.

Ishitsuka, R., and Kobayashi, T. (2004). Lysenin: a new tool for investigating membrane lipid organization. *Anatomical science international* 79, 184-190.

Ishitsuka, R., Yamaji-Hasegawa, A., Makino, A., Hirabayashi, Y., and Kobayashi, T. (2004). A lipid-specific toxin reveals heterogeneity of sphingomyelin-containing membranes. *Biophysical journal* 86, 296-307.

Iwabuchi, K., Zhang, Y., Handa, K., Withers, D.A., Sinay, P., and Hakomori, S. (2000). Reconstitution of membranes simulating "glycosignaling domain" and their susceptibility to lyso-GM3. *J Biol Chem* 275, 15174-15181.

Kabayama, K., Sato, T., Saito, K., Loberto, N., Prinetti, A., Sonnino, S., Kinjo, M., Igarashi, Y., and Inokuchi, J. (2007). Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. *Proc Natl Acad Sci U S A* 104, 13678-13683.

Kanazawa, T., Nakamura, S., Momoi, M., Yamaji, T., Takematsu, H., Yano, H., Sabe, H., Yamamoto, A., Kawasaki, T., and Kozutsumi, Y. (2000). Inhibition of cytokinesis by a lipid metabolite, psychosine. *J Cell Biol* 149, 943-950.

Kanazawa, T., Takematsu, H., Yamamoto, A., Yamamoto, H., and Kozutsumi, Y. (2008). Wheat germ agglutinin stains dispersed post-golgi vesicles after treatment with the cytokinesis inhibitor psychosine. *J Cell Physiol* 215, 517-525.

Kannagi, R., Papayannopoulou, T., Nakamoto, B., Cochran, N.A., Yokochi, T., Stamatoyannopoulos, G., and Hakomori, S. (1983). Carbohydrate antigen profiles of human erythroleukemia cell lines HEL and K562. *Blood* 62, 1230-1241.

Keusch, J.J., Manzella, S.M., Nyame, K.A., Cummings, R.D., and Baenziger, J.U. (2000). Cloning of Gb3 synthase, the key enzyme in globo-series glycosphingolipid synthesis, predicts a family of alpha 1, 4-glycosyltransferases conserved in plants, insects, and mammals. *J Biol Chem* 275, 25315-25321.

Kojima, N., and Hakomori, S. (1991). Cell adhesion, spreading, and motility of GM3-expressing

cells based on glycolipid-glycolipid interaction. *J Biol Chem* 266, 17552-17558.

Kojima, Y., Fukumoto, S., Furukawa, K., Okajima, T., Wiels, J., Yokoyama, K., Suzuki, Y., Urano, T., and Ohta, M. (2000). Molecular cloning of globotriaosylceramide/CD77 synthase, a glycosyltransferase that initiates the synthesis of globo series glycosphingolipids. *J Biol Chem* 275, 15152-15156.

Kozutsumi, Y., Kanazawa, T., Sun, Y., Yamaji, T., Yamamoto, H., and Takematsu, H. (2002). Sphingolipids involved in the induction of multinuclear cell formation. *Biochim Biophys Acta* 1582, 138-143.

Lloyd-Evans, E., Pelled, D., Riebeling, C., Bodennec, J., de-Morgan, A., Waller, H., Schiffmann, R., and Futerman, A.H. (2003a). Glucosylceramide and glucosylsphingosine modulate calcium mobilization from brain microsomes via different mechanisms. *J Biol Chem* 278, 23594-23599.

Lloyd-Evans, E., Pelled, D., Riebeling, C., and Futerman, A.H. (2003b). Lyso-glycosphingolipids mobilize calcium from brain microsomes via multiple mechanisms. *Biochem J* 375, 561-565.

Makino, A., et al. (2015). Visualization of the heterogeneous membrane distribution of sphingomyelin associated with cytokinesis, cell polarity, and sphingolipidosis. *FASEB J* 29, 477-493.

Marchesini, N., Luberto, C., and Hannun, Y.A. (2003). Biochemical properties of mammalian neutral sphingomyelinase 2 and its role in sphingolipid metabolism. *J Biol Chem* 278, 13775-13783.

Merrill Jr., A.H., and Sandhoff, K. (2002). Sphingolipids: metabolism and cell signaling. in *Biochemistry of Lipids, Lipoprotein and Membranes (4th Edn) Chapter 14*, 373-407.

Miyake, Y., Kozutsumi, Y., Nakamura, S., Fujita, T., and Kawasaki, T. (1995). Serine palmitoyltransferase is the primary target of a sphingosine-like immunosuppressant, ISP-1/myriocin. *Biochem Biophys Res Commun* 211, 396-403.

Miyatake, T., and Suzuki, K. (1972). Globoid cell leukodystrophy: additional deficiency of psychosine galactosidase. *Biochem Biophys Res Commun* 48, 539-543.

Morita, S., Kojima, T., and Kitamura, T. (2000). Plat-E: An efficient and stable system for transient packaging of retroviruses. *Gene Ther* 7, 1063-1066.

Ng, M.M., Chang, F., and Burgess, D.R. (2005). Movement of membrane domains and requirement of membrane signaling molecules for cytokinesis. *Dev Cell* 9, 781-790.

Ogiso, H., Taniguchi, M., Araya, S., Aoki, S., Wardhani, L.O., Yamashita, Y., Ueda, Y., and Okazaki, T. (2014). Comparative Analysis of Biological Sphingolipids with Glycerophospholipids and Diacylglycerol by LC-MS/MS. *Metabolites* 4, 98-114.

Okajima, F., and Kondo, Y. (1995). Pertussis toxin inhibits phospholipase C activation and Ca²⁺

mobilization by sphingosylphosphorylcholine and galactosylsphingosine in HL60 leukemia cells. Implications of GTP-binding protein-coupled receptors for lysosphingolipids. *J Biol Chem* 270, 26332-26340.

Prinetti, A., Loberto, N., Chigorno, V., and Sonnino, S. (2009). Glycosphingolipid behaviour in complex membranes. *Biochim Biophys Acta* 1788, 184-193.

Radu, C.G., Cheng, D., Nijagal, A., Riedinger, M., McLaughlin, J., Yang, L.V., Johnson, J., and Witte, O.N. (2006). Normal immune development and glucocorticoid-induced thymocyte apoptosis in mice deficient for the T-cell death-associated gene 8 receptor. *Mol Cell Biol* 26, 668-677.

Shakor, A.B., et al. (2011). Sphingomyelin synthase 1-generated sphingomyelin plays an important role in transferrin trafficking and cell proliferation. *J Biol Chem* 286, 36053-36062.

Shinoda, H., Kobayashi, T., Katayama, M., Goto, I., and Nagara, H. (1987). Accumulation of galactosylsphingosine (psychosine) in the twitcher mouse: determination by HPLC. *J Neurochem* 49, 92-99.

Sonnino, S., Mauri, L., Chigorno, V., and Prinetti, A. (2007). Gangliosides as components of lipid membrane domains. *Glycobiology* 17, 1R-13R.

Sprong, H., Degroote, S., Claessens, T., van Drunen, J., Oorschot, V., Westerink, B.H., Hirabayashi, Y., Klumperman, J., van der Sluijs, P., and van Meer, G. (2001). Glycosphingolipids are required for sorting melanosomal proteins in the Golgi complex. *J Cell Biol* 155, 369-380.

Steigemann, P., and Gerlich, D.W. (2009). Cytokinetic abscission: cellular dynamics at the midbody. *Trends Cell Biol* 19, 606-616.

Suzuki, K. (1998). Twenty five years of the "psychosine hypothesis": a personal perspective of its history and present status. *Neurochem Res* 23, 251-259.

Svennerholm, L., Vanier, M.T., and Mansson, J.E. (1980). Krabbe disease: a galactosylsphingosine (psychosine) lipidosis. *J Lipid Res* 21, 53-64.

Tafesse, F.G., Huitema, K., Hermansson, M., van der Poel, S., van den Dikkenberg, J., Uphoff, A., Somerharju, P., and Holthuis, J.C. (2007). Both sphingomyelin synthases SMS1 and SMS2 are required for sphingomyelin homeostasis and growth in human HeLa cells. *J Biol Chem* 282, 17537-17547.

Takematsu, H., Yamamoto, H., Naito-Matsui, Y., Fujinawa, R., Tanaka, K., Okuno, Y., Tanaka, Y., Kyogashima, M., Kannagi, R., and Kozutsumi, Y. (2011). Quantitative transcriptomic profiling of branching in a glycosphingolipid biosynthetic pathway. *J Biol Chem* 286, 27214-27224.

Tani, M., and Hannun, Y.A. (2007). Neutral sphingomyelinase 2 is palmitoylated on multiple

- cysteine residues. Role of palmitoylation in subcellular localization. *J Biol Chem* **282**, 10047-10056.
- Tidhar, R., and Futerman, A.H. (2013). The complexity of sphingolipid biosynthesis in the endoplasmic reticulum. *Biochim Biophys Acta* **1833**, 2511-2518.
- Varnai, P., and Balla, T. (1998). Visualization of phosphoinositides that bind pleckstrin homology domains: calcium- and agonist-induced dynamic changes and relationship to myo-[3H]inositol-labeled phosphoinositide pools. *J Cell Biol* **143**, 501-510.
- Veillon, L., Go, S., Matsuyama, W., Suzuki, A., Nagasaki, M., Yatomi, Y., and Inokuchi, J. (2015). Identification of Ganglioside GM3 Molecular Species in Human Serum Associated with Risk Factors of Metabolic Syndrome. *PloS one* **10**, e0129645.
- Wang, J.Q., et al. (2004). TDAG8 is a proton-sensing and psychosine-sensitive G-protein-coupled receptor. *J Biol Chem* **279**, 45626-45633.
- Wenger, D.A., Sattler, M., and Hiatt, W. (1974). Globoid cell leukodystrophy: deficiency of lactosyl ceramide beta-galactosidase. *Proc Natl Acad Sci U S A* **71**, 854-857.
- White, A.B., Givogri, M.I., Lopez-Rosas, A., Cao, H., van Breemen, R., Thinakaran, G., and Bongarzone, E.R. (2009). Psychosine accumulates in membrane microdomains in the brain of krabbe patients, disrupting the raft architecture. *J Neurosci* **29**, 6068-6077.
- Yamamoto, H., Takematsu, H., Fujinawa, R., Naito, Y., Okuno, Y., Tsujimoto, G., Suzuki, A., and Kozutsumi, Y. (2007). Correlation index-based responsible-enzyme gene screening (CIRES), a novel DNA microarray-based method for enzyme gene involved in glycan biosynthesis. *PloS one* **2**, e1232.
- Yamaoka, S., Miyaji, M., Kitano, T., Umehara, H., and Okazaki, T. (2004). Expression cloning of a human cDNA restoring sphingomyelin synthesis and cell growth in sphingomyelin synthase-defective lymphoid cells. *J Biol Chem* **279**, 18688-18693.

Figure Legends

Figure 1. Cross-cell profiling of psychosine-mediated polyploidization and cellular factors

A. Polyploidization of psychosine-treated cells.

U937, Namalwa and KMS12-PE cells were treated with 2.5 or 5 μ M psychosine for 2 days before harvesting and measuring cellular DNA content by propidium iodide staining.

Degree of multiploidy was expressed as average nuclear content value, where 2N represents normal diploid cells.

B. Expression of TDAG8.

The same set of cell lines was assessed for TDAG8 expression. Cells were stained with anti-TDAG8 antibody and evaluated using FCM.

C. Positive correlation between the cross-cell profiles for GM1 level and psychosine-mediated polyploidization.

The relative psychosine-mediated polyploidization profile among a set of six cell lines was plotted in the web-graph format. The relative PPIN values are expressed on the diagonal lines of a hexagon where the plots located at the edge of the hexagon indicate stronger polyploidization. Cells with the strongest value were set to 100% (top). The relative GM1 expression profile obtained by FCM staining using cholera toxin B subunit (CTxB) was also plotted in the web-graph format. Due to the use of fluorescent signals in the experiments, data were plotted on a log-scale (middle). Pearson's correlation coefficient between these profiles and associated *p* value were calculated (bottom).

Figure 2. Reduced polyploidization of Namalwa cells with decreased GM1.

