Supplemental Figure 1. ***PAL treatment increases ceramide-1-phosphate in a chain-length specific manner.*** C2C12 myotubes were treated with 1.25 mM uniformly labeled ^{13}C- PAL with or without 0.1 μM myriocin. After 14 h treatment, the lipid fraction was extracted from cell pellets and subjected to LC/MS analysis as described in Methods. Data are means(n=6) ± *S. E. M.* of four experiments. In all length of CER-1-P , PAL vs CTL, P>0.05. CER-1-P, ceramide-1-phosphate. CTL, control; PAL, ; MY, myriocin.

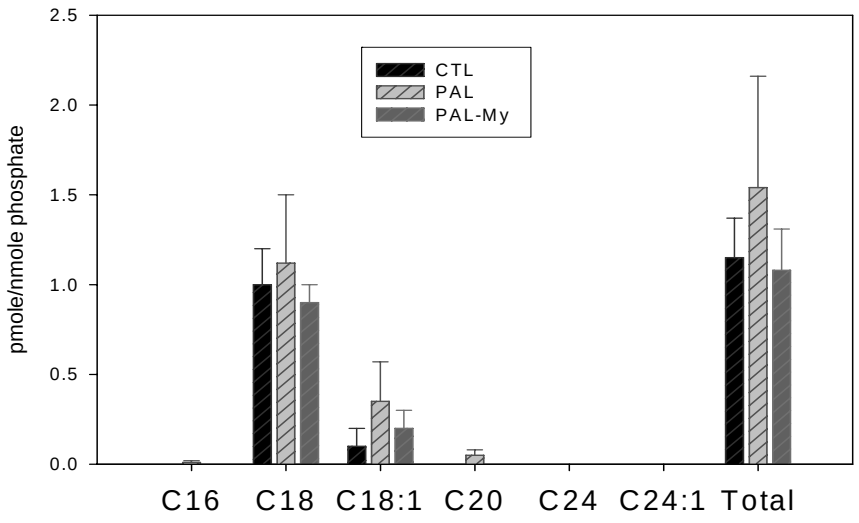
Supplemental Figure 2. ***Chain-length specificity of glycosphongolipid and sphingomyelin production from exogenous PAL:*** C2C12 myotubes were treated with 1.25 mM PAL with or without myriocin for 14 h. Lipid profiles were determent by LC/MS measurements. A. N-acyl chain lengths of glycosphingolipids; Data are mean(n=6)±*SEM*. For C16, C22, C24,C24:1 and total glycosphingolipid, CTL vs PAL, p>0.05. B. N-acyl chain lengths of sphingomyelins. Data are mean(n=6)±*SEM*. For both C16 and C22 SM, CTL vs PAL, p>0.05. for C24 SM, C24:1 SM and total SM, CTL vs PAL, p<0.05. CTL, control; PAL, palmitate; MY, myriocin ; SM, sphingomyelin;.

Supplemental Figure 3. ***The production of dihydroceramides, ceramide, sphingomyelin and diacylglycerol from exogenous PAL time course:*** C2C12 myotubes were treated with 1.25 mM PAL with or without 0.1 μM myriocin. At the indicated time point, cells were taken for lipid extraction, lipid fraction were subjected to LC/MS to measure diverse lipid species. Data are mean(n=6) ±*SEM*. A. dihydroceramide, B, ceramide, C. sphingomylin, D. diacylglycerol. CTL, control; PAL, palmitate; MY, myriocin . DHC, dihydroceramide, CER, ceramide; SM, sphingomylin; DAG, diacylglycerol.

