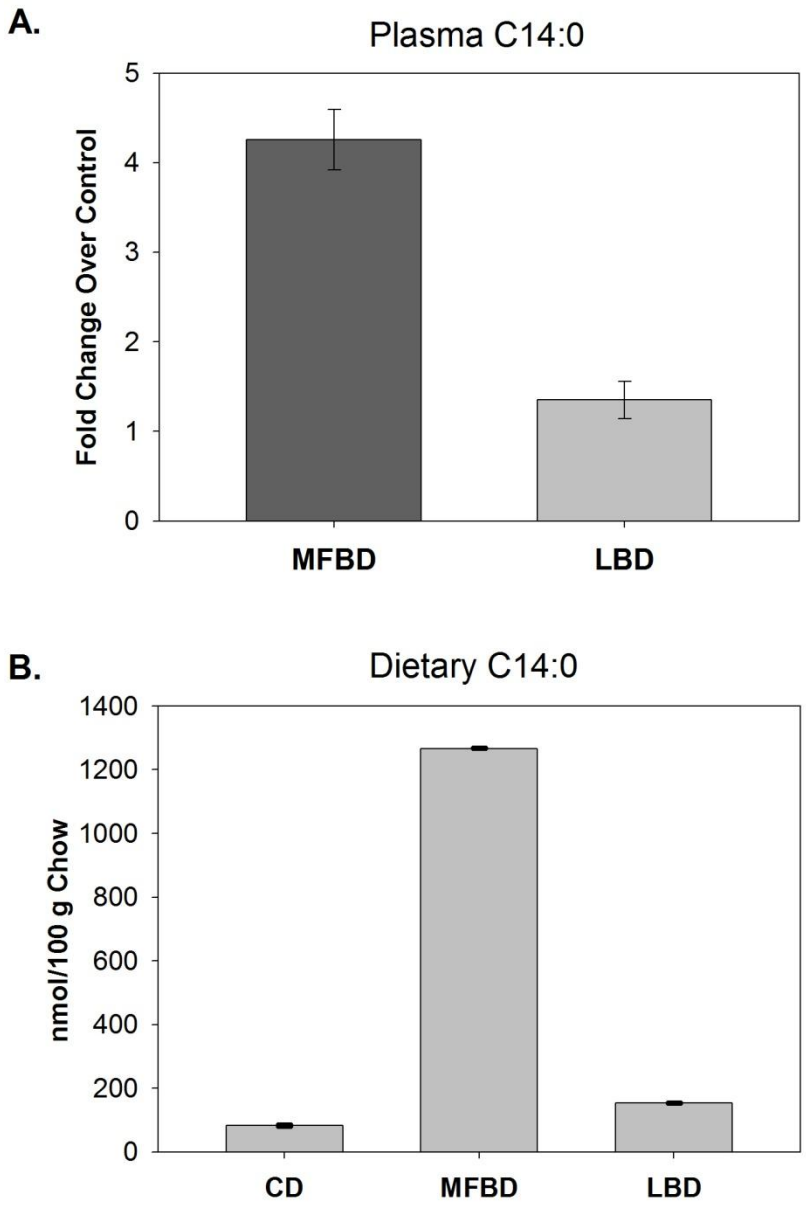
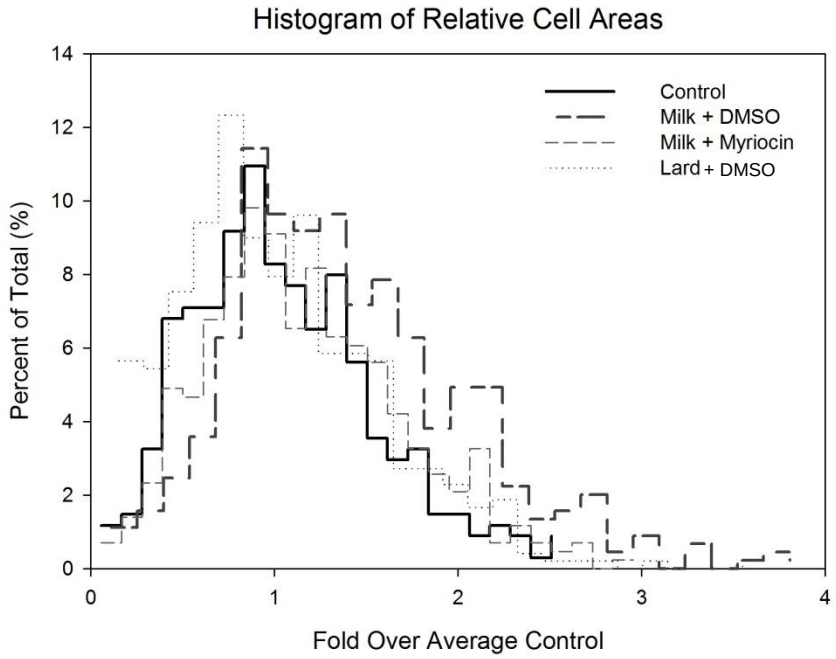
Supplemental Figure 1