A. Biosynthetic pathway of sphingolipids in Namalwa cells.

Lipids are indicated in white letters on black, and enzymes indicated by boxed black letters. Inhibitors used in this study are depicted in black letters.

B. CTxB staining of *GMISyn* knockdown Namalwa cells. Namalwa cells were infected with lentivirus encoding shRNA for control (*lamin*) and *GMISyn* (*B3GALT4*) was stained with CTxB. Shadow indicates control staining. Mean fluorescent intensity (MFI) values of the CTxB staining were depicted (bottom).

C. *GMISyn* knockdown cells were treated with psychosine and ploidy of the cells was analyzed as in Figure 1A.

D. Namalwa cells were treated with various doses of PDMP overnight. Cell surface GM1 level was examined using CTxB as in Figure 2B.

E. Namalwa cells were treated with a graded dose of PDMP and 5 μ M psychosine (Psy) for 2 days, and ploidy of the cells was analyzed as in Figure 1A.

Figure 3. Effects of molecular GSL species.

A. A flow chart of GSL biosynthetic pathway branching in Namalwa cells.

Gb3Syn dominantly regulates the biosynthetic pathway, thus could effectively alter GSL profiles in Namalwa cells.

B. TLC analysis of sphingolipid species in vector, Gb3Syn and Gb3Syn-TxT cells.

Namalwa cells transfected with MSCV-IRES-*EGFP* virus (vector), MSCV-*Gb3Syn*-IRES-*EGFP* virus (*Gb3Syn*) and MSCV-*Gb3Syn-TxT*-IRES-*EGFP* virus (*Gb3Syn-TxT*) were polyclonally sorted by GFP positivity. Sphingolipids purified from these cells were analyzed by TLC. Lipids were separated in chloroform, methanol and water (65:25:4). SM was visualized with primulin, and GSLs were visualized with orcinol-sulfate. The mobility of standard lipids was indicated. Relative mean band densities were plotted from three experimental replicates (bottom). Doublet

bands on TLC were calculated separately when possible.

C. Effect of expression of *Gb3Syn-TxT* and *Gb3Syn* on psychosine-induced polyploidization.

These cells were treated with psychosine, and polyploidization was measured as in Figure 1A.

Figure 4. Enhancement of psychosine-induced polyploidization by cellular GSL.

A. Enhanced polyploidization of Namalwa cells infected with *GlcCerSyn*.

Namalwa cells were transfected with MSCV-IRES-EGFP virus (*GFP*) and MSCV-*GlcCerSyn*-IRES-EGFP virus (*GlcCerSyn*) and were polyclonally sorted by GFP positivity. Psychosine-triggered polyploidization was measured by FCM as in Figure 1A.

B. Quantification of *GlcCerSyn*-enhanced nuclear ploidy triggered by psychosine.

The quantified mean nuclear ploidy from five independent experiments with graded psychosine (Psy) dose was plotted with SEM. Statistical significance was assessed using Student's *t*-test as indicated above the bars (*, $p < 0.05$).

C. TLC analyses of cells transfected with *GlcCerSyn* and *nSMase2*.

Namalwa cells used in Figure 3A were transfected with retrovirus MSCV-IRES-*Blast* virus (*Blast*) and MSCV-*nSMase2*-IRES-*Blast* virus (*nSMase2*) to produce four different types of cells. Sphingolipids were extracted from these cells and analyzed by TLC as in Figure 3B.

D. Additive effect of *GlcCerSyn* and *nSMase2* expression on psychosine-triggered polyploidization.

Psychosine-induced multiploidy was analyzed as in Figure 1A.

Figure 5. Suppressed psychosine-induced polyploidization by induction of SM.

A. Effect of SM and bacterial SMase (bSMase) treatment on psychosine-induced polyploidization.

Namalwa cells were treated with 10 or 20 μ M SM or 50 mU/ml bSMase with 5 μ M psychosine (Psy). Cellular DNA content was determined as in Figure 1A.

B. Expression of SMS1/2 as detected by Western blotting.

Namalwa cells were transfected with pSP72-EF1-IRES-*Blast* vector (vector) and pSP72-EF1 α -*SMS1/2*-IRES-*Blast* vector (*SMS1/2*); stable transfectants were selected as polyclonal mixtures. Whole cell lysates were prepared, and transgene-derived SMS1/2 was detected using anti-FLAG M2 antibody.

C. Effect of *SMS1/2* on cellular levels of sphingolipids.

Sphingolipids were isolated from these cells and were analyzed by TLC as in Figure 3B.

D. Detection of cell surface SM by NT-lysenin.

Induction of cell surface SM upon *SMS* introduction was detected using the GST-NT-lysenin probe by FCM. SM-bound lysenin was visualized as a complex with anti-GST and FITC-conjugated anti-goat IgG. Shadows indicate control staining, and solid dashed black lines indicate Namalwa cells transfected with control vectors.

E. Effect of SMS1/2 expression on psychosine-induced polyploidization.

SMS-transfected Namalwa cells were examined for psychosine-induced polyploidization as in Figure 1A.

F. Quantification of psychosine-mediated polyploidization in *SMS*-overexpressing cells.

Psychosine-induced polyploidization was determined, and mean ploidy values of six independent experiments were expressed as percentage decrease from vector control. Statistical significance of the difference between SMS1 compared to vector was $p < 0.05$ and SMS2 compared to vector was $p < 0.1$.

Figure 6. Alteration of cell surface SM expression upon psychosine treatment.

A. Cell surface expression of SM upon psychosine treatment.

Namalwa cells were treated with 5 μ M psychosine (Psy) overnight and stained with recombinant GST-NT-lysenin which detects cluster SM as in Figure 5D, or with NT-equinatoxin II-GFP-His, for total SM.

B. Cell surface localization of the NT-lysenin signal upon psychosine treatment.

Namalwa cells were treated with 5 μ M psychosine overnight and stained with recombinant EGFP-NT-lysenin. EGFP fluorescence was detected using fluorescence microscopy. Four independent cells in late mitotic phases (two anaphasic and two telophasic) are shown for each condition. To represent varied distribution found in the psychosine-treated samples, each of the four typical staining patterns was displayed.

C. Attenuated PIP₂-binding PH-GFP probe signals in psychosine-treated Namalwa cells

Namalwa cells expressing PH-GFP (PLC δ 1-PH-GFP) were treated with 5 μ M each lyso-sphingolipid overnight, and the GFP signal was observed in mitotic cells.

D. Calculation of ratio of cells observed in Figure 6C.

A total of 120 cells in late mitotic phases are counted. Incidence of anaphasic and telophasic cells was determined from cell body shapes and DAPI signal. Both anaphasic and telophasic cells were assessed for enrichment of PH-GFP signal in the cleavage furrow.

Figure legends for supplemental figures

Supplemental Figure S1

MS analyses of GM3 and GM2 from lipids isolated from shRNA-mediated *GM1Syn* knockdown Namalwa cells.

Namalwa cells were transfected with shRNA for control (*lamin*) or *GM1Syn*. Cellular lipid was purified and analyzed by LS-MS/MS. Amount of each gangliosides was expressed after normalization with the protein concentration.

Supplemental Figure S2

Effect of PDMP on cellular proliferation

Namalwa cells were pre-labeled with CFSE and treated with a graded dose of PDMP for 72 hr. Cellular proliferation was monitored by limited dilution of CFSE fluorochromes intensities detected by FCM.

Supplemental Figure S3

Lipidomic LC-MS/MS analyses of Namalwa cells.

Various lipid species were analyzed lipidomically with LC-MS/MS. Parental Namalwa cells and enzyme gene-transfected cells for *Gb3Syn-TxT*, *Gb3Syn*, *GlcCerSyn*, *nSMase2*, *GlcCerSyn/nSMase2*, *SMS1* and *SMS2* were analyzed. Complete lipidomic results differentiating each acyl-chain species are shown in **Supplemental Table S1**. Each lipid species with different

acyl-chains were combined and plotted for relatively abundant lipids as indicated.

Supplemental Figure S4

Analyses of lyso-sphingolipid-triggered multiploidy.

A, B. Namalwa cells transfected with *Gb3Syn-TxT* (A) and *GlcCerSyn/nSMase2* (B) were cultured with 5 μ M lyso-sphingolipids for 48 hr and cellular ploidy was examined as in Figure 1A.

C. Namalwa cells with *GlcCerSyn/nSMase2* were cultured with a graded dose of GlcPsy for 48 hr and cellular ploidy was examined as in Figure 1A.

Supplemental Figure S5

Introduction of GlcCerSyn in psychosine-insensitive KMS12-PE cells

KMS12-PE cells were infected with MSCV-IRES-*Blast* virus (Blast) and MSCV-*GlcCerSyn*-IRES-*Blast* virus (*GlcCerSyn*). Blasticidin resistant cells were polyclonally selected and assessed for psychosine-triggered multiploidization as in Figure 1A.

Supplemental Figure S6

Effect of GlcCer or LacCer treatment on psychosine-triggered polyploidization

A. Namalwa cells were treated with 10 μ M of GlcCer or LacCer and psychosine-mediated polyploidization was measured as in Figure 5A.

B. Effect of GlcCer treatment on the biosynthesis of GM1 was monitored by CTxB staining.

GlcCer treated cells did not induce CTxB staining (left). PDMP-mediated loss of GM1 was not rescued by GlcCer treatment (right).

Supplemental Figure S7

TLC analyses of SM or bacterial SMase (bSMase) treated Namalwa cells.

Namalwa cells were cultured with indicated concentrations of SM or bSMase, cellular lipids were purified and analyzed as in Figure 3B.

Supplemental Table S1

Lipidomic analyses for major cellular lipids from Namalwa and enzyme gene-transfected cells.

Lipidomic LC-MS data corresponding to various cellular lipids were detected with different acyl chains. Lipid species examined were sphingolipids; ceramide, hexosylceramide, lactosylceramide, GM3, SM and glycerolipid; PC (phosphatidycholine), PE (phosphatidylethanolamine), DAG (diacylglycerol), PS (phosphatidylserine), PA (phosphatidic acid), PI, PG (phosphatidylglycerol) and cholesterol. Data was composed of cell type and concentration (pmol) of each lipid species per microgram protein.

Supplemental Table S2

GM3 and GM2 analyses for lipids from Namalwa cells knockdown with *GM1Syn*.

Namalwa cells introduced with ShRNA construct were lysed and measured for gangliosides

content. GM3 and GM2 abundances with different acyl chains were differentially measured.

Due to a relatively limited expression, GM1 abundance was not reliably measured

.