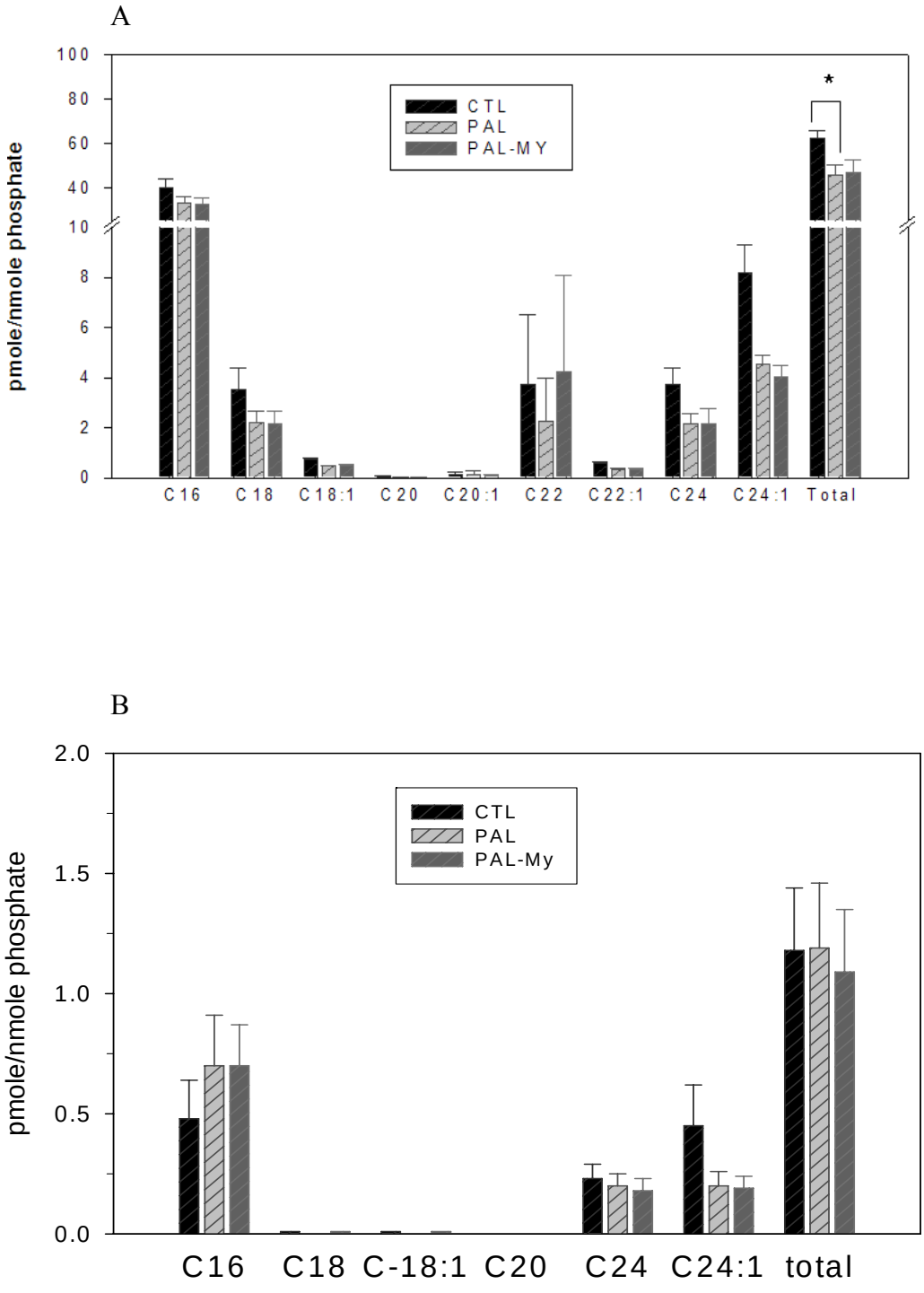
Supplemental Figure 4. ***The production of dihydrosphingosine, dihydrosphingosine-1-phosphate,***
sphingosine and S1P from exogenous PAL time course: C2C12 myotubes were treated with 1.25 mM PAL with or without 0.1 μM myriocin, at the indicated time point, cells were taken for lipid extraction, lipid fraction were subjected to LC/MS to measure diverse lipid species. Data are mean (n=6) ±*SEM*. A. dihydrosphingosine; B, dihydrosphingosine-1 phosphate; C.sphingosine; D. sphingosine-1 phosphate. CTL, control; PAL, palmitate; MY, myriocin. DHSph, dihydrosphingosine; B, DHSph-1p, dihydrosphingosine-1 phosphate; SPH, sphingosine; D. S1P, sphingosine-1 phosphate.