Supplemental Figure 1: Myristate levels in chow and plasma. (A) Plasma myristate levels were measured postprandially in mice fed the milk fat-based diet (MFBD) or lard-based diet (LBD) for 15 weeks. Samples were analyzed by high performance liquid chromatography/mass spectrometry (HPLC/MS). Data are presented as mean fold change over control diet (CD), ± SEM. (B) Myristate levels in chow were measured by LC/MS. Data are presented as mean ± SEM.

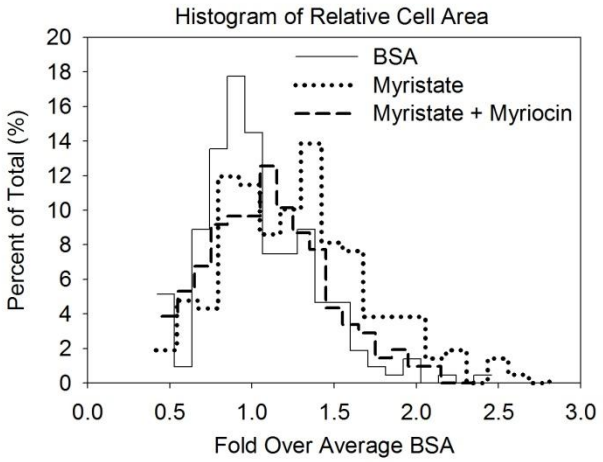
Supplemental Figure 2

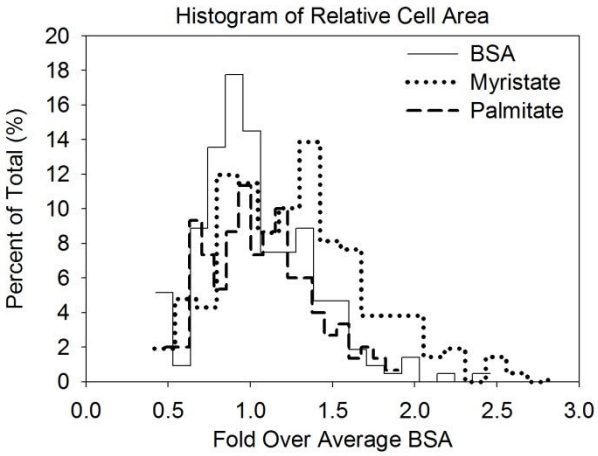


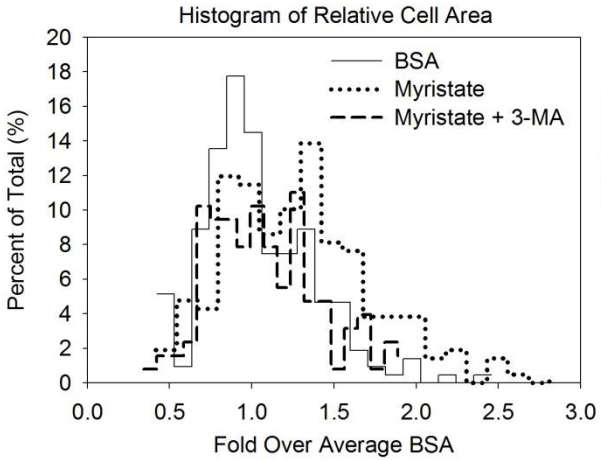
	Control	Milk + DMSO	Milk + Myriocin	Lard
Skewness	0.61	0.85	0.59	0.77
Kurtosis	3.13	3.92	3.08	3.94

Supplemental Figure 2: A milk fat-based high fat diet promotes sphingolipid-dependent cardiac hypertrophy. Mice fed a milk fat-based diet, but not those fed a lard-based diet, demonstrated increased cardiomyocyte cross-sectional area. Myriocin treatment attenuated this hypertrophy.

