Supplemental Materials

Molecular Biology of the Cell

He et al.

Supplemental Data

Legends

Supplemental Table

Titer of C24:0/24:1 and C16:0 ceramide-specific antibody (rbt IgG) assayed using ELISA: The serum was diluted at a concentration of 1/1,500 with PBS containing 1% BSA. The plate was coated with 25 ng of antigen (C16:0-, C18:0-, C24:0- and C24:1-Cer). After washing with PBS (3 times, 200 µl) the reaction was blocked using 1% donkey serum in PBS. The plate was washed 3 times with PBS and then incubated using 100 µl of the diluted serum over night at 4 ^{o}C. The excess antibody was washed using PBS (5-6 times) and incubated with secondary antibody (HRP-conjugated anti rabbit IgG at 1/2000) in PBS containing 1% BSA at RT for 1-2 h. The excess antibody was washed off and the color was developed using Sigma stain (5-10 min) at RT. The reaction was stopped using 3N H_2SO_4 and the OD was recorded at 492 nM.

Supplemental Figure 1

Structure of different ceramide species ($C_{16:0}$ and $C_{24:1}$ ceramide). Structures were generated with Sphingomaps drawing application.

Supplemental Figure 2

Undifferentiated human ES cells were cultivated feeder-free, deprived of KSR for 10 h, and then subjected to immunocytochemistry using antibodies against ceramide (mouse IgM, red) and acetylated tubulin (green). This figure is similar to Fig. 1A, but shows primary cilia at higher magnification. Arrow points at primary cilium, arrowhead at apoptotic cell. Bar = 5 µm.

Supplemental Figure 3

Neural differentiation of human ES cells. Immunocytochemistry was performed with antibodies against acetylated tubulin (rbt IgG), nestin (ms IgG), and ceramide (ms IgM). In right panel (low magnification), acetylated tubulin is pseudocolored in green and nestin in red. In right panel (high magnification and Z-scan), acetylated tubulin is pseudocolored in green and ceramide in red. A. Protocol developed by Chambers et al. using SMAD inhibitors (noggin, SB431542). B. Protocol developed by Swistovski et al. using suspension embryoid bodies, followed by attached embryoid bodies and replating of NPs after dissociation.

Supplemental Figure 4

Neural differentiation of human ES cells. A. Apical ceramide elevation (all Z-planes collapsed onto one). Bar = 20 μm. B. Z-scan. Note that during neural differentiation, Oct-4 expression decreases, while ceramide levels are going up. C. RT-PCR for Oct-4 and Nanog. D. Immunoblot analysis for Oct-4 and Sox1. E. Immunocytochemistry for Oct-4, Pax6, and Sox1. Bar = 10 μm.

Supplemental Figure 5

Quantitative HPTLC analysis of ceramide shown in Fig. 2B.

Supplemental Figure 6

Lipidomics analysis of ceramide, glycosylceramide, and sphingomyelin after incubation of human ES cells with FB1 and FB1+$C_{24:1}$ ceramide and differentiation to NPs. A. Ceramide. B. Glucosyl- and galactosylceramide. C. Sphingomyelin. Numbers on x-axis indicate length of fatty acids. Sphingolipid content was normalized to phospholipids. * non-saturated fatty acid.

Supplemental Figure 7

A. Co-labeling of untreated cells with anti-ceramide mouse IgM MAS0014(Glycobiotech) and anti C16 rabbit IgG (our laboratory). Bar = 5 µm. B. Treatment with FB1 and $C_{24:1}$ ceramide, followed by co-labeling with anti-ceramide mouse IgM (MAS0014) and anti-C24 rabbit IgG (anti-C24, our laboratory). Arrow points at cilium, which co-localizes with anti-C24 signal. After addition of exogenous $C_{24:1}$ ceramide to ceramide-depleted cells, there is occasional labeling of rescued cilia with mouse IgM as well, possibly due to a small proportion of exogenous ceramide taken up by the cells and metabolically converted to $C_{16/18}$ ceramide (see also Suppl. Fig. 5 for lipidomics analysis).

Supplemental Figure 8

Quantification of band intensities of immunoblot shown in Fig. 5A using ImageJ. A. Total aPKC in cytosol. B. Phosphorylated aPKC in cytosol.

Supplemental Figure 9

Ceramide restores sequestration of aPKC to the cell membrane and rescues ciliogenesis

FB1 treated differentiating human ES cells were incubated with $C_{16:0}$ or $C_{24:1}$ ceramide, followed by immunocytochemistry using antibodies against ceramide (mouse IgM, labels $C_{16:0}$ ceramide, but does not label $C_{24:1}$ ceramide in immunocytochemistry), acetylated tubulin (mouse IgG), and aPKC (C20, rabbit IgG). A and B shows that $C_{16:0}$ ceramide partially rescues ciliogenesis, but only $C_{24:1}$ ceramide restores sequestration of aPKC to the cell membrane concurrent with nearly complete rescue of primary cilia (C). Arrowheads: primary cilium, arrows: apicolateral cell membrane. Bars = 10 µm.