Figure 1

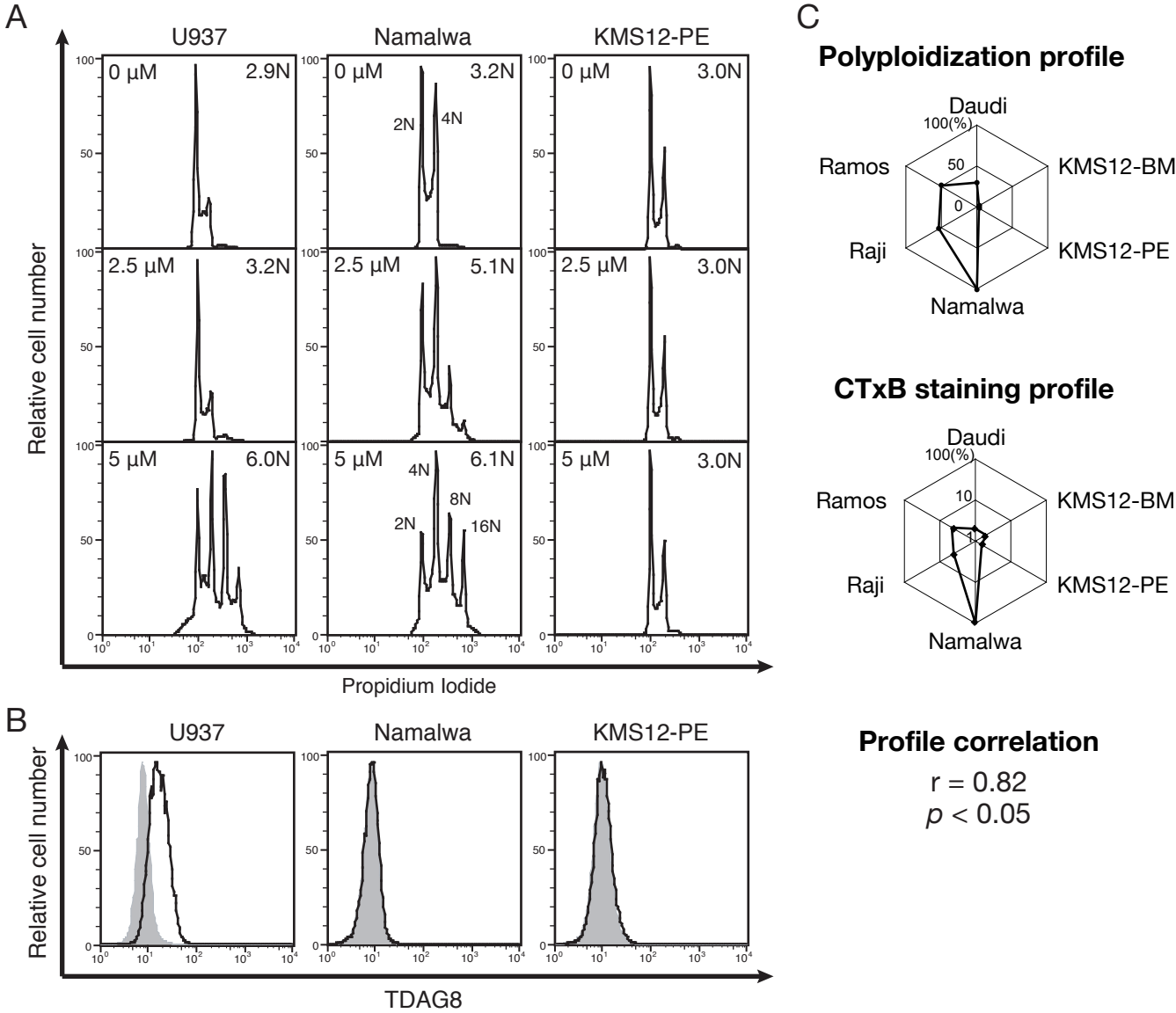


Figure 2

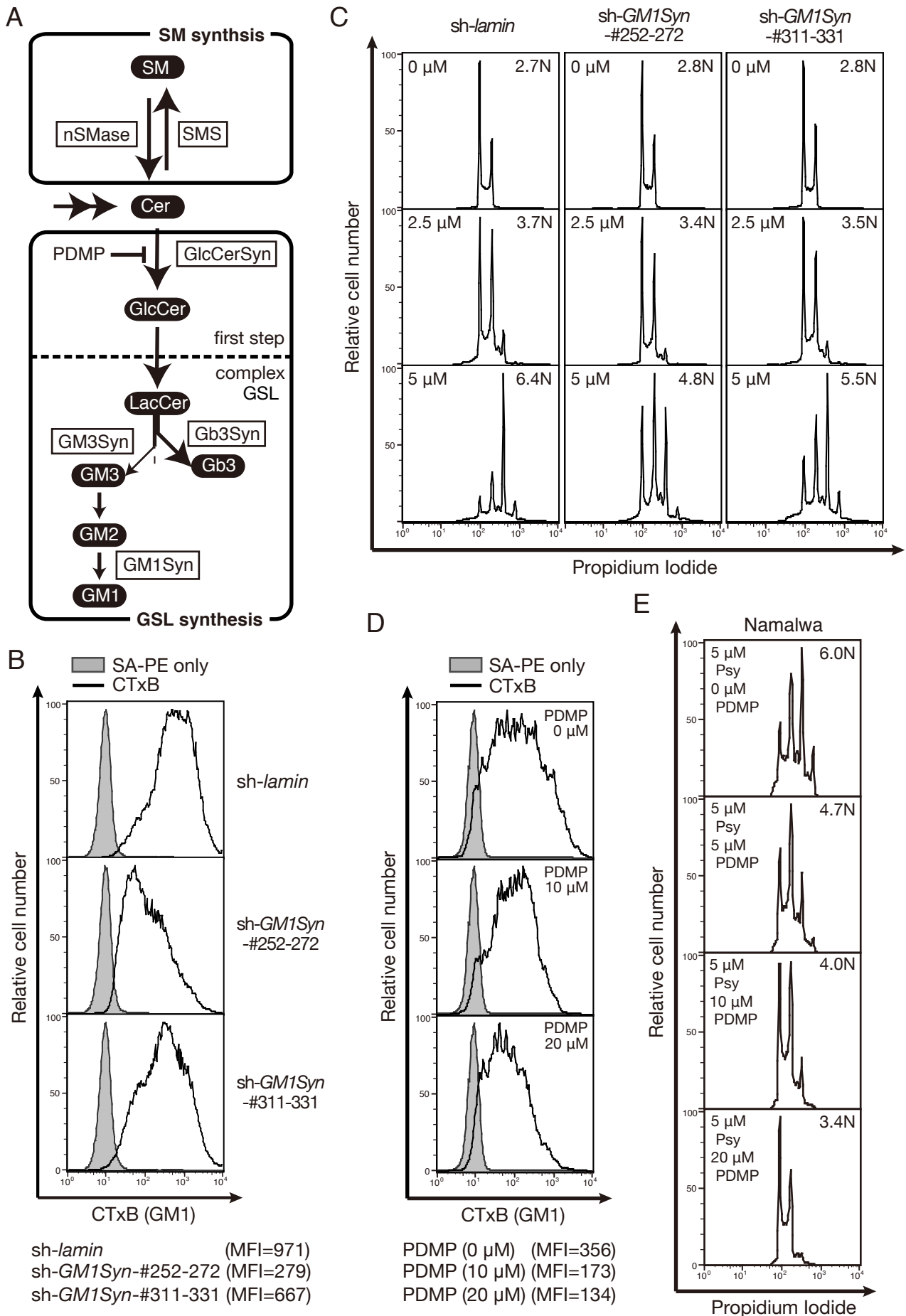


Figure 3

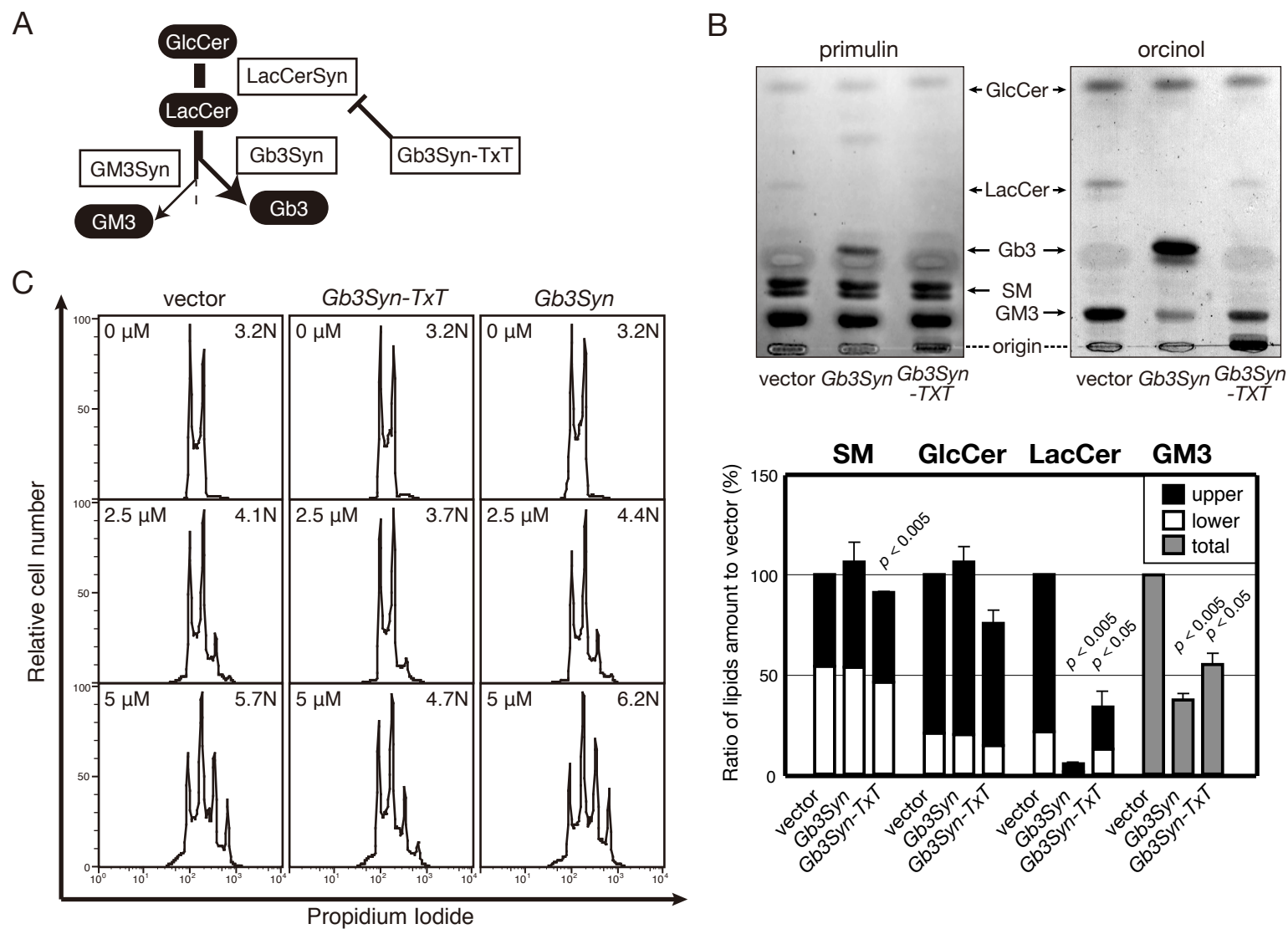


Figure 4