Supplemental Figure 3



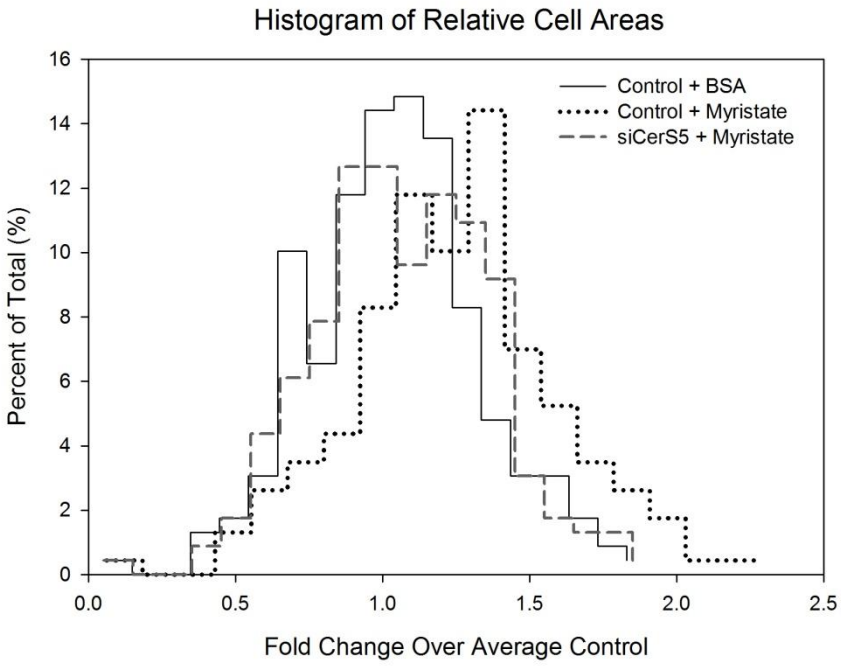




	BSA	Myristate	Myristate + Myriocin	Myristate + 3-MA	Palmitate
Skewness	1.09	0.69	0.58	0.46	0.46
Kurtosis	5.08	3.41	3.27	2.73	2.79

Supplemental Figure 3: Myristate induced hypertrophy in a sphingolipid- and autophagy-dependent dependent manner. Myristate, but not palmitate, treatment induced hypertrophy in cardiomyocytes. This was blocked by inhibition of *de novo* sphingolipid synthesis with myriocin and by inhibition of autophagy with 3-methyladenine (3-MA).