Supplemental Figure 5. ***PAL treatment significantly reduced the expression of myogenin, a key marker of myogenic differentiation***. C2C12 myoblasts were plated in 60 mm diameter dishes and switch with 10% horse serum when confluent. At the day of confluence, and after myogenic differentiation for 1, 2, 3, 4 and 5 days with 10% horse serum, the cells were treated with 1.25 mM PAL for 14h. Cells were then pelleted and resuspended in buffer containing 50mM Tris, pH7.5, 120 mM NaCl, 1 mM EDTA, 15mM $Na_4P_2O_7$, 20mM NaF, 1% Nonidet, 0.1% phenylmethyl sulfluoride and protease inhibitors (0.08 uM aprotinin, 0.02 uM leupeptin, 0.04 uM bestatin and 15 uM pepstaitin). Equal protein (10ug) from cell lysates were used for immunoblot analysis for myogenic marker expression. Antibodies against to myogenin and β-actin were from Santa Cruz Biotechnology, Inc (Santa Cruz, CA, USA), mouse anti-caveolin 3 antibody was from BD Biosciences, Pharmingen (San Diego, CA, USA).

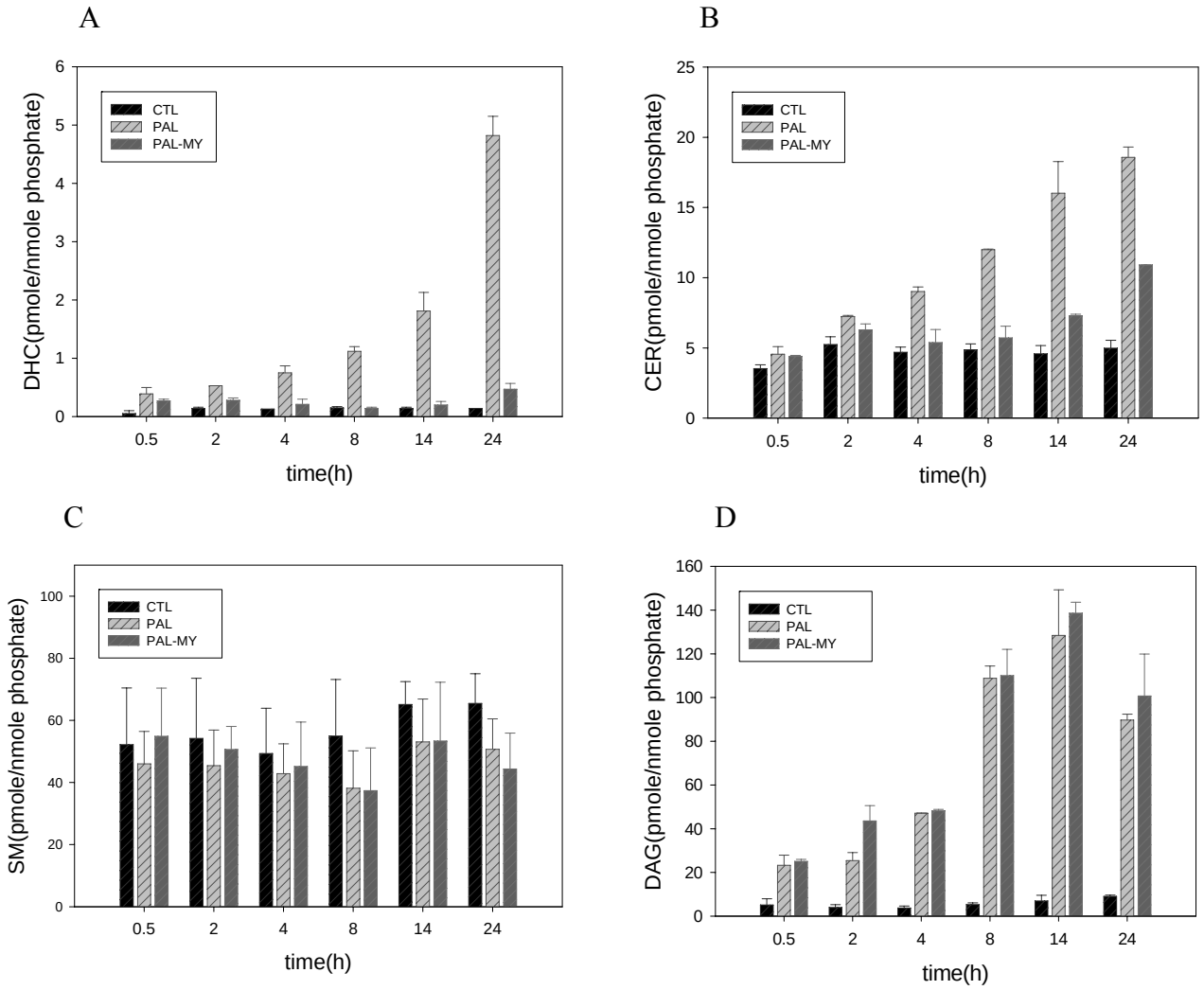
Supplemental Figure 1.