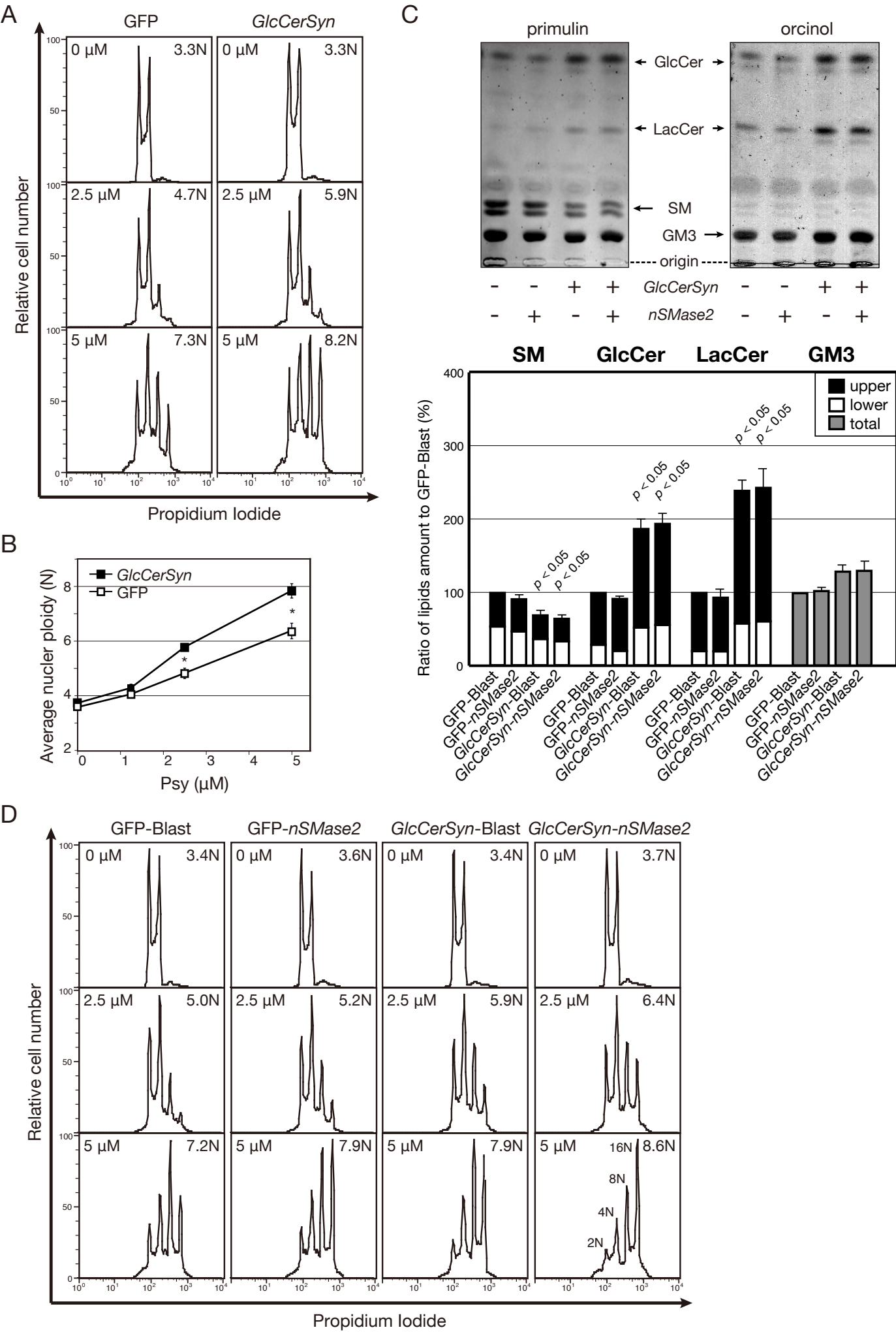


Figure 5

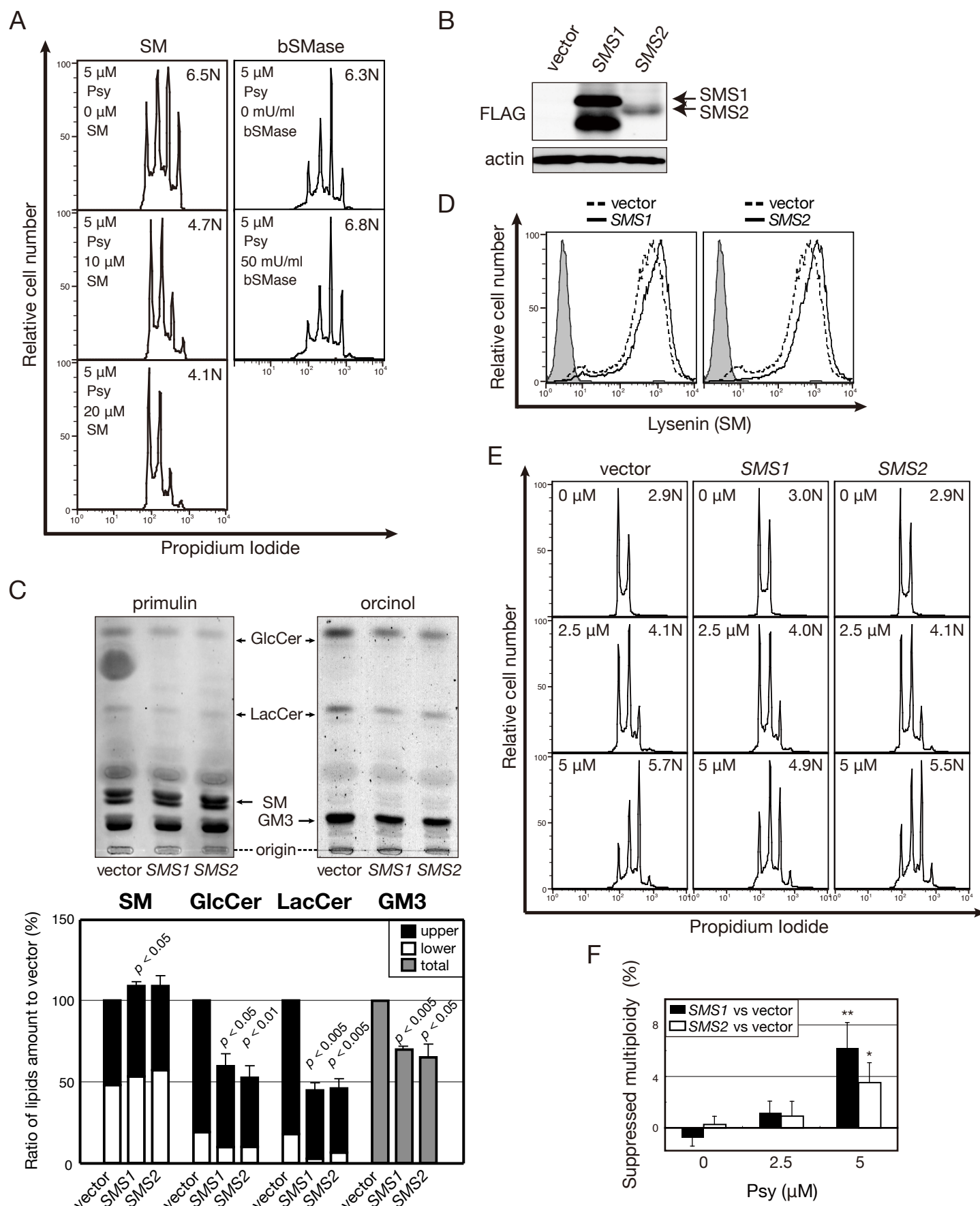
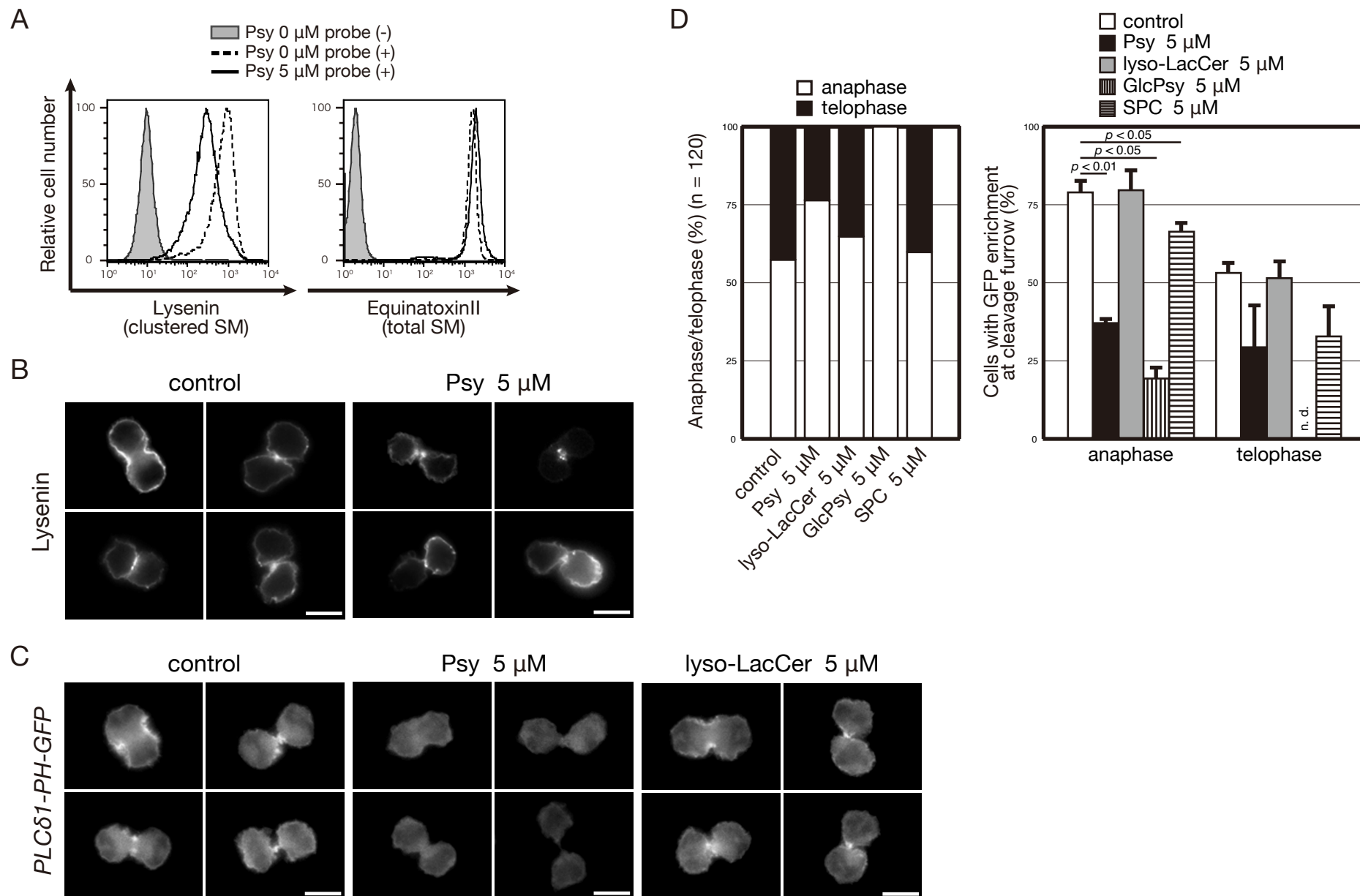
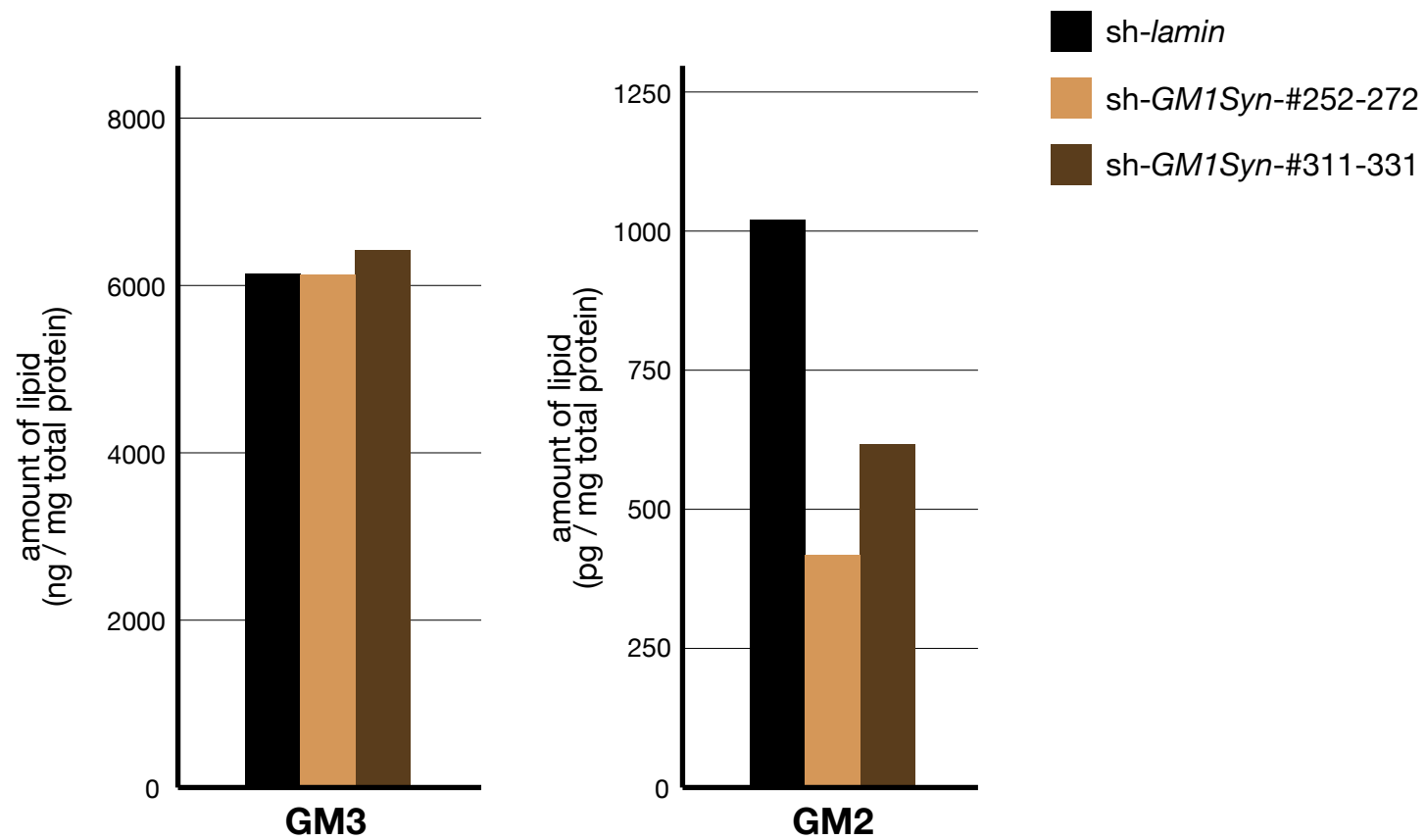


