

# **SPTLC1 variants associated with ALS produce distinct sphingolipid signatures through impaired interaction with ORMDL proteins**

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## Legends to supplementary figures:

### Figure S1. The de novo sphingolipid synthesis pathway.

(A) Sphingolipid synthesis starts with the formation of aliphatic amino alcohols or long chain bases (LCB), the identifying structural units of all SLs. This reaction is carried out by the serine-palmitoyltransferase (SPT) using palmitoyl-CoA and L-serine. Therefore, majority of SL in mammals are composed of canonical C18 LCBs, sphingosine (d18:1) and sphinganine (d18:0). Dihydroceramide synthases CerS1-6 conjugate LCB with activated fatty acids (acyl-CoA's) of variable chain lengths ( $C_{16}$  to  $C_{26}$ ) to form dihydroceramide (dhCer, d18:0) species. Desaturases, DEGS1 and FADS3 introduce double bonds at C4-C5 and C14-C15 positions in LCB of dhCer to form mono- (d18:1) and di-unsaturated (d18:2) ceramides, respectively. Ceramides are converted to complex sphingolipids, such as sphingomyelin and glycosphingolipids with addition of specific head group structures. In a catabolic pathway, ceramides are degraded back to release LCB sphingosine (SO) and fatty acid by ceramidases. Phosphorylation of the primary hydroxyl group by sphingosine kinases SK1/2 lead to formation of a sphingosine-1-phosphate (S1P) which is degraded terminally by S1P lyase SGPL1.

(B) Schematic representation of SPTLC1-ALS and -HSAN1 variants relative to the ER luminal and cytosolic side.

### Figure S2. ORMDLs associate with SPT-complex.

(A) SPTLC1, SPTLC2 and ORMDL protein levels in blue native PAGE samples from Figure 2B. Solubilized membrane fractions from Figure 2B were analyzed on a denaturing SDS-PAGE and immunoblotted with anti-SPTLC1, anti-SPTLC2 and anti-ORMDL antibodies. TOMM40 was used as a loading control. \* = carryover signal from SPTLC2 antibody.

(B-C) ORMDL-protein levels in SPTLC1-KO and SPTLC2-KO cells. Whole cell lysates from Flp-In T-REx 293 control, SPTLC1-KO and SPTLC2-KO cells were analyzed by SDS-PAGE and immunoblotting (B), and ORMDL levels were quantified (C). ORMDL signals were normalized to ACTIN signal. Mean  $\pm$  SD, n=4 independent replicates, unpaired two-sided Welch's t-test, \*\*\*  $p < 0.001$

(D) SPTLC1, SPTLC2 and ORMDL protein levels in blue native PAGE samples from Figure 2C. Samples from Fig 2C were analyzed on denaturing SDS-PAGE and immunoblotted with anti-SPTLC1, anti-SPTLC2 and anti-ORMDL antibodies. TOMM40 was used as a loading control.

(E-F) Analysis of SPT complex in patient fibroblasts by blue native PAGE. Membrane fractions from immortalized control fibroblasts (3 different control cells) and patient fibroblasts derived from S331Y and L39del patients were analyzed by blue native PAGE and immunoblotted with anti-SPTLC1, anti-SPTLC2 and anti-ORMDL antibodies (E). Protein levels in these samples were analyzed on a denaturing SDS-PAGE (F) where TOMM40 and HSPA5 were used as loading controls. \* = carryover signal from SPTLC2 antibody.

### Figure S3. Plasma sphingolipid levels in ALS and HSAN1 patients

(A-D) Circulating sphingolipid levels in ALS patients, carrying SPTLC1p.Y23F, L39del and F40S41del mutations. Total ceramides (A), total sphingomyelins (B), total monohexosylceramides (C) and 1-deoxysphingolipids (D). n=4 (independent replicates), Data are represented as mean  $\pm$  SD, two-way ANOVA with Dunnett's adjustment for multiple comparisons. \*  $p < 0.05$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .

**Figure S4. Sphingolipid species levels in ALS and HSAN1 variant expressing cells.**

(A-B) Levels of indicated de novo formed dihydroceramides (dhCer, A) and ceramides (Cer, B) with conjugated intermediate chain length fatty acids. HEK293 control and variant expressing SPTLC1-KO cells were grown in the presence of D<sub>3</sub>, <sup>15</sup>N-L-serine and D<sub>4</sub>-L-alanine to label SLs. Absolute levels of each SL species were quantified relative to internal lipid standard by high resolution mass spectrometry. (C) Pie charts displaying relative abundance of de novo synthesized sphingolipid subclasses and ceramides species in SPTLC1-KO cells expressing the WT-SPTLC1 or the ex2del variant, represented as average of three independent experiments.

Data are represented as mean ± SD, n=3 independent replicates, one-way ANOVA followed by Bonferroni adjustment for multiple comparison, \* p< 0.05, \*\* p<0.01, \*\*\* p<0.001, \*\*\*\* p<0.0001.

**Figure S5. Sphingolipid species profiles in patient derived primary fibroblasts carrying SPTLC1-ALS disease variants.**

(A and B) Dihydroceramide (A) and ceramide (B) profiles based on conjugated fatty acids (FA) in control and patient derived primary fibroblasts carrying SPTLC1p.L39del and F40S41del mutations. Cells were grown in presence of D<sub>3</sub>, <sup>15</sup>N-L-serine and D<sub>4</sub>-L-alanine for 16 hours.

(C) Ceramide species profiles based on conjugated fatty acids (FA) in patient derived fibroblasts after transfection with scramble and mutant specific siRNA and in comparison to scramble transfected control cells. Isotope labelling was performed 72 hours post SiRNA (10 nM) transfection.