Supplemental Table

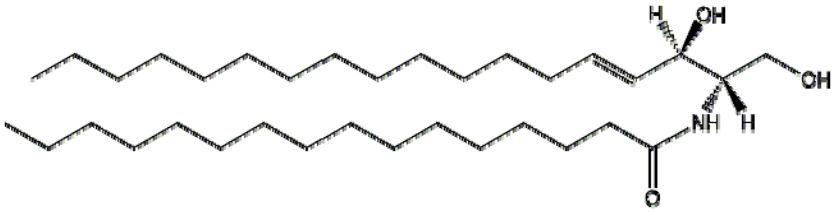
C24:/24:1 ceramide antibody		*C16:0 ceramide antibody*	*MAS0014 (Glycobiotech)**
Antigen	OD at 492	OD at 492	OD at 492
C16-Cer	0.25	0.83	0.86
C18-Cer	0.29	0.34	0.59
C24-Cer	0.81	0.45	0.53
C24:1-Cer	0.78	Not tested	Not tested

Background OD: 0.078: OD presented after subtracting the background OD

*MAS0014 (highly concentrated IgM, dilution 1:12,000)

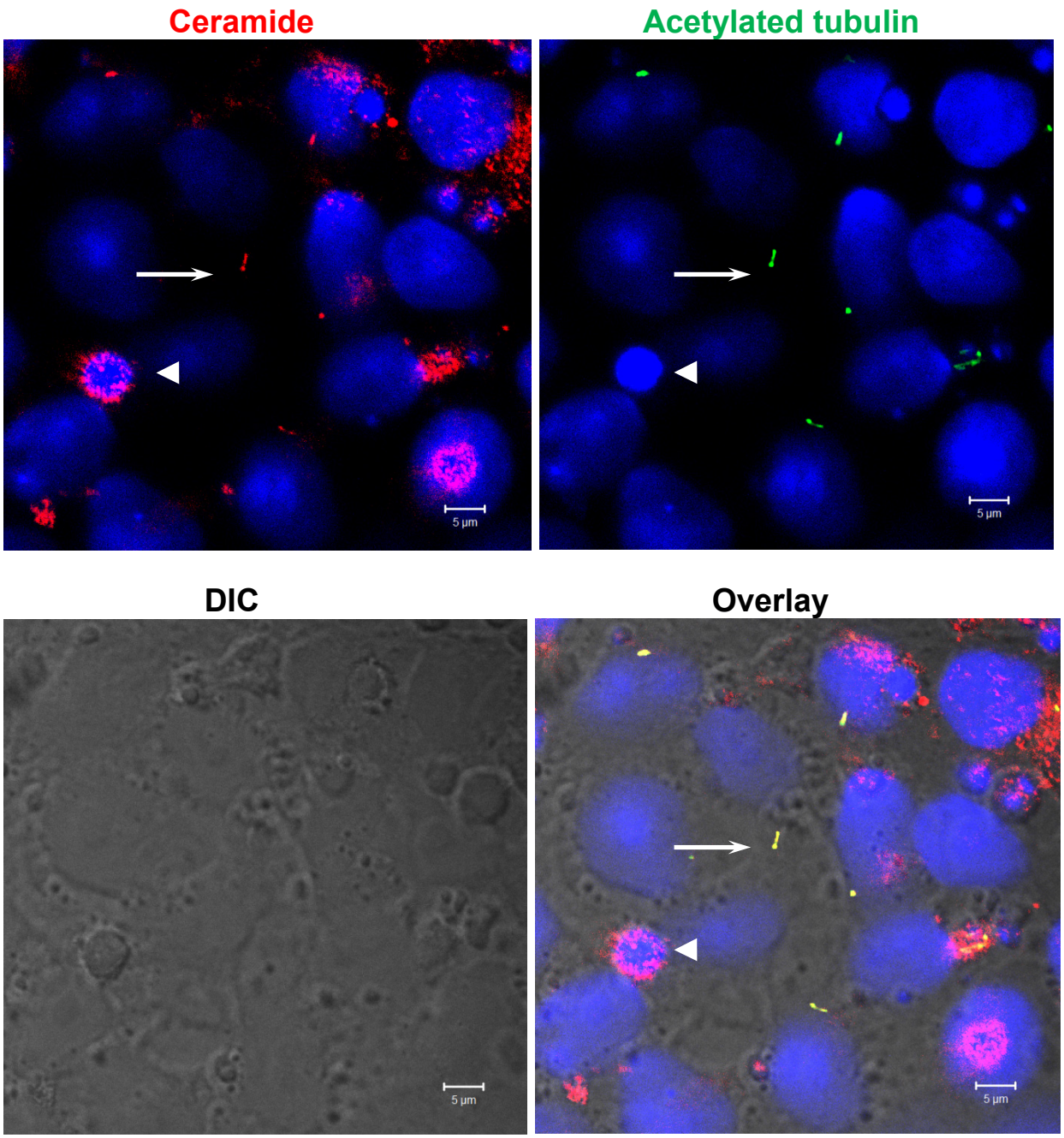
Supplemental Figure 1

$C_{16:0}$ ceramide



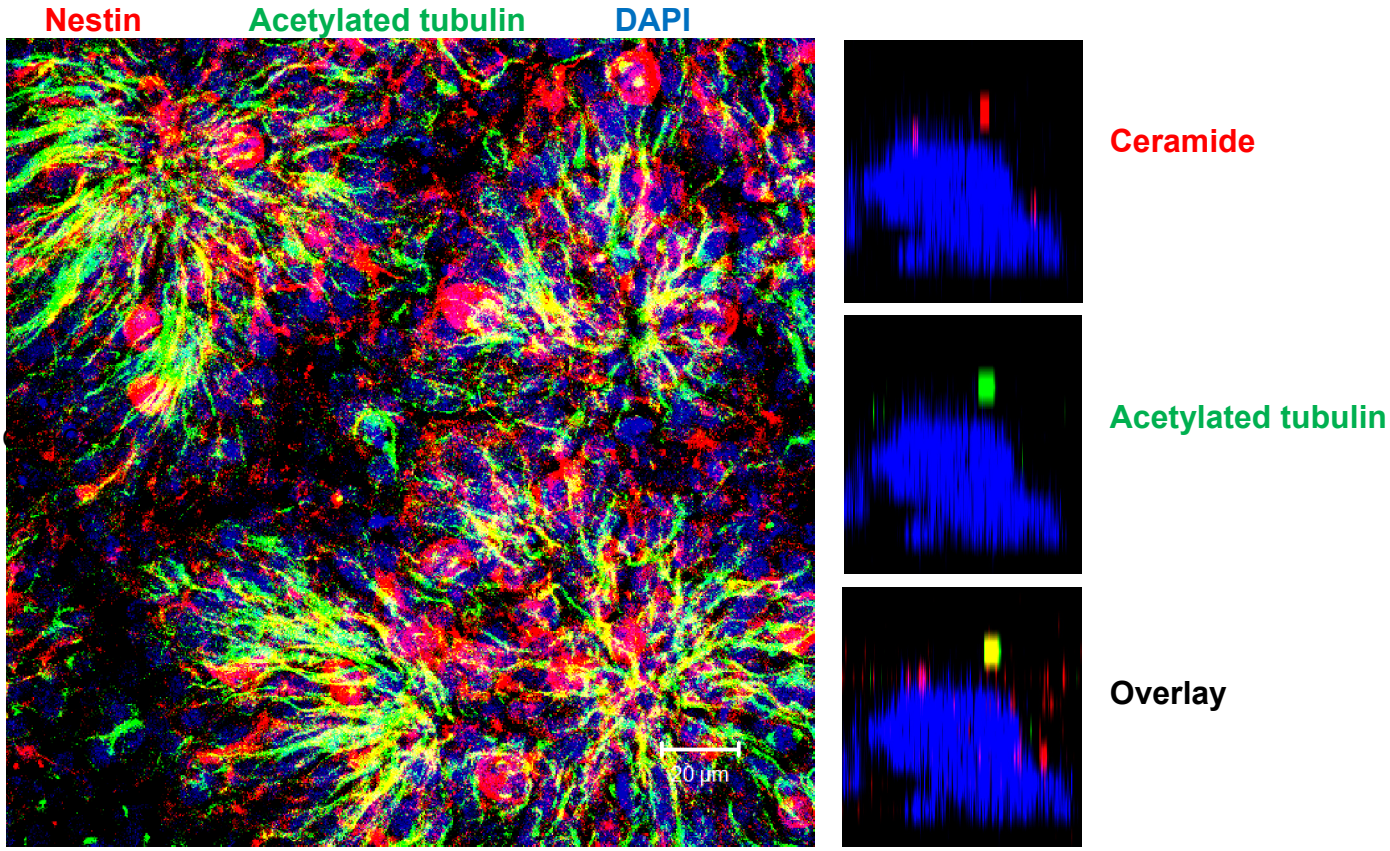
$C_{24:1}$ ceramide

Supplemental Figure 2 (Detailed cilia from Fig. 1A)

