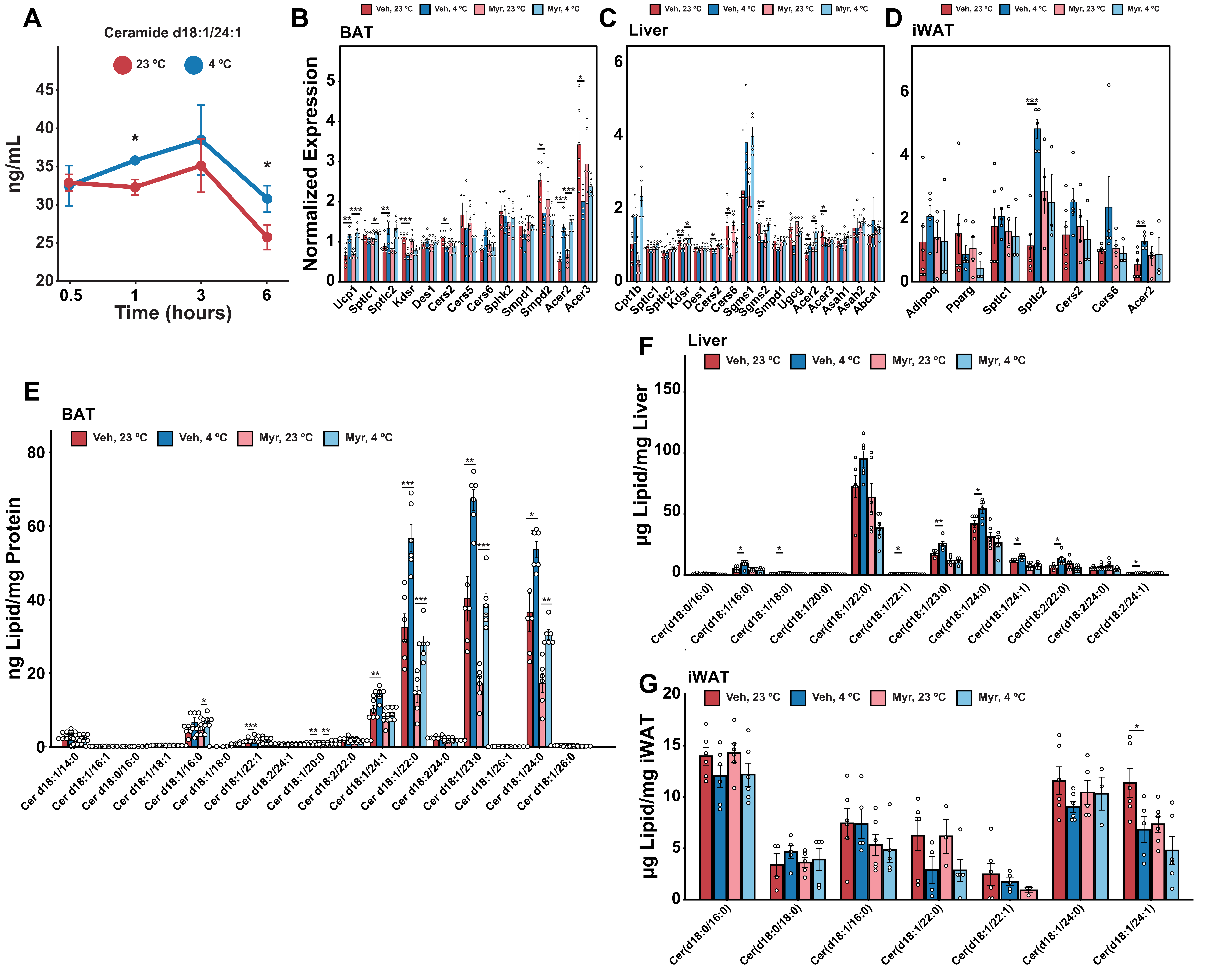
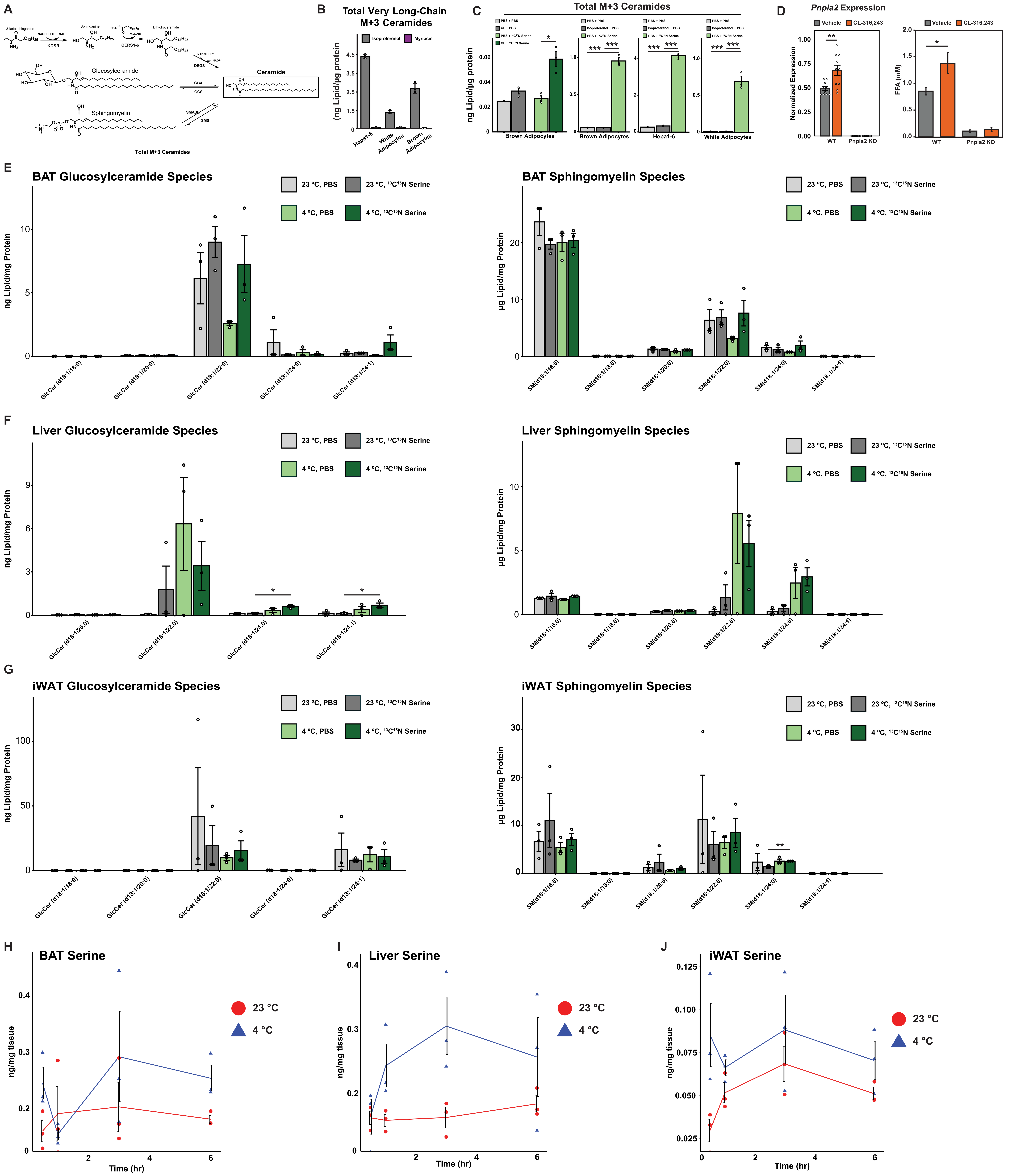
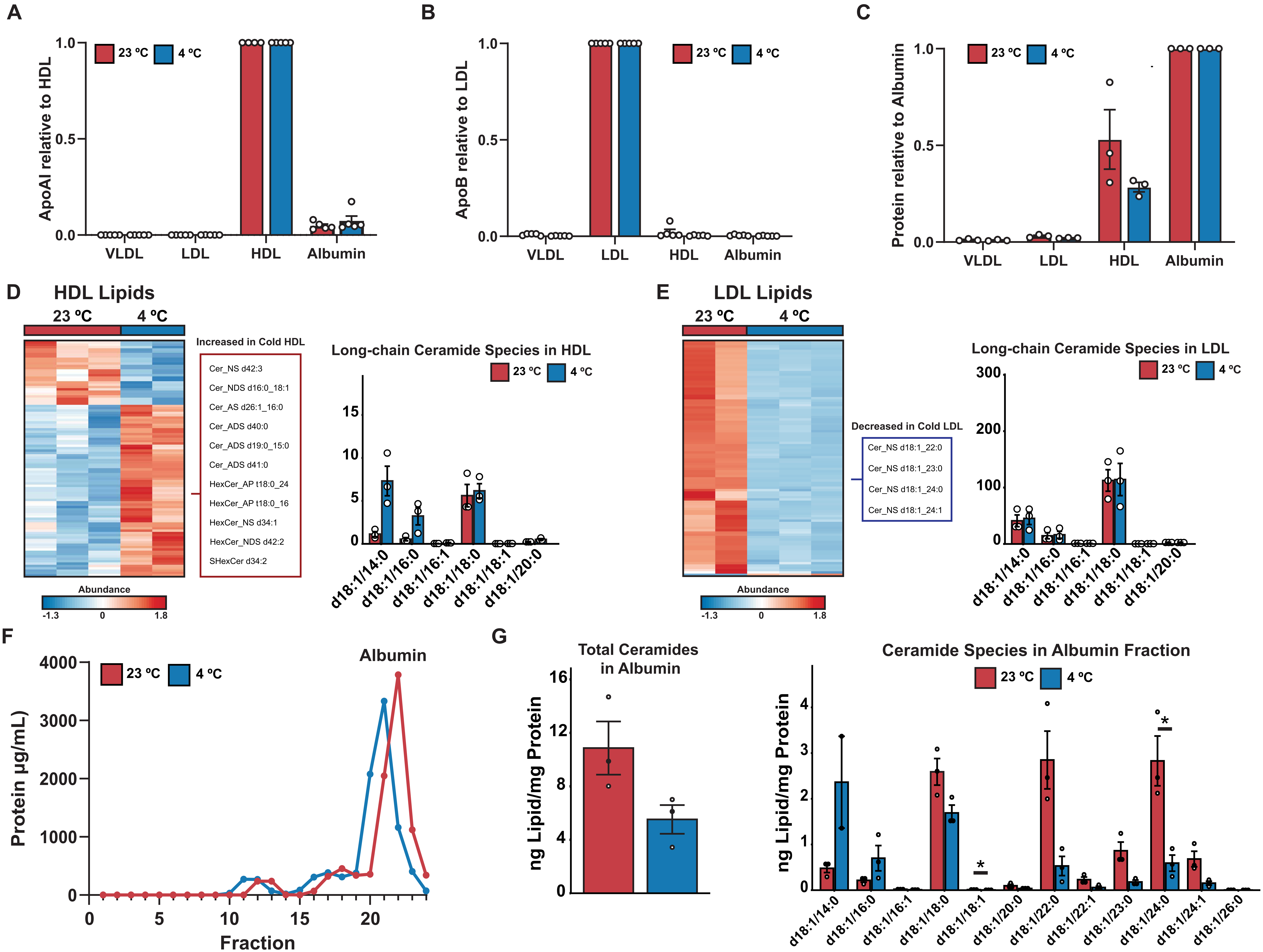


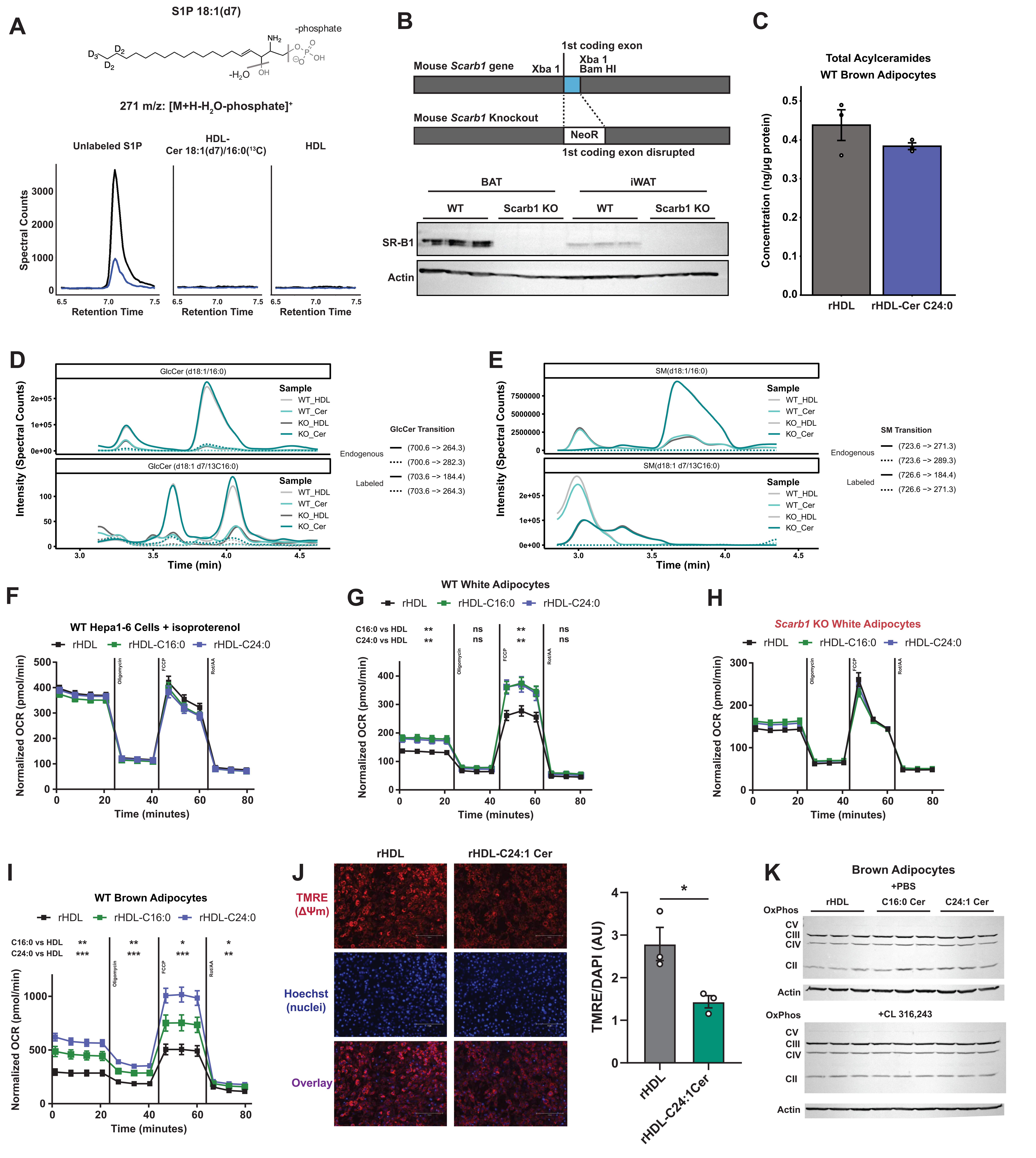
Supplementary Figure 1. Ceramide lipid and transcriptional changes in tissues in response to cold exposure and myriocin. A) Ceramide d18:1/24:1 levels in plasma of male C57Bl6J mice (n=6/group) exposed to 23 ºC or 4 ºC for 30 min, 1 hr, 3hr, or 6 hr. Expression of thermogenic, ceramide producing, and ceramide catabolizing targets in mice exposed to 23 ºC or 4 ºC and given PBS or myriocin by intraperitoneal injection in brown adipose tissue (B), liver (C), and white adipose tissue (D). Targeted measurement of ceramide species in brown adipose tissue (E), liver (F) and white adipose tissue (G). *p<0.05 **p<0.01 ***p<0.001.