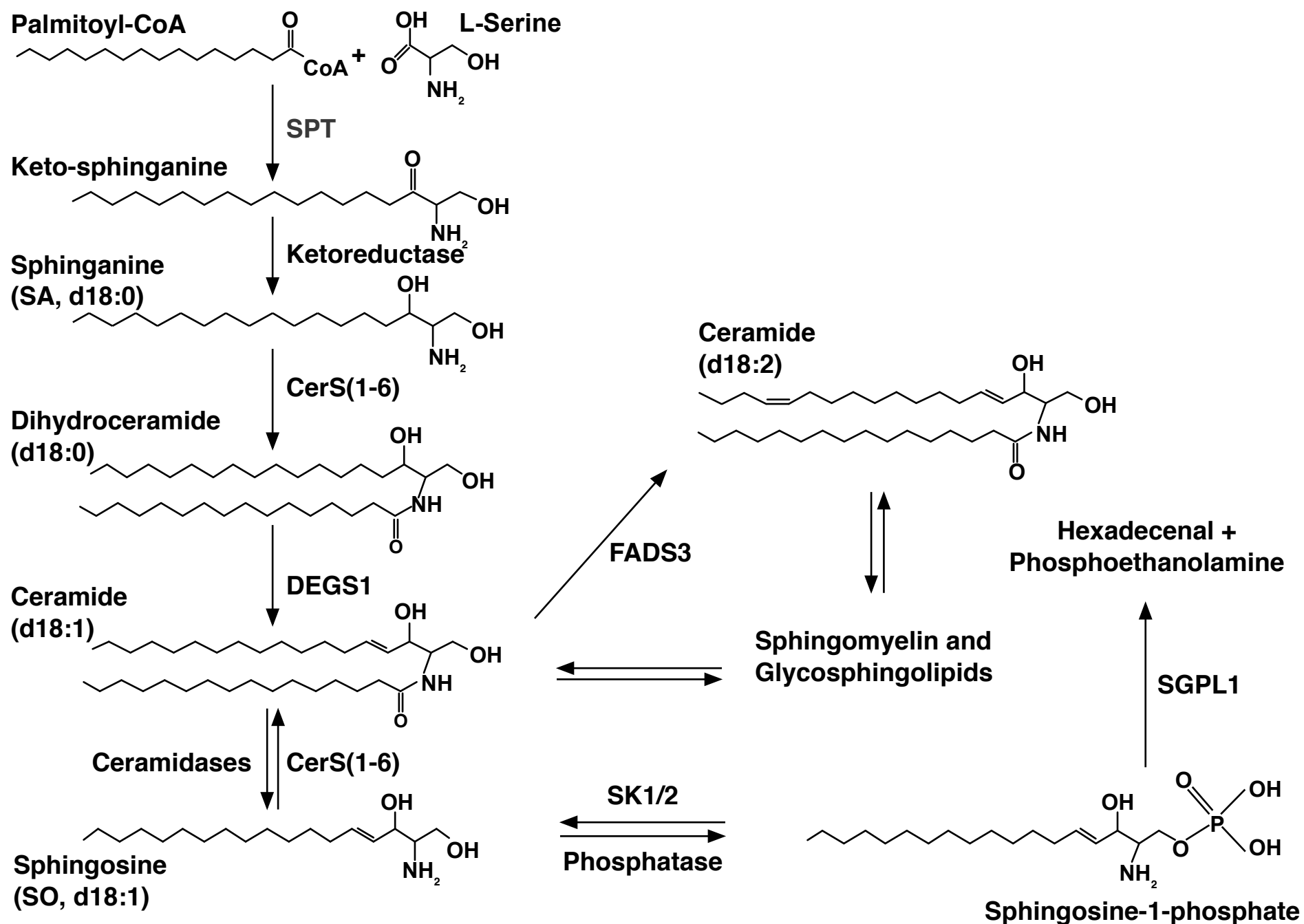
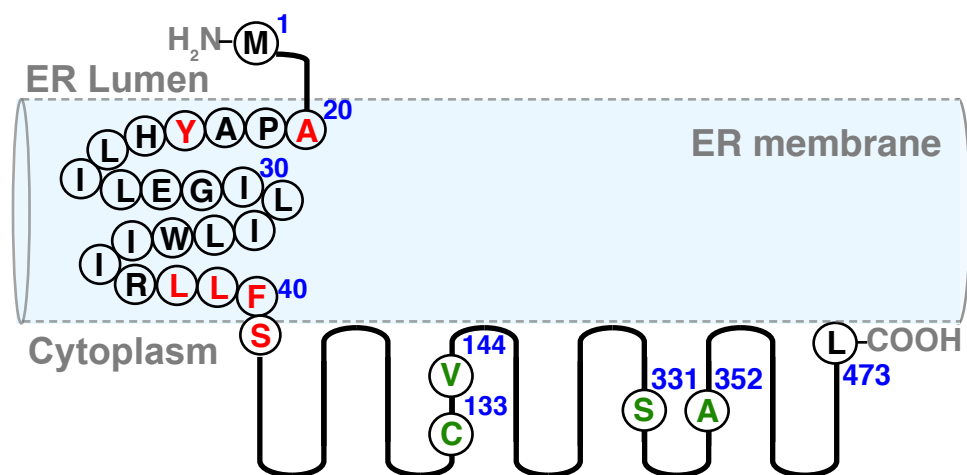
Data are represented as mean ± SD, n=3 and one-way or two-way ANOVA followed, respectively, by Bonferroni or Dunnett's adjustment for multiple comparison, \* p< 0.05, \*\* p<0.01, \*\*\* p<0.001, \*\*\*\* p<0.0001.