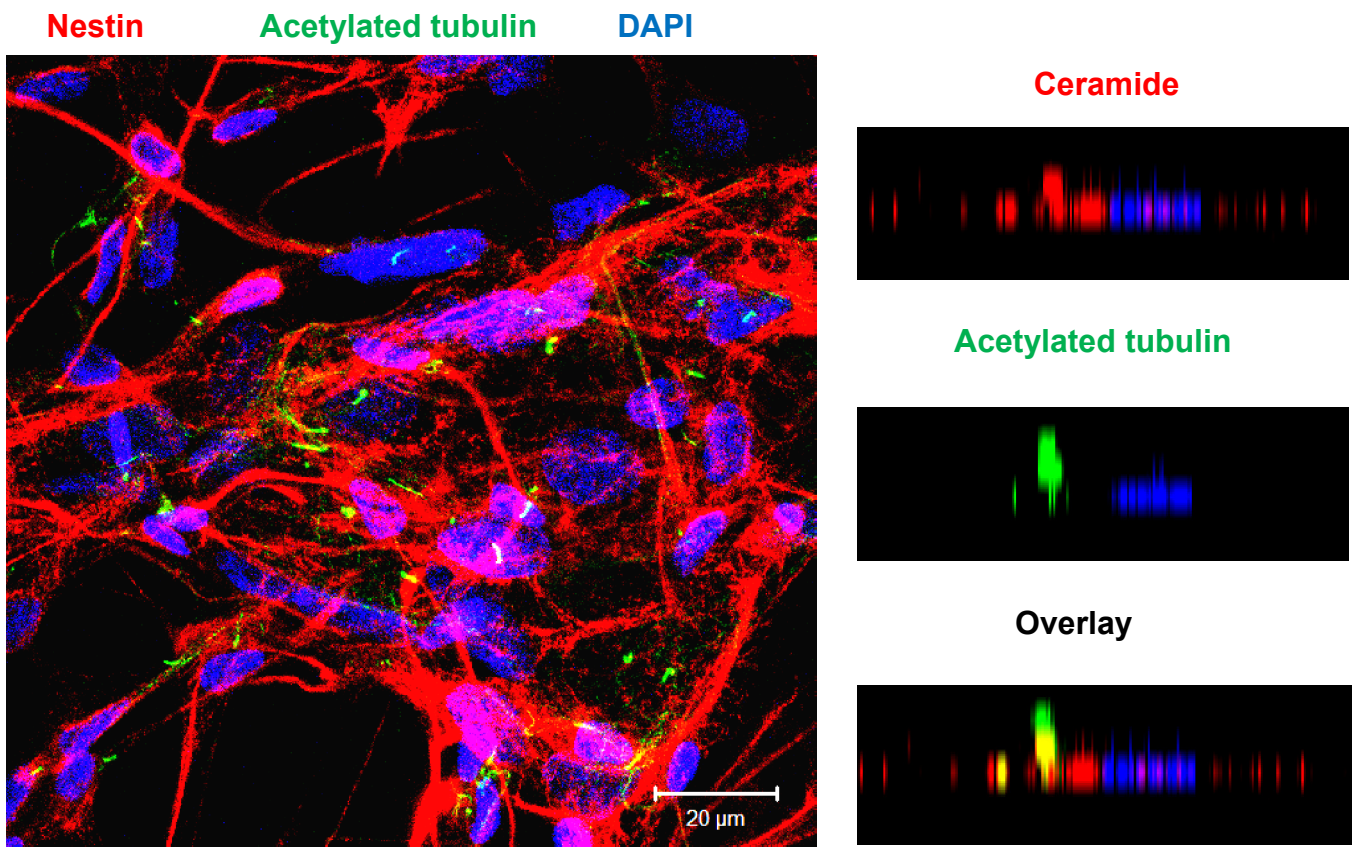


Supplemental Figure 3

A

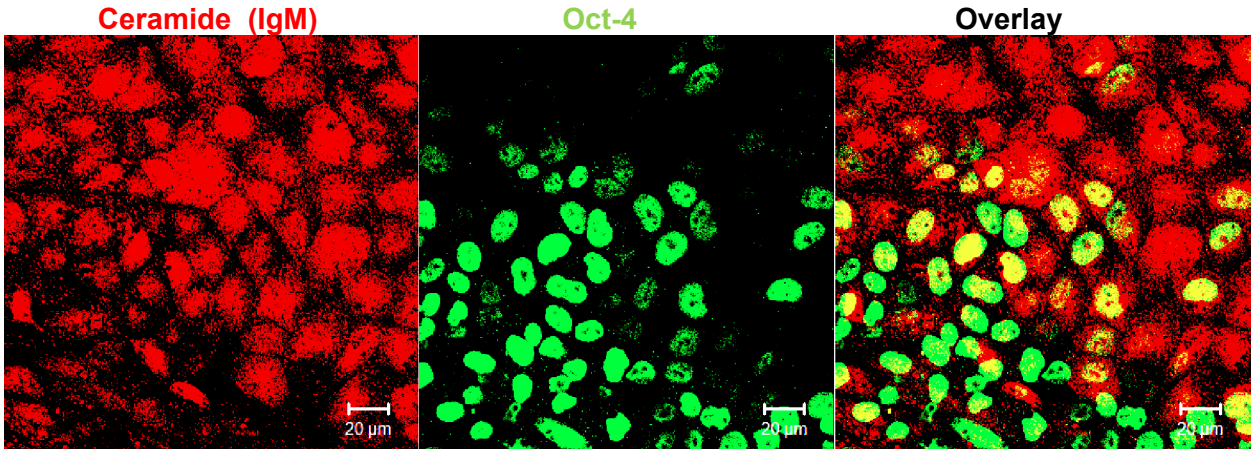


B



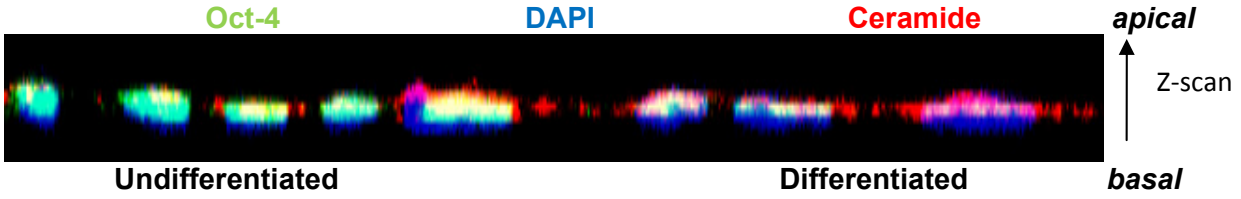
Supplemental Figure 4

A



All Z-planes collapsed onto one

B



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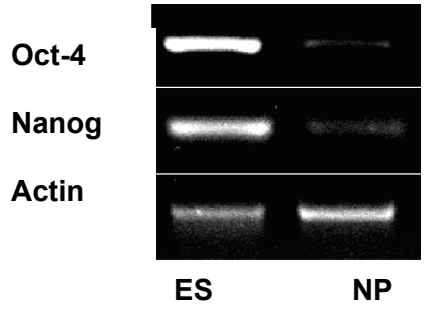