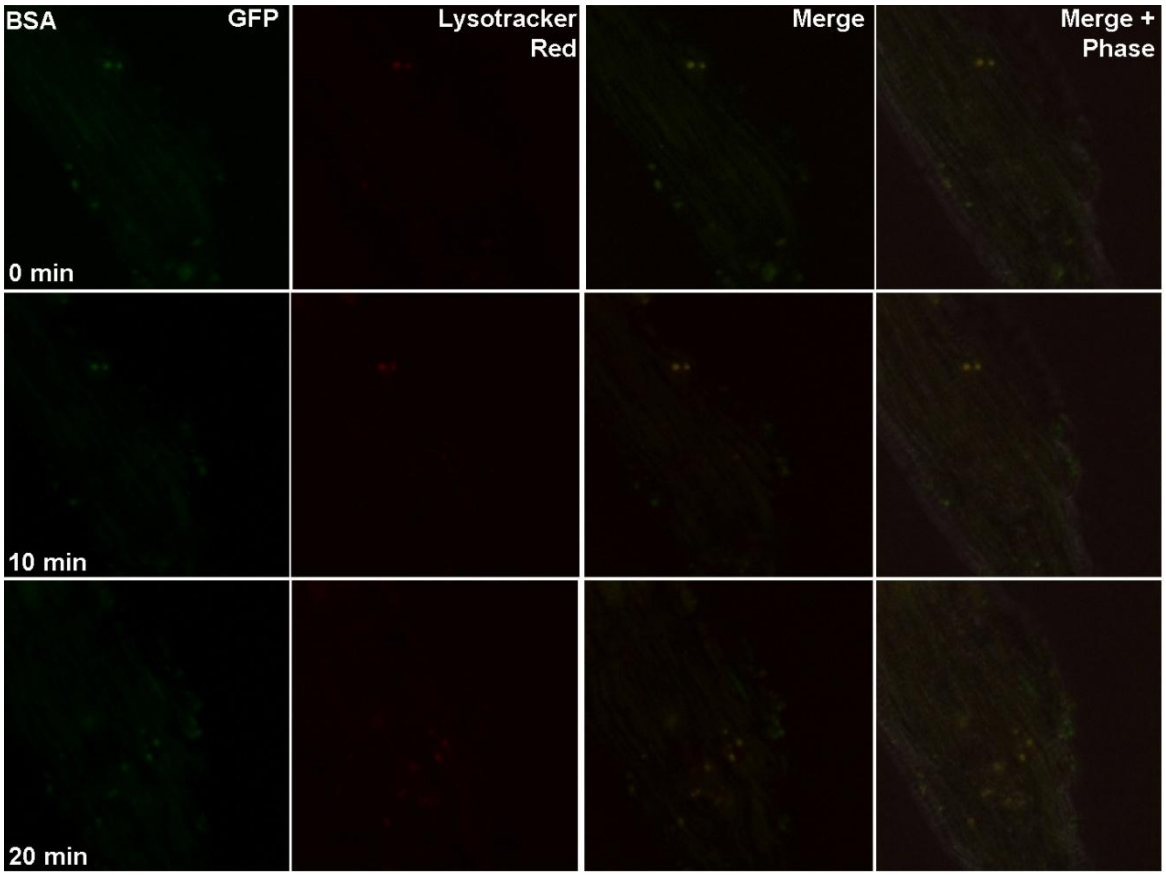
Supplemental Figure 4



	Control BSA	Control Myristate	siCerS5 Myristate
Skewness	0.23	0.24	0.19
Kurtosis	3.07	3.07	2.88

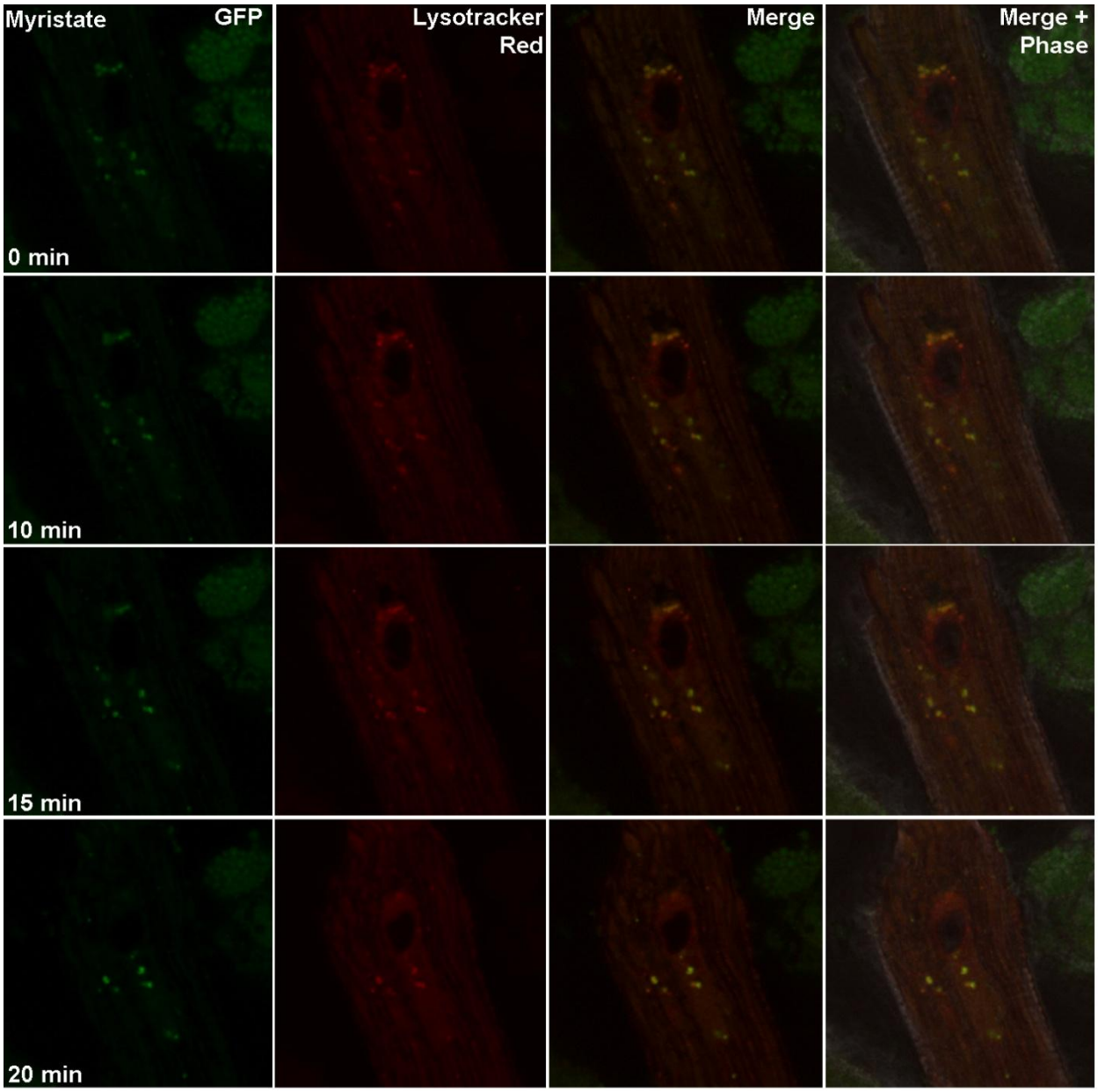
Supplemental Figure 4: Myristate induced hypertrophy in a CerS5-dependent manner. siRNA-mediated knockdown of CerS5 was sufficient to prevent induction of hypertrophy by myristate.