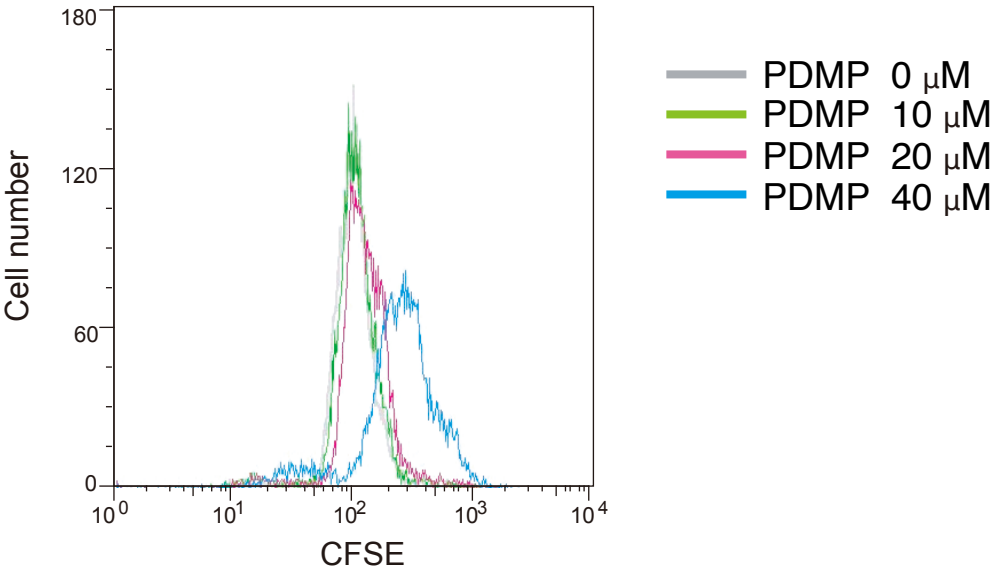
Figure 6



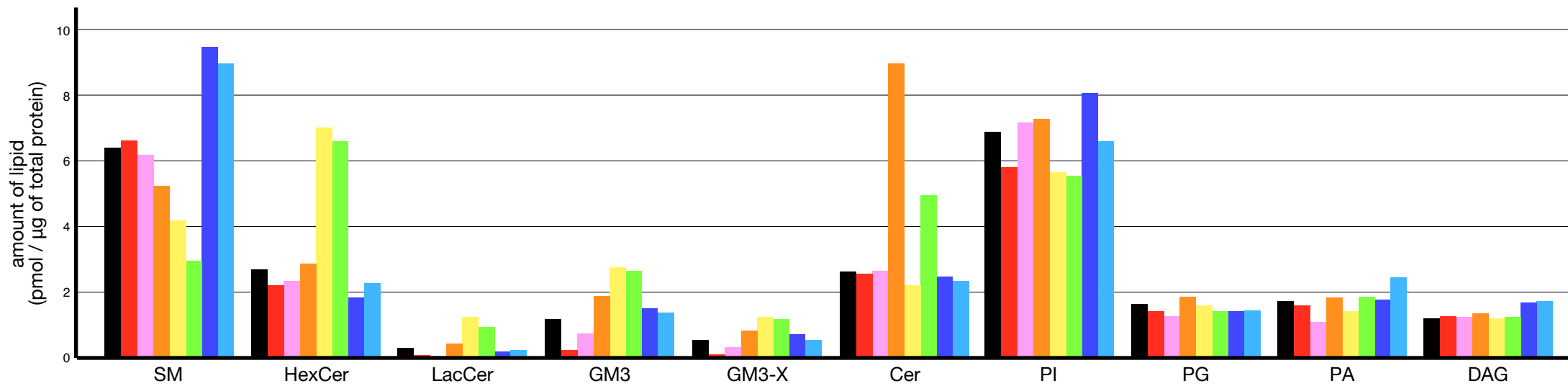
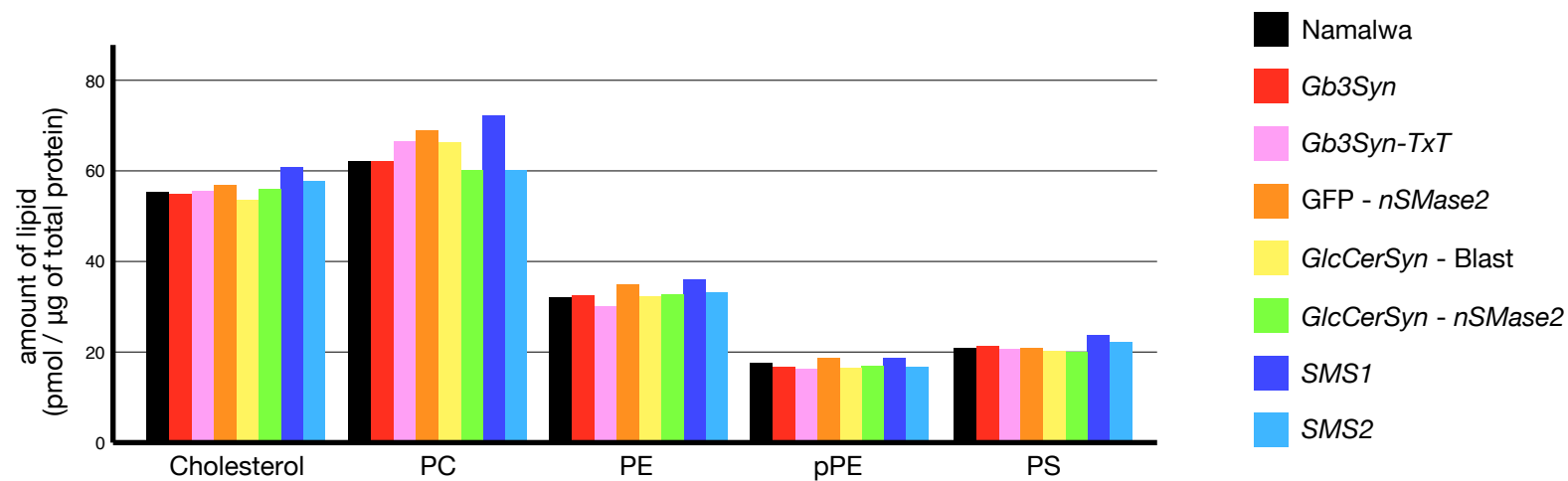
Supplemental Figure S1