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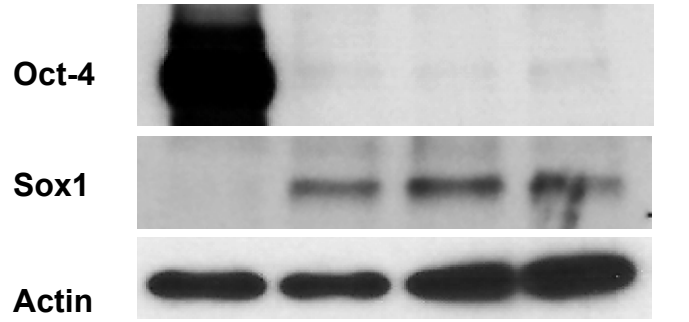

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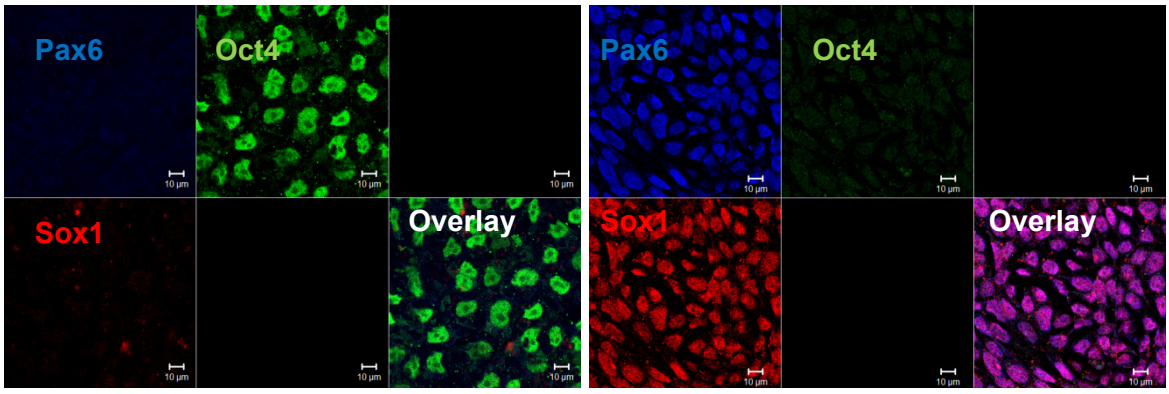
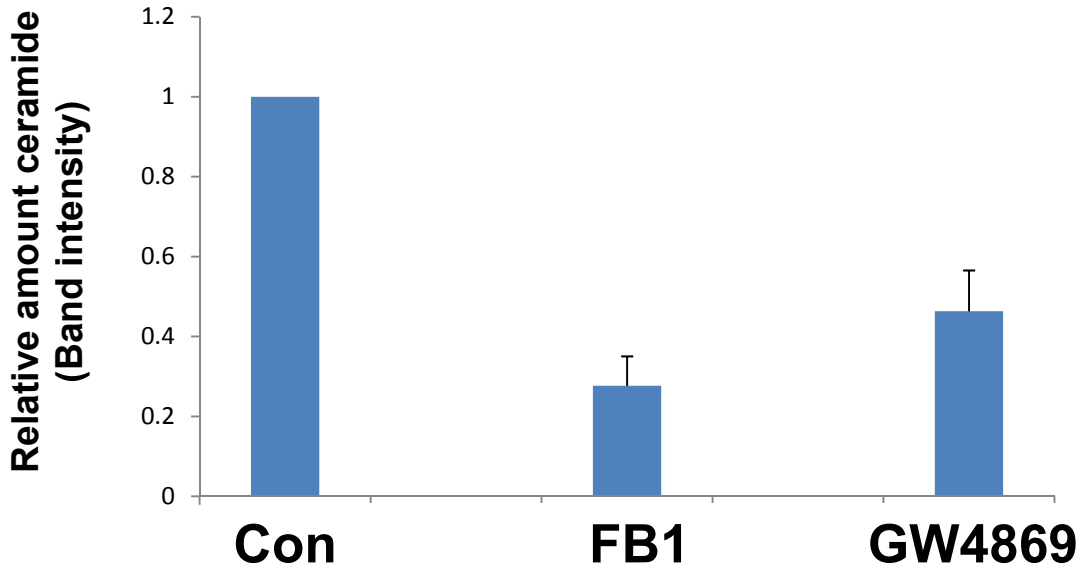
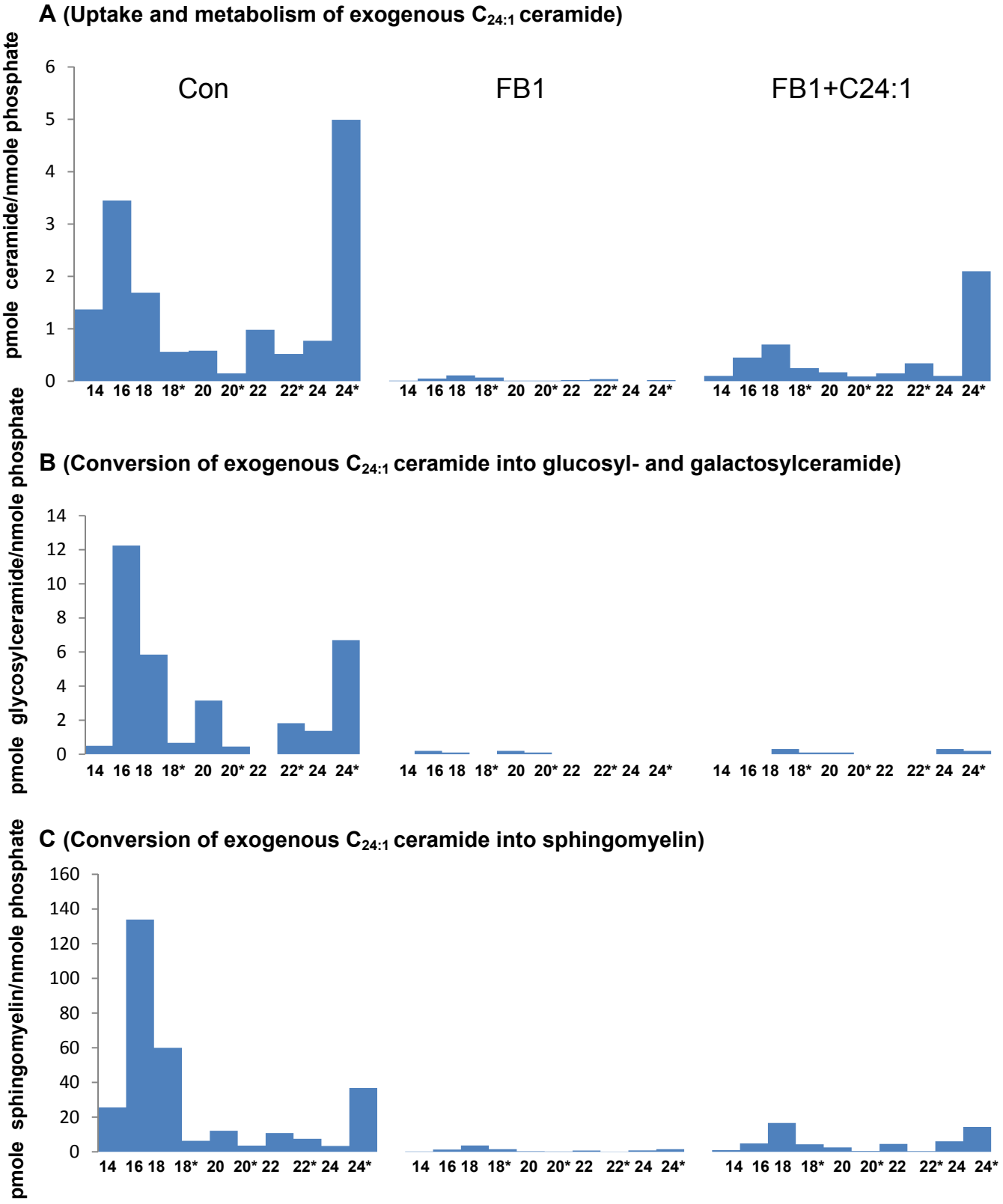