Supplemental Figure 5



Supplemental Figure 5: Autophagosome dynamics in BSA-treated cardiomyocytes. After 16 hours of treatment with BSA, cells expressing GFP-LC3B were loaded with LysoTracker Red, and autophagosome dynamics were examined by fluorescent confocal microscopy for 20 minutes. Autophagosomes can be observed to colocalize with acidic compartments (lysosomes) and disappear, and new autophagosomes can be observed when comparing the 20 minute time point to the 10 minute time point. Although cells were imaged with identical laser and sensitivity settings as those used with myristate-treated cells (Supplemental Figure 5) and processed identically for presentation, GFP- and LysoTracker-labeled puncta were less brilliant and less numerous than those observed in myristate-treated cells.

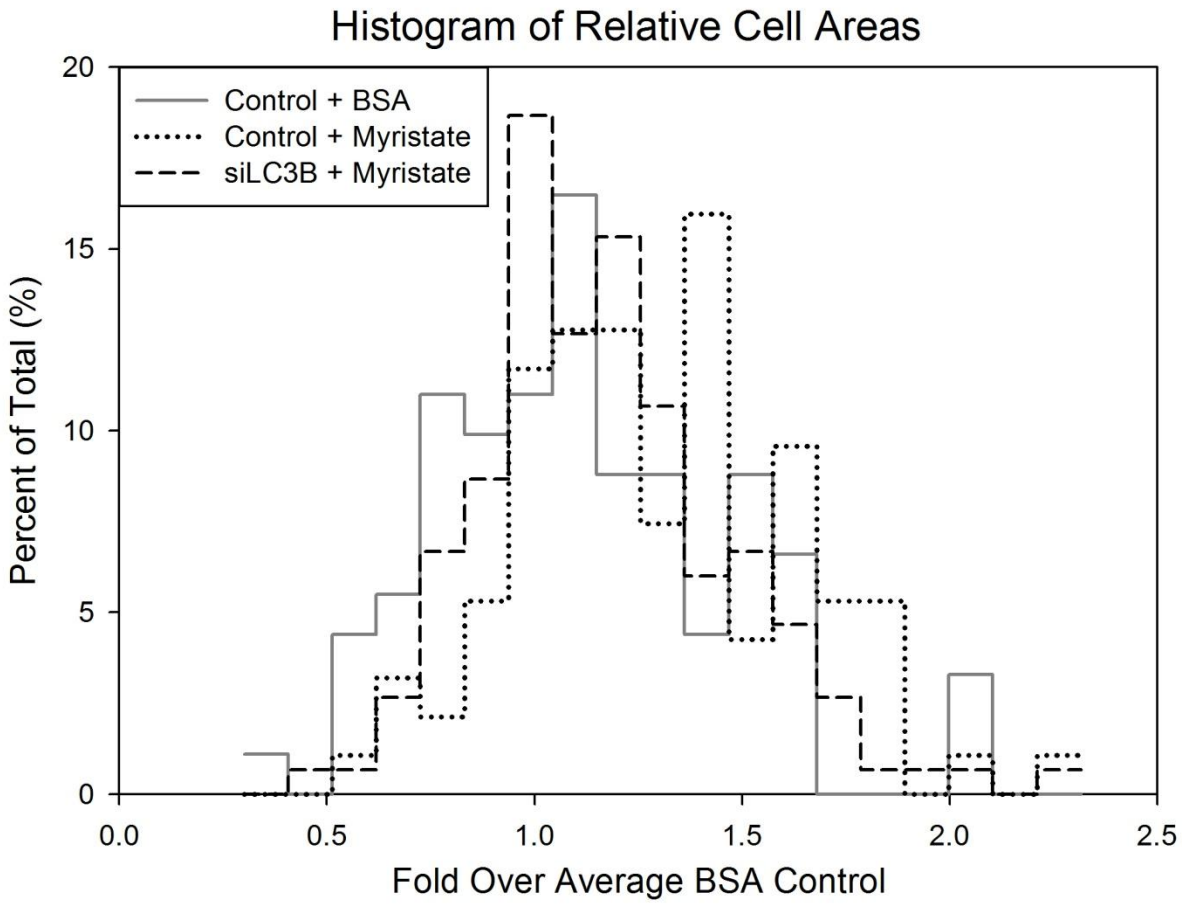
Supplemental Figure 6



Supplemental Figure 6: Autophagosomal turnover was unimpaired in myristate-treated cells. After 16 hours of treatment with myristate, cells expressing GFP-LC3B were loaded with LysoTracker Red, and autophagosome dynamics were examined by fluorescent confocal microscopy for 20 minutes. Autophagosomes can be observed to colocalize with acidic compartments (lysosomes) and disappear, and

new autophagosomes can be seen to form and grow throughout the time course. No aberrant accumulation of non-acidified autophagosomes was observed (compare to BSA controls in Supplemental Figure 4). Photographs were taken at a magnification of 60x, and images are presented with a gain of 0.7 for both red and green channels.

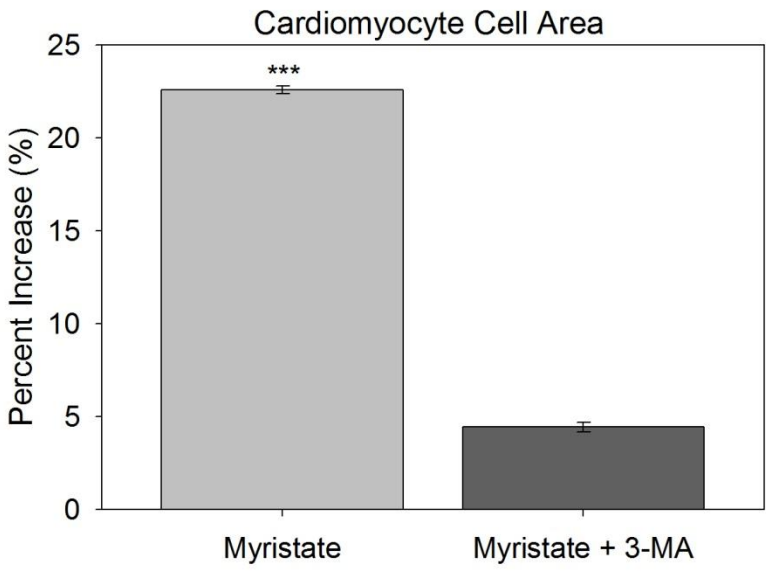
Supplemental Figure 7



	Control + BSA	Control + Myristate	siLC3B + Myristate
Skewness	0.59	0.87	0.95
Kurtosis	3.35	5.12	4.98