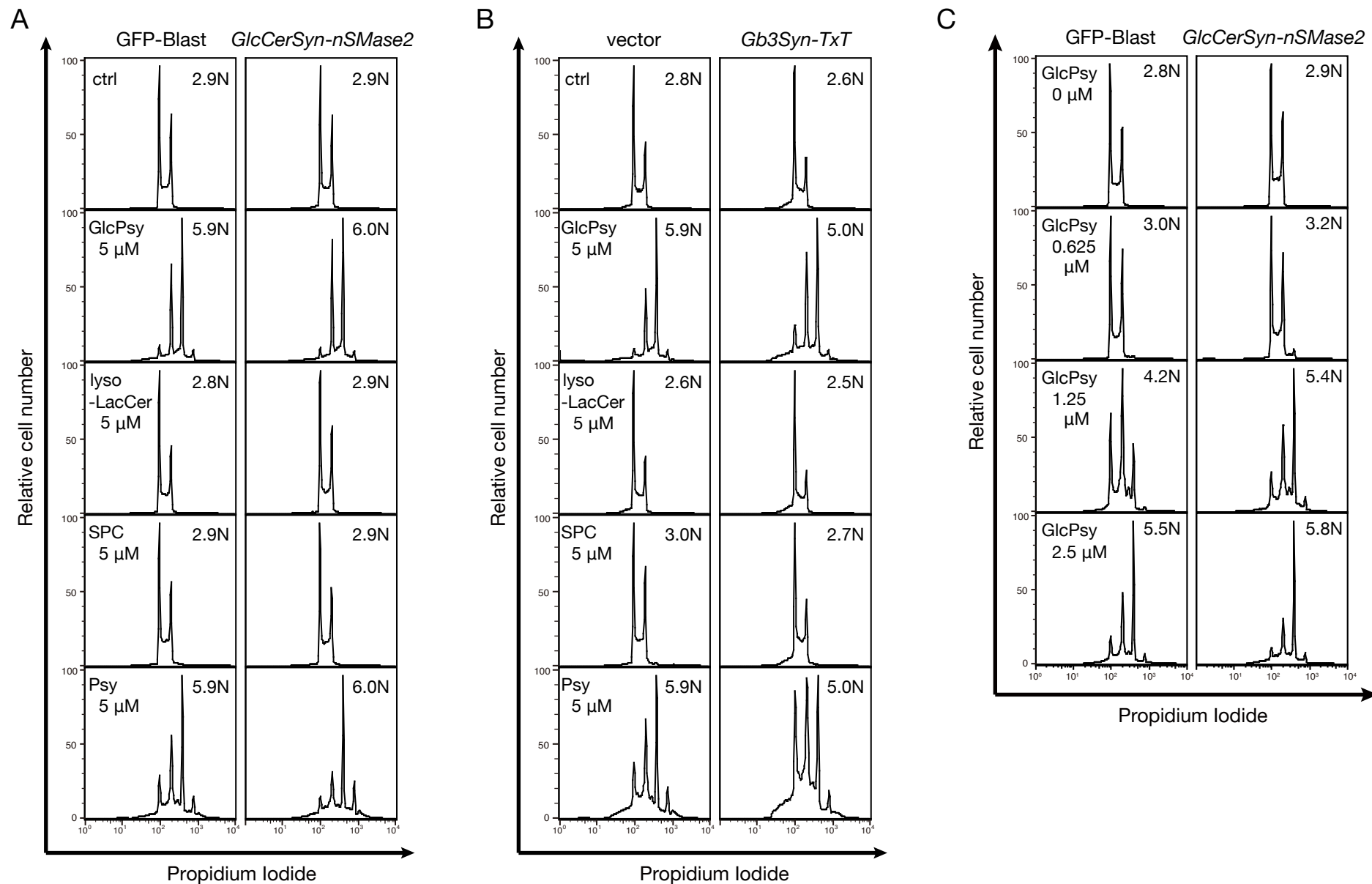


Supplemental Figure S2

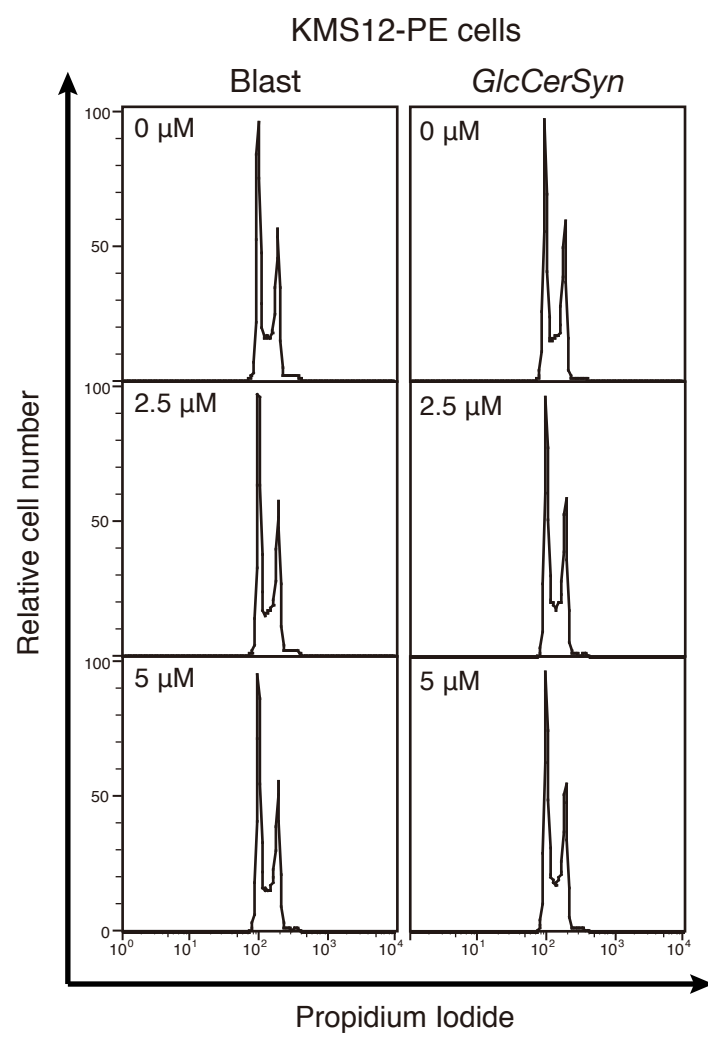


Supplemental Figure S3

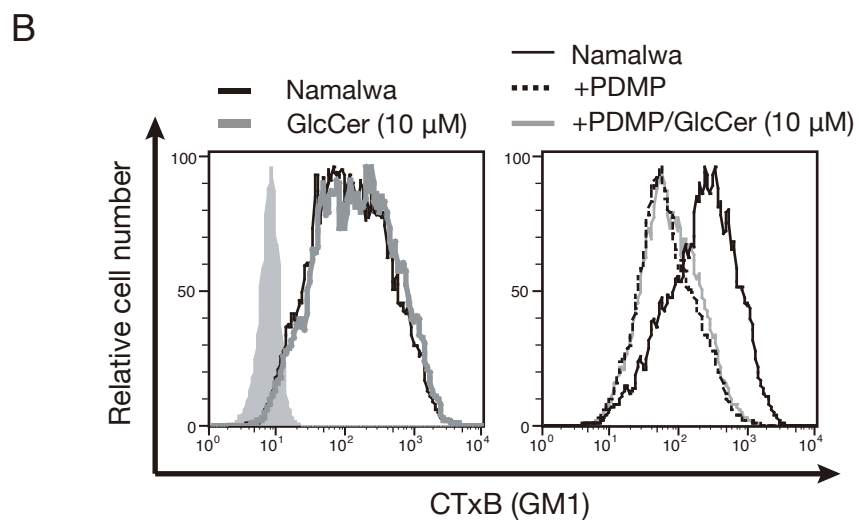
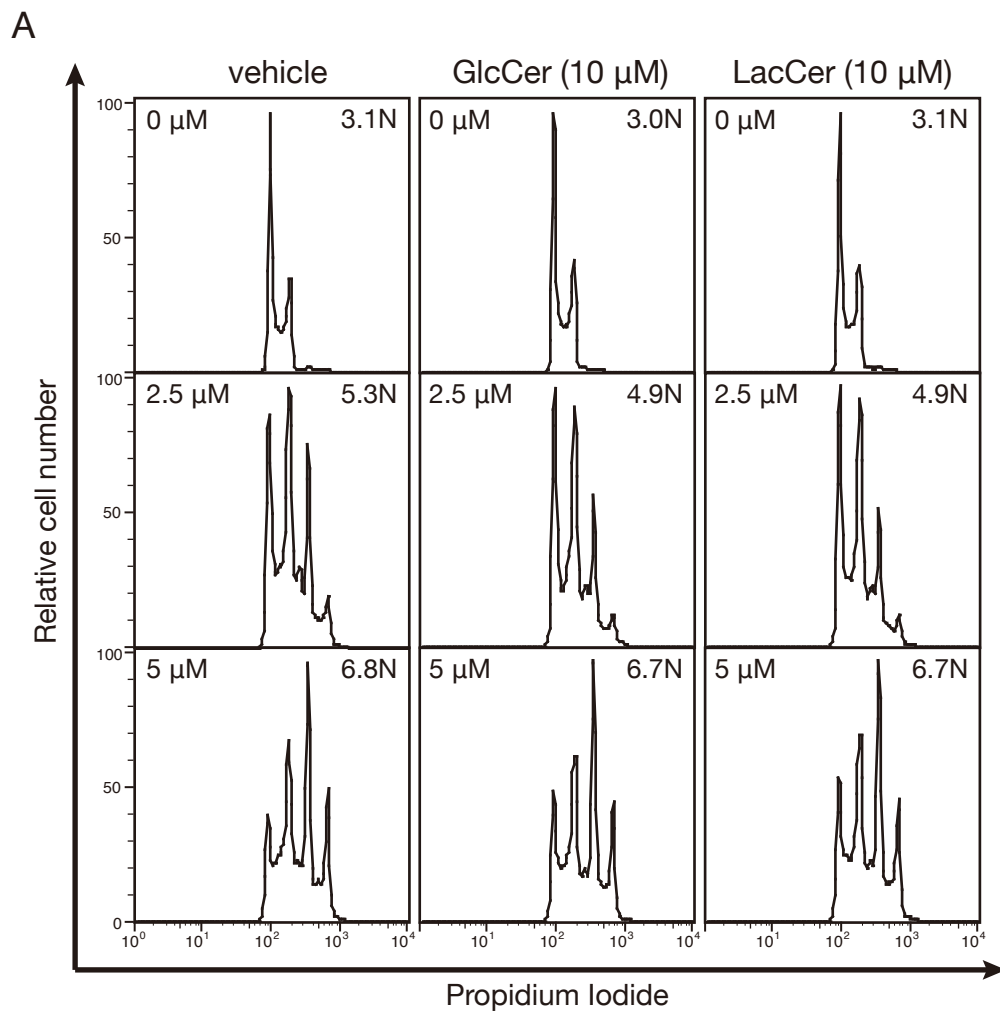




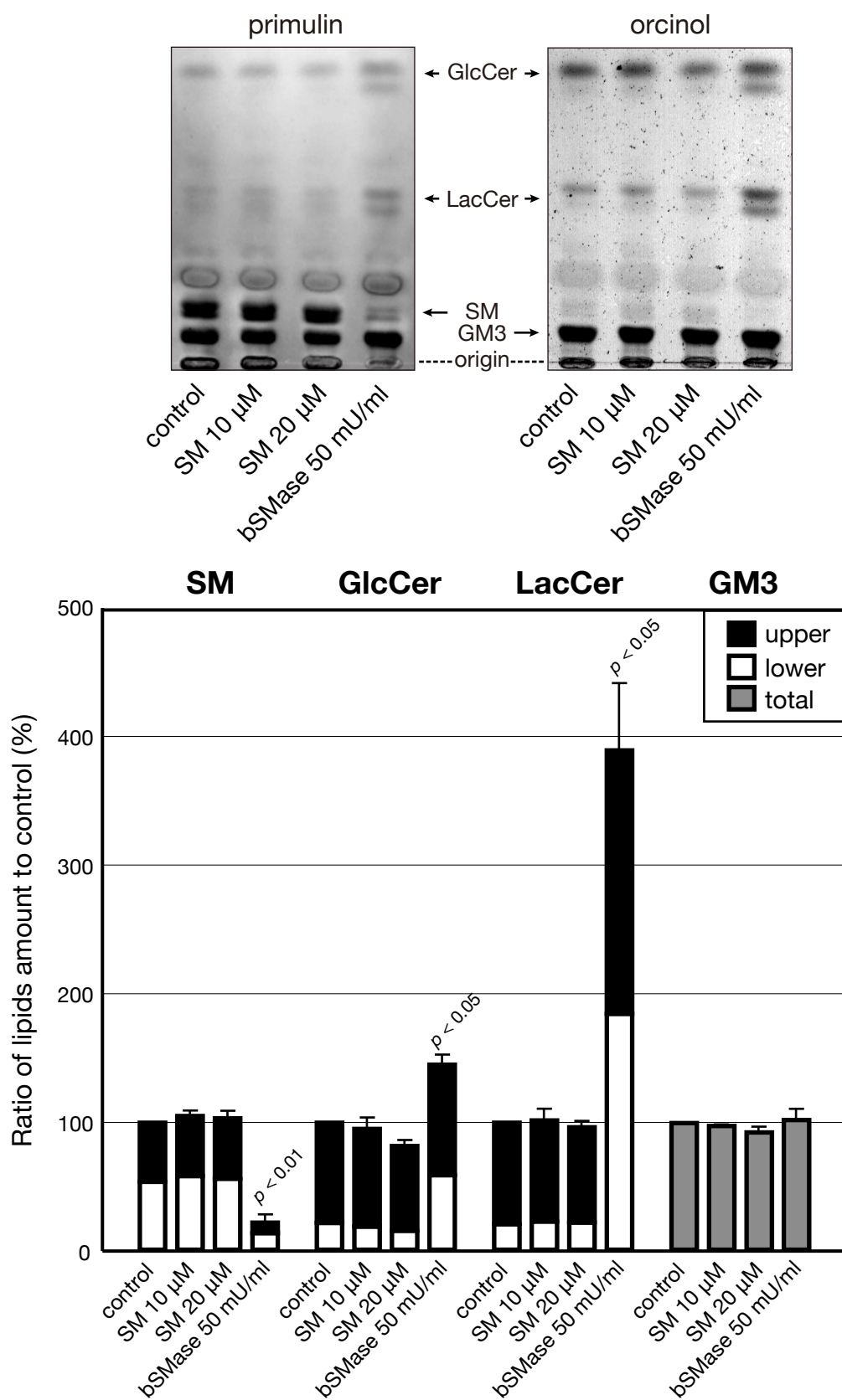
Supplemental Figure S5



Supplemental Figure S6



Supplemental Figure S7



Supplemental Table 1

Ceramide	Namalwa	GFP-nSMase2	GlcCerSyn-Blast	GlcCerSyn-nSMase2	Gb3syn	Gb3syn-TxT	SMS1	SMS2
d18:1/16:0	0.325983879	2.115712157	0.333390081	1.495958499	0.279338526	0.320512821	0.260516865	0.211349075
d18:1/18:1	n.d.	0.004657228	0.000709341	0.003920859	n.d.	n.d.	n.d.	0.000370788
d18:1/18:0	0.051861072	0.163002981	0.03546703	0.120641814	0.047886605	0.039086929	0.048095421	0.035595634
d18:1/20:0	0.015558321	0.053225463	0.016314834	0.033176499	0.012370706	0.014071295	0.012424651	0.014831514
d18:1/22:1	0.006297416	0.066531829	0.009930768	0.042224635	0.007582046	0.009380863	0.008416699	0.006303393
d18:1/22:0	0.203739924	0.538907813	0.180881852	0.301604536	0.199527519	0.195434647	0.200397589	0.196517561
d18:1/24:1	0.663080844	3.492921013	0.638406537	1.698033538	0.610554208	0.656660413	0.617224574	0.552473897
d18:1/24:0	1.179098506	2.229814243	0.870006242	1.05652069	1.21432448	1.232410882	1.131444786	1.0986444
d18:1/26:1	0.085200332	0.159676389	0.060293951	0.078417179	0.083801558	0.089899937	0.084166987	0.085281206
d18:1/26:0	0.059269796	0.079838195	0.03546703	0.036192544	0.059858256	0.054721701	0.064127228	0.07415757
d16:1/18:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
d16:1/20:0	0.000370436	0.001995955	n.d.	0.000904814	n.d.	n.d.	n.d.	n.d.
d20:1/18:0	n.d.	0.000332659	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
d20:1/24:1	0.001111309	0.002328614	n.d.	0.000301605	n.d.	0.000390869	0.001202386	n.d.
d20:1/24:0	0.001111309	0.002993932	0.00035467	0.000301605	0.00399055	0.002736085	0.001202386	0.002224727
d18:0/16:0	0.006297416	0.026612732	0.006384065	0.033176499	0.001995275	0.001954346	0.006813518	0.007044969
d18:0/18:0	n.d.	0.000665318	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
d18:0/22:0	0.001852181	0.004324569	0.003901373	0.005428882	0.00079811	0.003126954	0.004809542	0.007786545
d18:0/24:1	0.002222617	0.024949436	0.003546703	0.026239595	0.00399055	0.005863039	0.003607157	0.006303393
d18:0/24:0	0.013335704	0.012973707	0.007802747	0.009048136	0.015962202	0.009380863	0.010420675	0.022247271
d18:0/26:0	n.d.	0.000332659	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
d18:1/16:0h	0.002593054	0.00765116	0.001064011	0.008746532	0.001995275	0.001172608	0.001202386	0.000741576
d18:0/16:0h	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
total	2.618984116	8.989448052	2.203921235	4.950838461	2.543975865	2.636804253	2.456072849	2.321873517