Supplementary Figure 2. Serine incorporation into cellular ceramides and complex sphingolipids in tissues following cold exposure. A) Schematic of ceramide production by de novo synthesis and complex sphingolipid catabolism. B) $^{13}C^{15}N$ serine incorporation into very long-chain M+3 ceramides in Hepa1-6 cells, white adipocyte, and brown adipocytes after isoproterenol or myriocin treatment. C) Total M+3 ceramdies in brown adipocytes, Hepa1-6 cells, and white adipocytes treated with PBS or CL 316,243 and PBS or $^{13}C^{15}N$ serine. D) *Pnpla2* expression in WT and AAKO KO iWAT and free fatty acid concentration in serum of WT and AAKO mice after PBS or CL 316,243 intraperitoneal injection. M+3 Glucosylceramide (GlcCer) and M+3 sphingomyelin (SM) species in brown adipose tissue (E), liver (F), and inguinal white adipose tissue (G) after PBS or $^{13}C^{15}N$ serine IV injection and exposure to 23 ºC or 4 ºC. Serine levels in BAT (H), liver (I), or iWAT (J) of male C57BL6J mice exposed to 23 ºC or 4 ºC (n=6/group) for 6 hours. *$p<0.05$ **$p<0.01$ ***$p<0.001$

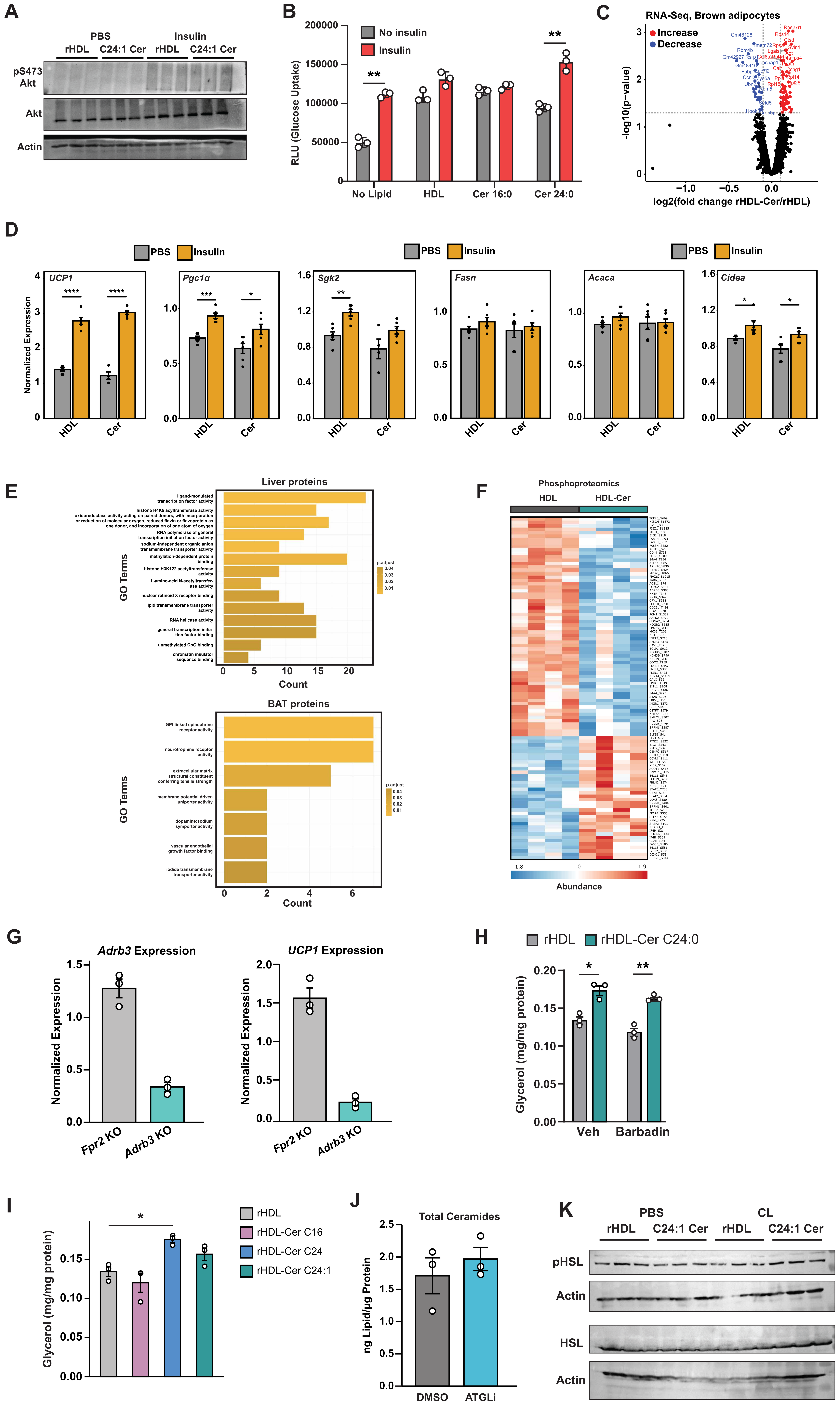


Supplementary Figure 3. Ceramides in the albumin-rich plasma fraction are modestly reduced after acute cold exposure. Validation of lipoprotein separation by ELISA for ApoAI (HDL, A), ApoB (LDL, B), and by total protein measurement for albumin in male C57Bl6J mice (n=3/group after pooling) exposed to 23 ºC or 4 ºC for 6 hours. (C).Heatmaps of relative lipid changes in HDL (D) or LDL (E) after exposure to 23 ºC or 4 ºC for 6 hours measured by untargeted lipidomics and targeted measurement of long-chain ceramide species in HDL or LDL. F) FPLC separation of albumin. G) Total ceramides and ceramide species in albumin following exposure to 23 ºC or 4 ºC for 6 hours. $p<0.05$ $^{**}p<0.01$ $^{***}p<0.001$.