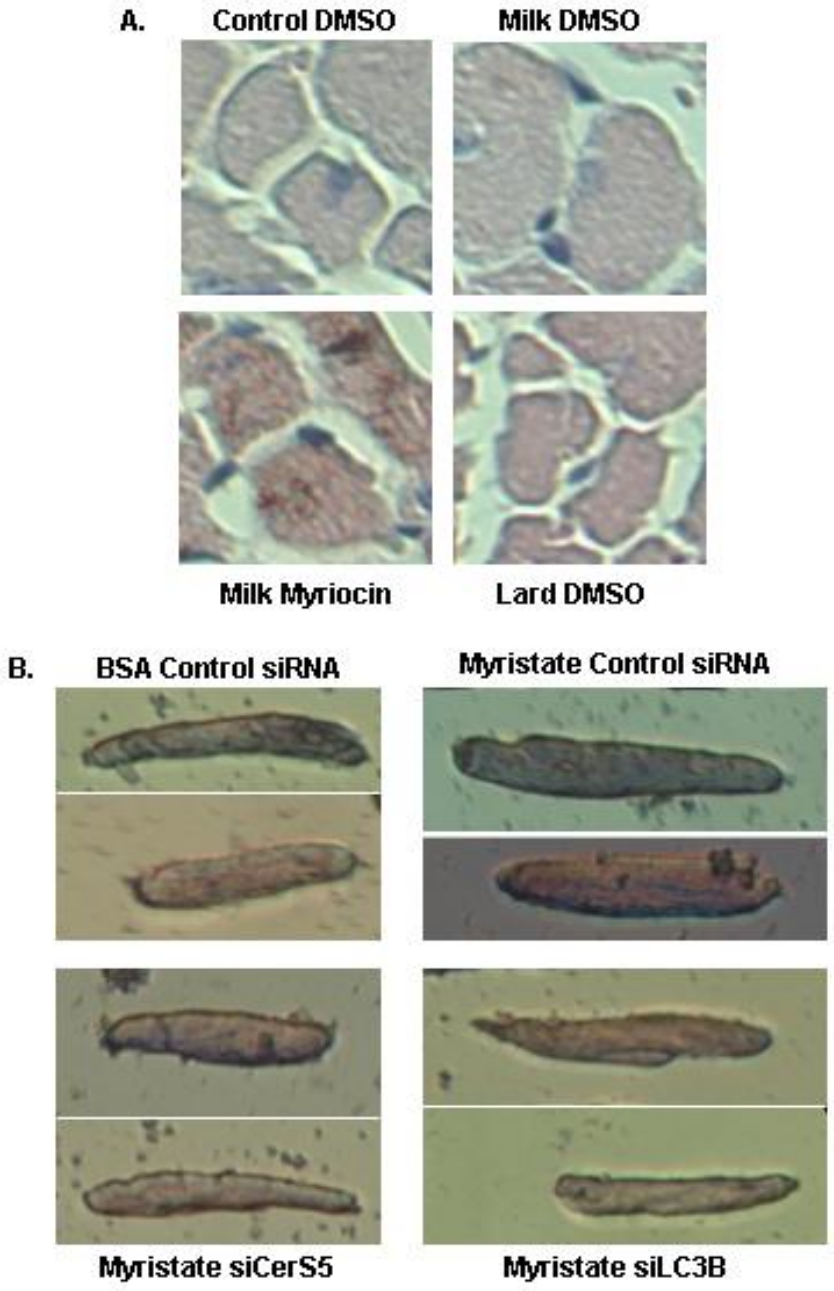
Supplemental Figure 7: Knockdown of LC3B prevented myristate-induced autophagy in isolated cardiomyocytes. siRNA-mediated knockdown of LC3B was sufficient to prevent induction of hypertrophy by myristate.

Supplemental Figure 8



Supplemental Figure 8: Inhibition of autophagy with 3-methyladenine (3-MA) prevented myristate-induced hypertrophy in isolated cardiomyocytes. Treatment with the type III phosphoinositide 3-kinase inhibitor 3-MA was sufficient to prevent induction of hypertrophy by myristate treatment. Histograms and a table showing skewness and kurtosis are provided in Supplemental Figure 2. These results complement those obtained with knockdown of LC3B. *** $p < 0.001$ *vs.* BSA.

Supplemental Figure 9



Supplemental Figure 9: Exogenous myristate induces cardiomyocyte hypertrophy in a CerS5- and autophagy-dependent manner. (A) Feeding with a diet based on milk fat, which is high in myristate, induced an increase in cardiomyocyte cross-sectional area; this was prevented by administration of the *de novo* sphingolipid synthesis inhibitor myriocin. In contrast, a lard-based diet, which is not high in

myristate, did not induce hypertrophy. Images were taken at a magnification of 20x. (B) Treatment with myristate induced hypertrophy of isolated adult cardiomyocytes. This hypertrophy was prevented by knockdown of CerS5, which synthesizes C_{14}-ceramide, and by knockdown of the autophagy protein LC3B. Two representative cells are shown per treatment group at a magnification of 10x.