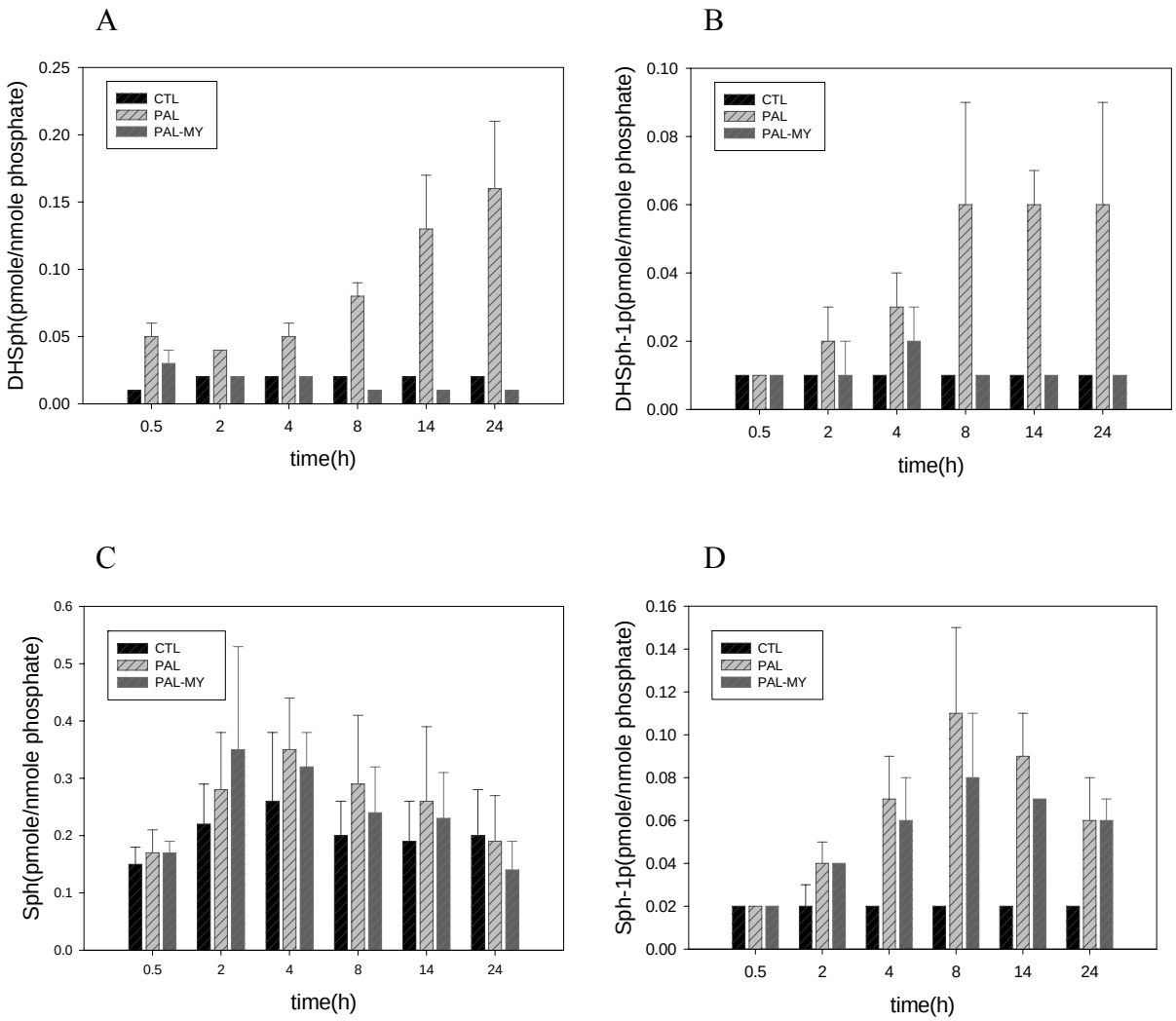
Supplemental Figure 2.



Supplemental Figure 3.



Supplemental Figure 4.



Supplemental Figure 5.