Supplementary Figure 4. Regulation of brown adipocyte energy expenditure by HDL-ceramides is species dependent and independent of electron transport chain protein abundance. A) Fragmentation of S1P(d7). B) Knockout strategy of *Scarb1* knockout mice and western blot validation of knockout in brown and white adipose tissue from male mice. C) Total acylceramides in WT brown adipocytes treated with rHDL or rHDL-Cer 24:0. Comparison of endogenous, unlabeled glucosylceramide (GlcCer) d18:1/16:0 (D) and sphingomyelin (SM) d18:1/16:0 (E) to labeled GlcCer and SM after treatment of Cer 18:1(d7)/16:0(^{13}C). Oxygen consumption rates (OCR) of Hepa1-6 cells (F), WT white adipocytes (G), *Scarb1* KO white adipocytes (H), and WT brown adipocytes (I) treated with rHDL-ceramide d18:1/16:0 or 24:0. J) TMRE, Hoechst, and overlayed stain of brown adipocytes treated with isoproterenol and rHDL or rHDL-ceramide d18:1/24:1 and quantified relative fluorescence. K) Abundance of mitochondrial electron tranpsort chain complex II (CII), complex III (CIII), complex IV (CIV), and complex V (CV) in brown adipocytes treated with PBS or CL 316,243 and rHDL, ceramide d18:1/16:0, or ceramide d18:1/24:1.

Adipocytes from all experiments were treated with 1 µM CL 316,243 unless otherwise stated. *$p<0.05$ **$p<0.01$ ***$p<0.001$.



Supplementary Figure 5. HDL-ceramides do not regulate lipolysis through ATGL function, phosphorylation of HSL, or internalization of β3AR. A) Abundance of phosphorylated Akt (S473) and AKT in brown adipocytes treated with rHDL or rHDL-Cer d18:1/24:1 and PBS or insulin. B) Insulin-stimulated glucose uptake of brown adipocytes treated with rHDL or rHDL-ceramide (d18:1/16:0 or 24:0). Volcano plot of global gene expression by RNA-seq in brown adipocytes treated with rHDL or rHDL-Cer d18:1/24:1 (C) and qPCR measurement of thermohgenic or insulin regulatory gene expression in response to insulin and rHDL or rHDL-Cer d18:1/24:1 (D). E) Molecular function gene ontology (GO) analysis of proteins unique to the liver and BAT from male C57BL6J mice. F) Heatmap of the top 100 significantly different phosphorylated protein by relative abubndance in brown adipocytes treated with rHDL or rHDL-Cer d18:1/24:1. G) Normalized expression of *Adrb3* and *Ucp1* in *Fpr2*$^{-/-}$ and *Adrb3*$^{-/-}$ brown adipocytes. Glycerol release in brown adipocytes in response to barbadin (H) and rHDL or varying rHDL-ceramide species (I). J) Total ceramides in brown adipocytes treated CL 316,243 and DMSO or ATGLi. K) Western blot of phosphorylated HSL (pHSL) and HSL in brown adipocytes after treatment with PBS or CL 316,243 and rHDL or rHDL-ceramide d18:1/24:1. p<0.05 **p<0.01 ***p<0.001.