**Figure S6. Plasma ceramide species profiles from patients carrying SPTLC1-ALS disease variants.**

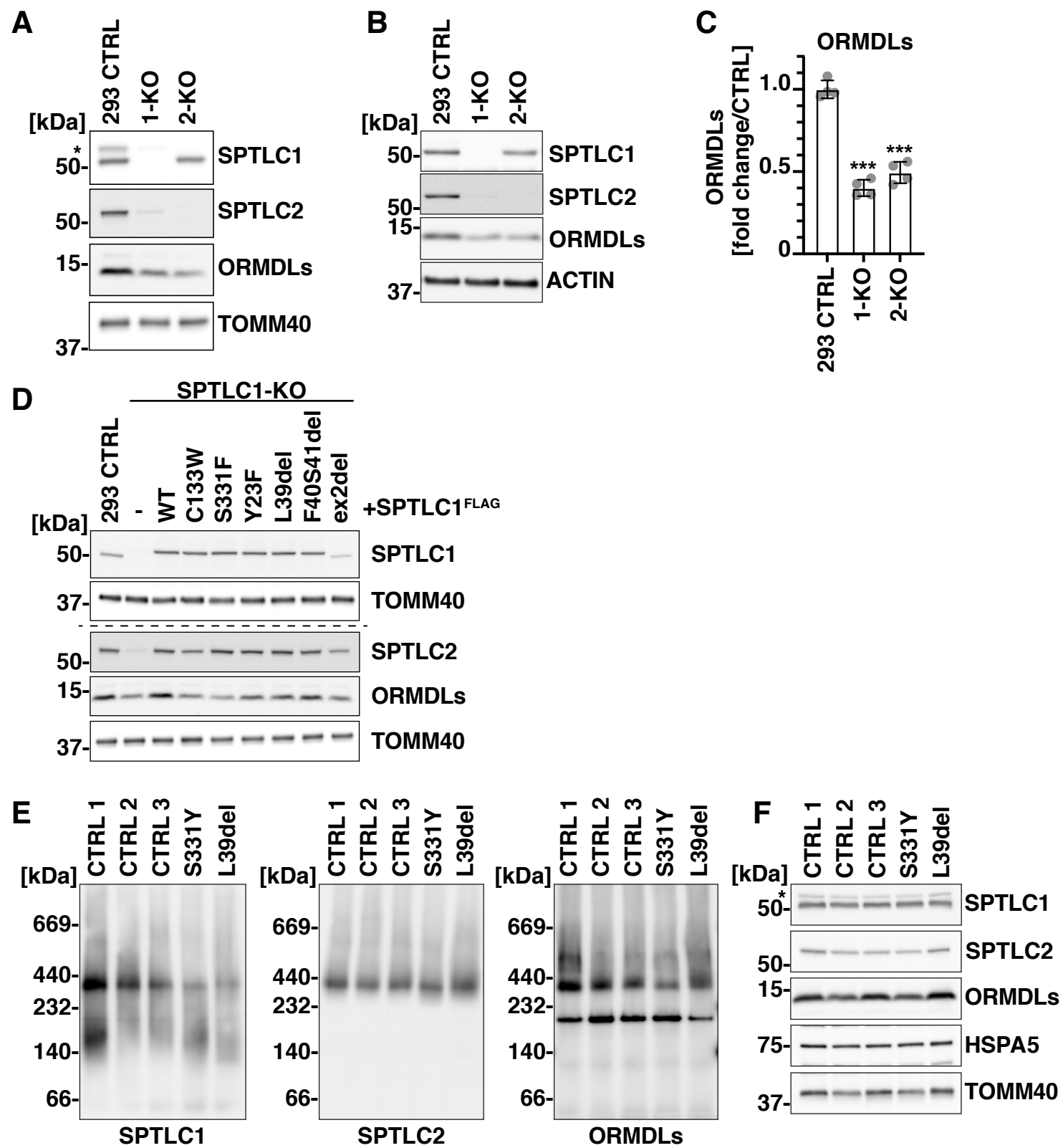
(A and B) Dihydroceramide (A) and ceramide (B) profiles based on conjugated fatty acids (FA) in plasma of healthy controls, ALS patients carrying SPTLC1p.Y23F, L39del and F40S41del mutations and HSAN1 C133W patients. n=4 (independent replicates). Data are represented as mean ± SD, two-way ANOVA with Dunnett's adjustment for multiple comparisons. \* p< 0.05, \*\*\* p<0.001, \*\*\*\* p<0.0001.