Supplemental Figure 5

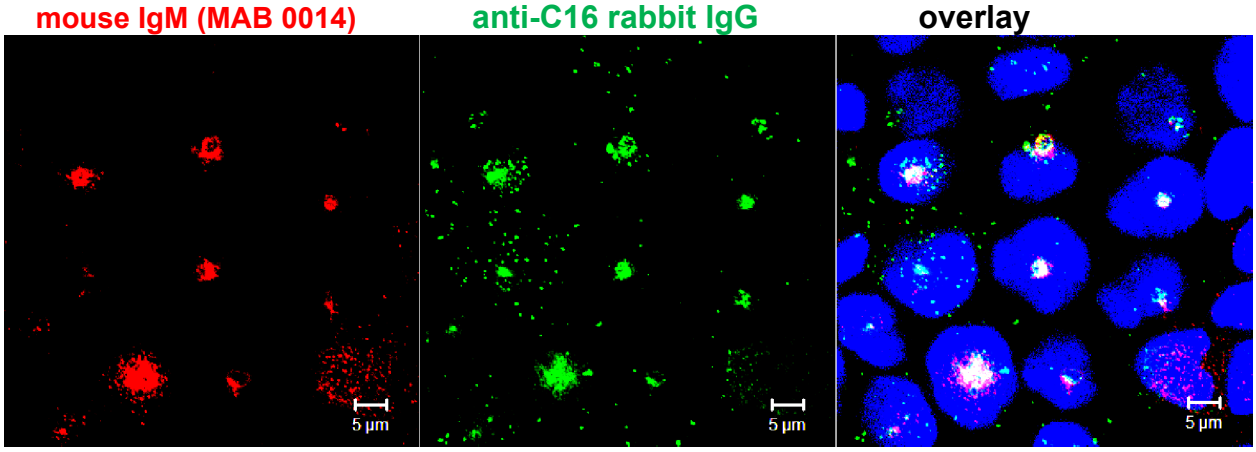


Supplemental Figure 6

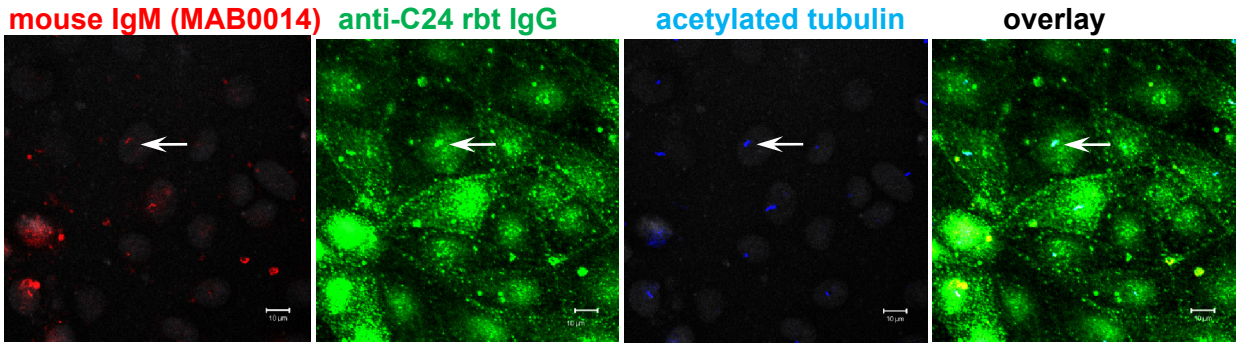


Supplemental Figure 7

A (untreated cells)

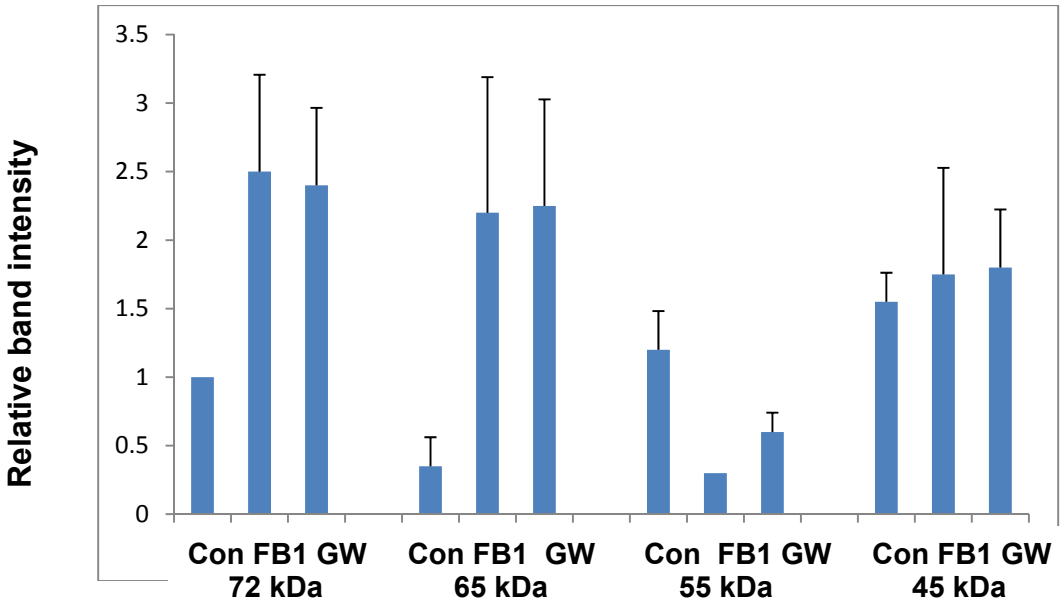


B (FB1 + C24:1 ceramide-treated cells)