HexCer	Namalwa	GFP-nSMase2	GlcCerSyn-Blast	GlcCerSyn-nSMase2	Gb3syn	Gb3syn-TxT	SMS1	SMS2
d18:1/16:0	0.25127924	0.206248669	0.873907615	0.882193268	0.18592337	0.203962704	0.120238553	0.184613319
d18:1/18:1	0.004568713	0.007983819	0.044475655	0.054967427	0.009668015	0.013111888	0.007214313	0.004195757
d18:1/18:0	0.076145224	0.073185012	0.405742821	0.39359392	0.072881961	0.072843823	0.050500192	0.082236842
d18:1/20:0	0.031980994	0.026612732	0.117041199	0.115363735	0.025285578	0.02039627	0.017634988	0.031887755
d18:1/22:1	0.015990497	0.013306366	0.078027466	0.074647123	0.015617563	0.009469697	0.012825446	0.015943878
d18:1/22:0	0.373111598	0.332659144	0.959737828	0.916123779	0.275166587	0.269522145	0.240477107	0.360835124
d18:1/24:1	0.639619883	0.625399191	1.771223471	1.621878393	0.498274631	0.568181818	0.432858792	0.528665414
d18:1/24:0	1.142178363	1.390515222	2.372034956	2.225841477	0.981675393	1.034382284	0.817622162	0.897892052
d18:1/26:1	0.114217836	0.133063658	0.29650437	0.244299674	0.104117087	0.101981352	0.096190843	0.109089689
d18:1/26:0	0.04416423	0.0545561	0.101435705	0.088219327	0.040903141	0.040064103	0.034468385	0.051188238
total	2.693256579	2.863529913	7.020131086	6.617128122	2.209513327	2.333916084	1.830030781	2.266548067

LacCer	Namalwa	GFP-nSMase2	GlcCerSyn-Blast	GlcCerSyn-nSMase2	Gb3syn	Gb3syn-TxT	SMS1	SMS2
d18:1/16:0	0.025889376	0.057361225	0.214575531	0.141962135	n.d.	0.010272847	0.003922676	0.02823395
d18:1/18:0	0.003045809	0.003955947	0.015605493	0.023400352	n.d.	n.d.	n.d.	0.003321641
d18:1/20:0	0.001522904	0.001977973	0.003901373	0.021840328	n.d.	n.d.	n.d.	n.d.
d18:1/22:0	0.033503899	0.059339199	0.146301498	0.120121807	n.d.	n.d.	0.019613381	0.044842156
d18:1/24:1	0.106603314	0.16219381	0.54619226	0.390005866	0.052166325	0.024654832	0.084337538	0.073076105
d18:1/24:0	0.103557505	0.136480157	0.312109863	0.218403285	0.016598376	0.008218277	0.066685496	0.073076105
total	0.274122807	0.421308311	1.238686017	0.915733773	0.068764701	0.043145957	0.174559091	0.222549957

GM3	Namalwa	GFP-nSMase2	GlcCerSyn-Blast	GlcCerSyn-nSMase2	Gb3syn	Gb3syn-TxT	SMS1	SMS2
d18:1/16:0	0.138901154	0.181299234	0.325042592	0.314191182	0.019831826	0.068895279	0.117507861	0.123676807
d18:0/16:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
d18:1/18:1	0.005519251	0.008316479	0.012932729	0.013892807	0.000743693	0.016474958	0.005470194	0.002980164
d18:1/18:0	0.018397504	0.029939323	0.061214918	0.057708584	0.004338212	0.014977235	0.022285974	0.023841312
d18:1/20:1	0.001931738	0.002162284	0.00431091	0.003526636	n.d.	n.d.	0.001215599	n.d.
d18:1/20:0	0.017477629	0.024949436	0.038798187	0.040609745	0.002478978	0.00763839	0.017423579	0.02235123
d18:1/22:1	0.013798128	0.021622844	0.049144371	0.044884455	0.002107132	0.0070393	0.01722098	0.019371066
d18:1/22:0	0.126022901	0.207911965	0.3198695	0.32380928	0.02850825	0.08237479	0.184365782	0.201161072
d18:1/24:1	0.358751325	0.554709123	0.993233596	0.904101157	0.063213946	0.212676731	0.465979448	0.411262636
d18:1/24:0	0.424062463	0.734345061	0.789758658	0.810057538	0.08552475	0.284567457	0.579435314	0.482786573
d18:1/26:1	0.040474508	0.08150149	0.118118926	0.105799072	0.010411709	0.025461299	0.064831923	0.058113199
d18:1/26:0	0.013798128	0.027444379	0.029314186	0.030991647	0.001859234	0.009136113	0.020057376	0.02235123
total	1.159134729	1.874201618	2.741738573	2.649572102	0.21901773	0.729241553	1.495794029	1.367895289

SM	Namalwa	GFP-nSMase2	GlcCerSyn-Blast	GlcCerSyn-nSMase2	Gb3syn	Gb3syn-TxT	SMS1	SMS2
d18:1/16:0	1.773709376	1.48135757	1.505383398	1.070885197	1.868571985	1.781121399	2.276771082	2.01526651
d18:0/16:0	0.195481018	0.161239359	0.160527843	0.162526081	0.20014741	0.170148781	0.47700988	0.361460578
d18:1/18:1	0.04628631	0.032062005	0.050693003	0.030207727	0.050301598	0.049956474	0.066137566	0.051495754
d18:1/18:0	0.216152574	0.169138694	0.157545902	0.141253034	0.232446331	0.220599873	0.268647282	0.27827513
d18:1/20:0	0.206266178	0.199806699	0.125241537	0.12593644	0.165201037	0.198342039	0.293814674	0.247575738
d18:1/22:1	0.199076071	0.144511356	0.156551922	0.096579634	0.204383334	0.192406616	0.293814674	0.240643618
d18:1/22:0	0.503756831	0.509739415	0.294218214	0.247192809	0.532137956	0.517371003	0.981528305	0.981390227
d18:1/24:1	2.042888985	1.465094234	1.085923646	0.63053312	2.081427168	1.899335233	2.813480358	2.535670712
d18:1/24:0	0.895168248	0.838258801	0.363796846	0.278251458	0.962613735	0.846786958	1.616565997	1.806312587
d18:1/26:1	0.062464049	0.051113342	0.026837472	0.01786936	0.06353886	0.059848845	0.101254858	0.143593928
d18:1/26:0	0.019323411	0.015334002	0.006957863	0.00468007	0.020120639	0.018795505	0.036873156	0.064864843

d16:1/18:1	0.174809462	0.086428014	0.156551922	0.074881126	0.16890747	0.168170307	0.196071546	0.170827259
d18:1/16:0h	0.072350446	0.081781346	0.082997368	0.074030204	0.086306952	0.074687401	0.081940347	0.0836806
total	6.407732959	5.235864838	4.173226938	2.954826259	6.636104475	6.197570434	9.503909725	8.981057485

PC	Namalwa	GFP-nSMase2	GlcCerSyn-Blast	GlcCerSyn-nSMase2	Gb3syn	Gb3syn-TxT	SMS1	SMS2
(32:0)	4.595061615	4.875651701	4.716159387	4.111482889	4.589072934	4.581979054	4.706617194	4.462930975
(34:2)	5.642291965	6.424068438	6.009852581	5.611363342	5.784618067	5.98666787	6.578487066	5.548824423
(34:1)	27.90248757	30.87037416	30.23056952	27.12921855	28.09531063	29.89772682	31.70809325	27.30756912
(36:4)	0.647859143	0.674535178	0.786958212	0.648025536	0.716589089	0.73106015	0.808275386	0.689281902
(36:3)	1.605782319	1.73132218	1.775638262	1.550786482	1.613493936	1.842572529	1.97230479	1.52127856
(36:2)	14.53588917	16.71358423	15.22683937	14.36042651	14.35761146	15.90225111	18.01001189	13.23650157
(36:1)	4.129528856	4.807832829	4.333373969	4.083771946	4.08674718	4.467366879	5.014692838	4.497500179
(38:6)	0.403519952	0.426779366	0.508193212	0.398216515	0.434184601	0.457947113	0.513177287	0.462479894
(38:5)	0.680612386	0.706732016	0.812720278	0.655209854	0.709701175	0.765167931	0.874573716	0.708668686
(38:4)	0.759875234	0.528621847	0.558016076	0.49202319	0.50822968	0.599644878	0.679628413	0.485136737
(38:3)	0.477542281	0.492314775	0.512324864	0.433111777	0.483629986	0.524657919	0.579757737	0.524611031
(40:7)	0.282332953	0.30324398	0.319595444	0.274646236	0.293474351	0.325027086	0.36675672	0.305049869
(40:6)	0.257003779	0.267621946	0.290673879	0.252066949	0.264446712	0.305716063	0.339108905	0.259969758
(40:5)	0.190842228	0.201401499	0.228456059	0.181250094	0.191139623	0.207906986	0.244034279	0.208115952
(40:4)	0.077297653	0.079921123	0.090653309	0.071022121	0.076997043	0.083012319	0.099306435	0.081985072
total	62.1879271	69.10400538	66.40002442	60.25262199	62.20524647	66.67870471	72.49482591	60.29990373

PE	Namalwa	GFP-nSMase2	GlcCerSyn-Blast	GlcCerSyn-nSMase2	Gb3syn	Gb3syn-TxT	SMS1	SMS2
(32:0)	0.137061404	0.164666276	0.175502506	0.141735043	0.162092944	0.127262443	0.165529649	0.159923392
(34:2)	1.142178363	1.3310524	1.128908014	1.176830358	1.165043036	1.051093514	1.175831301	1.211540847
(34:1)	6.917032164	7.574648712	6.692811797	6.996557127	7.278986271	6.240573152	7.58011635	7.434014639
(36:4)	0.178179825	0.187536593	0.19447575	0.180390055	0.192485371	0.174396682	0.239732595	0.203538862
(36:3)	0.680738304	0.75014637	0.683036782	0.721560219	0.714222035	0.707013575	0.833356165	0.722078345
(36:2)	6.122076023	6.737595141	5.786839399	6.38237194	5.956915695	5.948340875	7.083527403	5.330779728
(36:1)	8.72624269	9.317366803	8.556933013	9.14405778	8.61625306	7.942119155	9.406650411	9.110787172
(38:6)	0.219298246	0.233277225	0.232422238	0.214750065	0.222877798	0.197963801	0.234024676	0.242308169
(38:5)	1.151315789	1.184682377	1.219030923	1.078045328	1.119454395	1.126508296	1.381316383	1.14369456
(38:4)	2.275219298	2.424253513	2.4617784	2.211925672	2.325020667	2.323717949	2.711261496	2.515158799
(38:3)	0.973135965	1.006293911	1.06250166	0.936310285	0.987753878	0.994532428	1.090212517	1.066155946
(40:7)	0.456871345	0.489424766	0.460101165	0.450975137	0.445755596	0.438348416	0.525128542	0.465231685
(40:6)	1.868603801	2.017161885	2.106030076	1.791015544	1.950180734	1.696832579	2.117637926	2.137158055
(40:5)	1.174159357	1.262441452	1.323383764	1.15106035	1.235958699	1.074660633	1.341360951	1.371464239
(40:4)	0.127923977	0.146370023	0.151785951	0.103080031	0.121569708	0.103695324	0.136990055	0.159923392
total	32.15003655	34.82691745	32.23554144	32.68066493	32.49456989	30.14705882	36.02267642	33.27375783