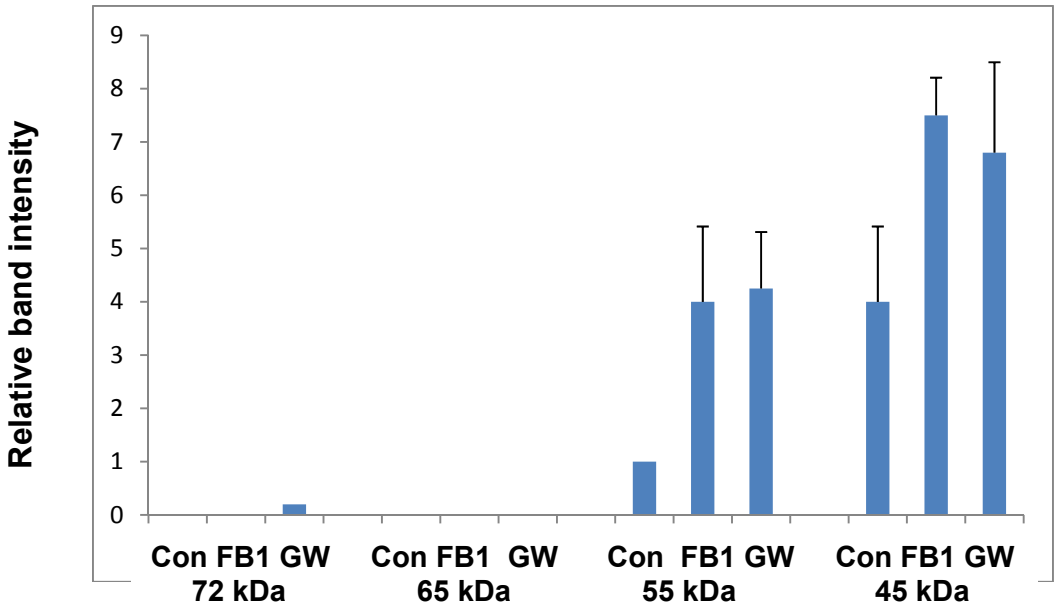


Supplemental Figure 8 (Quantitation western blot for aPKC)

A (Total aPKC in cytosol)

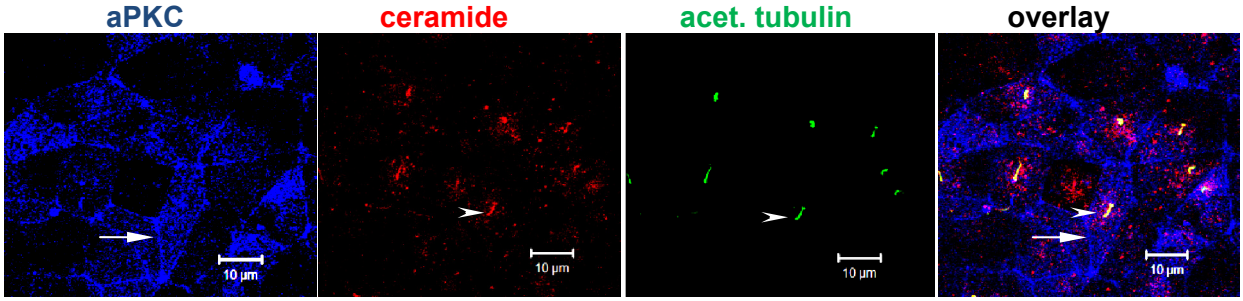


B (phosphoylated aPKC in cytosol)

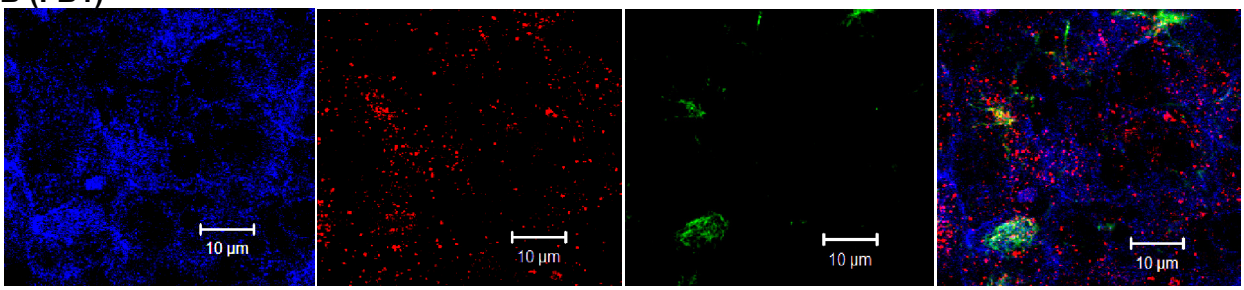


Supplemental Figure 9

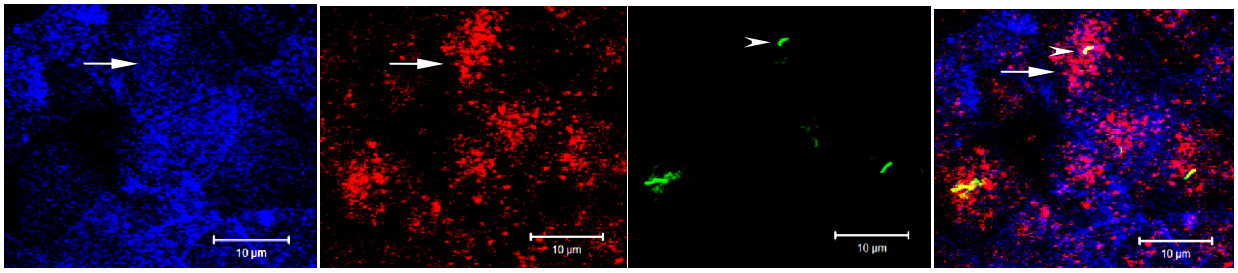
A (Con)



B (FB1)



C (FB1 + C16:0 ceramide)



D (FB1 + C24:1 ceramide)