DAQ	Namalwa	GFP-nSMase2	GlcCerSyn-Blast	GlcCerSyn-nSMase2	Gb3syn	Gb3syn-TxT	SMS1	SMS2
32:1(16:0/16:1)	0.173533675	0.174444274	0.173481065	0.17065378	0.196617169	0.18493081	0.239675516	0.257977166
34:3(16:1/18:2)	0.00092923	0.000719853	n.d.	0.001334971	n.d.	0.000763126	0.001874906	0.001829625
34:2(16:0/18:2)	0.016726137	0.017516413	0.014565127	0.016242146	0.010155263	0.010683761	0.023748813	0.026137504
34:2(16:1/18:1)	0.073641466	0.085662456	0.07204536	0.079430768	0.072497292	0.078093203	0.102182391	0.09331089
34:1(16:0/18:1)	0.477391838	0.533890659	0.492873491	0.472802193	0.517636306	0.497303622	0.657779611	0.714860739
36:4(16:0/20:4)	0.005575379	0.004319115	0.00676238	0.006229864	0.006205994	0.006613757	0.009374531	0.011500502
36:4(18:2/18:2)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
36:3(18:1/18:2)	0.010918451	0.013197297	0.011444028	0.012904719	0.010155263	0.012210012	0.017811609	0.014375627
36:2(18:1/18:1)	0.292475097	0.359686336	0.280638785	0.335745181	0.287450352	0.312627188	0.446540173	0.399642439
36:1(18:0/18:1)	0.103609129	0.121415134	0.102996255	0.105907691	0.108886983	0.100732601	0.136243188	0.150552024
38:6(16:0/22:6)	0.011383066	0.011757592	0.013784852	0.013794699	0.012976169	0.011701262	0.015311734	0.016728003
38:4(16:0/20:4)	0.009524606	0.013197297	0.016125676	0.013572204	0.01777171	0.01500814	0.017499125	0.018296253
40:7(18:1/22:6)	0.003949227	0.004559066	0.004681648	0.004672398	0.002820906	0.003306878	0.005624719	0.005227501
40:6(18:0/22:6)	0.004878457	0.007198526	0.005461923	0.005339884	0.002820906	0.003815629	0.007187141	0.006795751
total	1.184535757	1.347564019	1.194860591	1.238630498	1.245994313	1.237789988	1.680853457	1.717234025

PS	Namalwa	GFP-nSMase2	GlcCerSyn-Blast	GlcCerSyn-nSMase2	Gb3syn	Gb3syn-TxT	SMS1	SMS2
(32:0)	0.039391663	0.039434642	0.062030947	0.045185306	0.041761249	0.036062292	0.039998	0.029564806
(34:2)	0.241566227	0.252950138	0.231949528	0.236890613	0.240270201	0.229253143	0.245821042	0.251652813
(34:1)	3.262852834	3.202554796	2.986372388	3.054161234	3.405925445	3.05995908	3.485867373	3.563615016
(36:4)	0.024462403	0.021848923	0.023106083	0.020765306	0.023836329	0.025574789	0.022915521	0.027277053
(36:3)	0.148033511	0.149922694	0.140236151	0.143862041	0.149692149	0.151424829	0.171449761	0.15134365
(36:2)	2.30360288	2.286321082	2.063017754	2.157266129	2.22308669	2.280939975	2.684449111	2.1401048
(36:1)	10.69888365	10.76370336	10.20471279	10.37667269	10.8293212	10.60139392	12.04398113	11.2661269
(38:6)	0.026261109	0.027177929	0.030571126	0.027077959	0.029175667	0.028518649	0.028331917	0.029212844
(38:5)	0.099828188	0.101251108	0.098822941	0.09967347	0.104307778	0.107818894	0.118119094	0.107348403
(38:4)	0.42575373	0.373208033	0.400268457	0.397531022	0.425430808	0.442683035	0.443102845	0.454382912
(38:3)	0.838017164	0.836120995	0.88656264	0.780443268	0.862112364	0.883894139	1.003074846	0.884656429
(40:7)	0.104324953	0.104270878	0.098111984	0.098510613	0.100875346	0.103771085	0.10353649	0.10858027
(40:6)	1.084979509	1.113762193	1.171122941	1.022151432	1.12793511	1.069357355	1.293893639	1.20898939
(40:5)	1.405149192	1.44380528	1.569969486	1.338282453	1.49692149	1.433844093	1.703456494	1.759809884
(40:4)	0.181309573	0.191311305	0.21577527	0.174760817	0.186686132	0.179023521	0.204364782	0.225431646
total	20.88441659	20.90764336	20.18263048	19.97323435	21.24655994	20.6335188	23.59236205	22.20809681

PA	Namalwa	GFP-nSMase2	GlcCerSyn-Blast	GlcCerSyn-nSMase2	Gb3syn	Gb3syn-TxT	SMS1	SMS2
(32:0)	0.586495308	0.578987875	0.518606792	0.643914346	0.652121257	0.36550789	0.666279427	1.246042972
(34:2)	0.148217564	0.185913538	0.122426933	0.170928135	0.121690485	0.092455621	0.147350258	0.111554523
(34:1)	0.510527166	0.537083554	0.394603547	0.51839697	0.458813885	0.362426036	0.511098962	0.713527985
(36:4)	0.005843703	0.008853026	0.002417012	0.007143265	0.004425109	0.004191321	0.003915103	0.005262006
(36:3)	0.032405991	0.036002304	0.028899061	0.040308426	0.023872296	0.019723866	0.031320828	0.022626625
(36:2)	0.287403951	0.301593072	0.20229343	0.317875307	0.197383131	0.161489152	0.254125807	0.182591601
(36:1)	0.097749218	0.112728526	0.085646309	0.102046648	0.085008664	0.049309665	0.104640038	0.125235738
(38:6)	0.005843703	0.005783977	0.004886569	0.003928796	0.004599784	0.00191075	0.001779592	0.004420085
(38:5)	0.012749898	0.013574639	0.013135937	0.015306997	0.007569265	0.005362426	0.012101229	0.007366808
(38:4)	0.016999864	0.010033429	0.005779812	0.010714898	0.01048052	0.006163708	0.009253881	0.010524012
(38:3)	0.009031178	0.011213832	0.011559625	0.007653499	0.004192208	0.002095661	0.007830207	0.005788206
(40:7)	0.004674963	0.007672622	0.005201831	0.006633032	0.002794805	0.002896943	0.004413389	0.003420304
(40:6)	0.001381239	0.005902017	0.002574644	0.005102332	0.003435282	0.001849112	0.002349082	0.003104583
(40:5)	0.005843703	0.005724957	0.005254375	0.004183913	0.003260606	0.002095661	0.003772736	0.004420085
(40:4)	0.000796869	0.001003343	0.000893244	0.00030614	0.000349351	0.000493097	0.000213551	0.00021048
total	1.725964317	1.822070711	1.40417912	1.854442703	1.579996646	1.077970907	1.760444072	2.446096013

PI	Namalwa	GFP-nSMase2	GlcCerSyn-Blast	GlcCerSyn-nSMase2	Gb3syn	Gb3syn-TxT	SMS1	SMS2
(34:2)	0.341501732	0.368873905	0.282296383	0.311941788	0.299307114	0.344103866	0.38921665	0.286737719
(34:1)	0.732529854	0.75276012	0.648443177	0.638659767	0.674577204	0.705290902	0.840864041	0.848239493
(36:4)	0.138789068	0.135110791	0.135558164	0.111095058	0.124657193	0.138292334	0.153150411	0.165110511
(36:3)	0.146851504	0.157629256	0.130899808	0.122040384	0.136343805	0.150494598	0.176561939	0.155657619
(36:2)	1.294596786	1.382204837	0.961950547	1.025029771	1.056989113	1.253579331	1.460294049	1.072588126
(36:1)	0.871894811	0.982234003	0.71831852	0.719655178	0.761577537	0.918423793	1.004744736	0.912519158
(38:6)	0.038584513	0.041283853	0.035403507	0.032288711	0.031164298	0.0414877	0.050724977	0.041592724
(38:5)	0.591437233	0.61496856	0.538971808	0.48597247	0.49538249	0.598724457	0.68576267	0.594901992
(38:4)	1.671227702	1.751078742	1.329960683	1.260901545	1.308251267	1.847422882	2.038753882	1.569180044
(38:3)	0.664575041	0.703970113	0.55574189	0.519355714	0.588875384	0.767111571	0.818427994	0.622630475
(40:7)	0.021307865	0.020373849	0.016770082	0.014228924	0.015582149	0.019523624	0.023411528	0.018275591
(40:6)	0.099052779	0.097043862	0.071738685	0.07388095	0.082455539	0.104939477	0.11803312	0.087596798
(40:5)	0.211350988	0.224112344	0.172359178	0.174030682	0.187635045	0.222894703	0.248747483	0.180235138
(40:4)	0.067378925	0.067555395	0.054502767	0.050895765	0.060380828	0.074027073	0.081940347	0.057977737
total	6.8910788	7.299199629	5.652915199	5.539976708	5.823178966	7.186320448	8.090633828	6.613243123

PQ	Namalwa	GFP-nSMase2	GlcCerSyn-Blast	GlcCerSyn-nSMase2	Gb3syn	Gb3syn-TxT	SMS1	SMS2
(32:0)	0.078019568	0.087822014	0.092789419	0.080427876	0.095671754	0.070986061	0.072835864	0.094436813
(34:2)	0.067476383	0.086358314	0.068537639	0.065347649	0.061008075	0.055928412	0.063731381	0.062549058
(34:1)	1.166076248	1.280737705	1.126126126	1.004343105	0.996927411	0.884099122	1.021978222	1.002011381
(36:4)	0.001476046	0.004244731	0.004428586	0.001910162	0.00263444	0.002796421	0.003414181	0.005028454
(36:3)	0.016869096	0.020491803	0.014761953	0.015080227	0.013865472	0.010755464	0.012973888	0.015943878
(36:2)	0.080128205	0.099531616	0.068537639	0.067358346	0.06378117	0.059155051	0.077388106	0.072360675
(36:1)	0.210863698	0.25029274	0.194014239	0.167893192	0.173318396	0.153803132	0.15022397	0.155759419
(38:6)	n.d.	0.00131733	0.000948983	0.002211767	n.d.	0.000430219	n.d.	0.000367936
(38:5)	0.000632591	0.00307377	0.00126531	0.001910162	0.001109238	0.002151093	0.00113806	0.001349097
(38:4)	0.001897773	0.005122951	0.003268718	0.003016045	0.00263444	0.003334194	0.002731345	0.004047292
(38:3)	0.000632591	0.0029274	0.003163276	0.001306953	0.000831928	0.001075546	0.001593285	0.001471743
(40:7)	0.000632591	0.00043911	0.000527213	0.000904814	0.000138655	0.000537773	0.00113806	0.000490581
(40:6)	0.001476046	0.00087822	0.00168708	0.001709092	0.001247892	0.000322664	0.00113806	0.000735871
(40:5)	0.000632591	0.00073185	0.001159868	0.001206418	0.000831928	0.000967992	n.d.	0.000613226
(40:4)	n.d.	0.00014637	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
total	1.626813428	1.844115925	1.581216048	1.414625809	1.414000799	1.246343142	1.410284424	1.417165424

Cholesterol	Namalwa	GFP-nSMase2	GlcCerSyn-Blast	GlcCerSyn-nSMase2	Gb3syn	Gb3syn-TxT	SMS1	SMS2
	55.43826918	57.08430913	53.7274835	56.08513764	55.05341729	55.64458689	60.96361849	57.76767229

Supplemental Table 2

GM3 (ng / mg total protein)	sh-lamin	sh-GM1Syn-#252-272	sh-GM1Syn-#311-331
d18:1/16:0	698.3783784	450.877193	547.4747475
d18:1/18:0	50.81081081	30.40935673	41.41414141
d18:1/20:0	81.08108108	60.23391813	78.28282828
d18:1/22:0	650.2702703	454.3859649	736.8686869
d18:1/23:0	134.5945946	138.5964912	145.959596
d18:1/24:0	2067.027027	3304.678363	2614.141414
d18:1/24:1	2457.297297	1695.906433	2263.636364

GM2 (pg / mg total protein)	sh-lamin	sh-GM1Syn-#252-272	sh-GM1Syn-#311-331
d18:1/16:0	664.2931302	315.9787755	231.7644429
d18:1/18:0	14.71137002	17.5772086	13.06555855
d18:1/20:0	13.43247959	5.206232262	7.092087835
d18:1/22:0	75.42400557	7.324045004	102.643521
d18:1/24:0	55.11468663	64.12367026	83.94622348
d18:1/24:1	194.4583252	5.607680831	175.1327398