**Figure S7. Plasma L-serine and L-alanine levels in Leu39del family.**

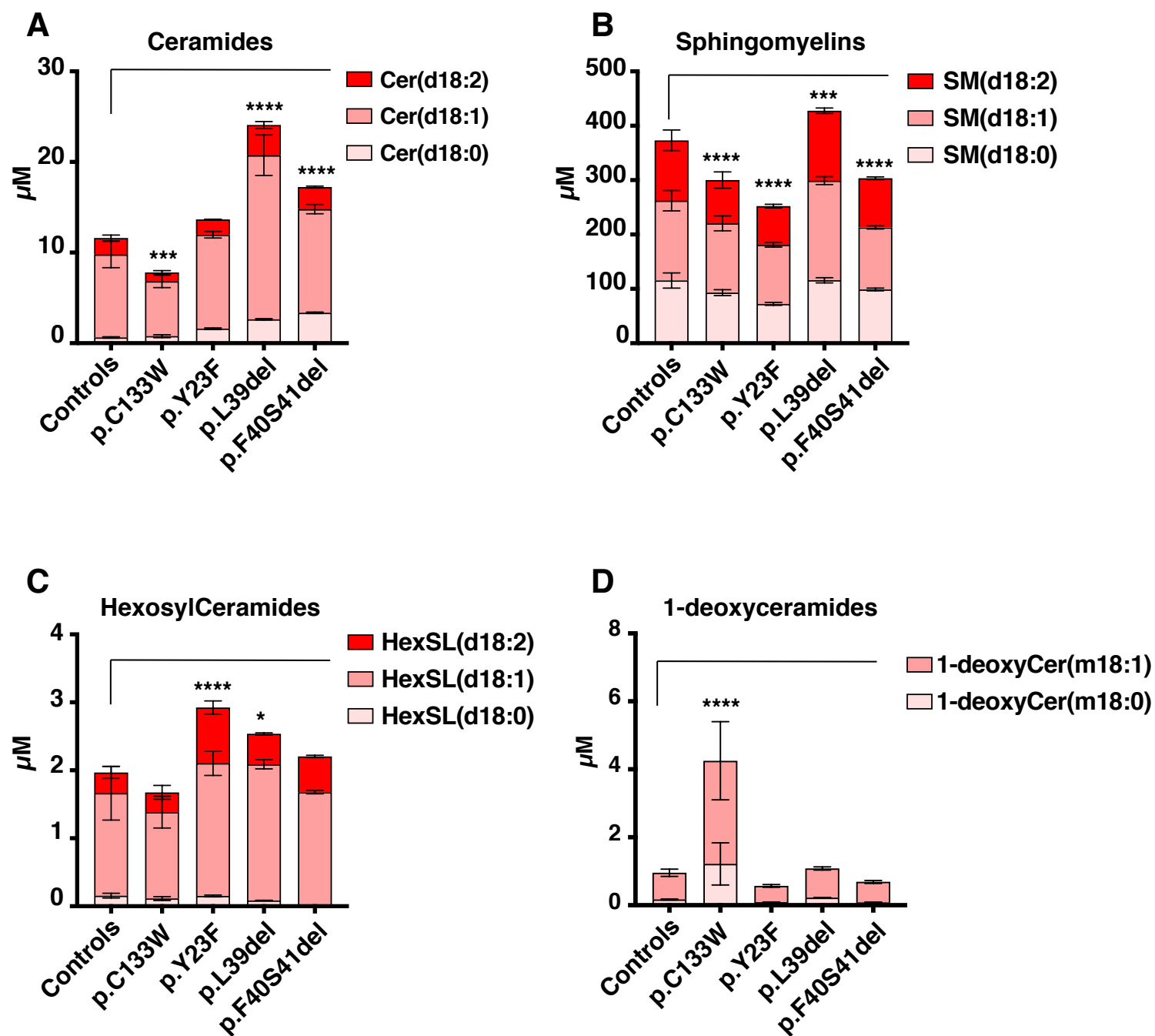
(A and B) Levels of L-serine (A) and L-alanine (B) in plasma of SPTLC1p.Leu39del family members. Amino acids were extracted from plasma and quantified relative to isotope labelled internal standards. Data are represented as mean ± SD, n=3 (independent replicates), one-way ANOVA followed by Bonferroni correction for multiple comparison, \* p< 0.05, \*\*\* p<0.001, \*\*\*\* p<0.0001.

**A****B****Figure S1**





**Figure S2**



**FIGURE S3**

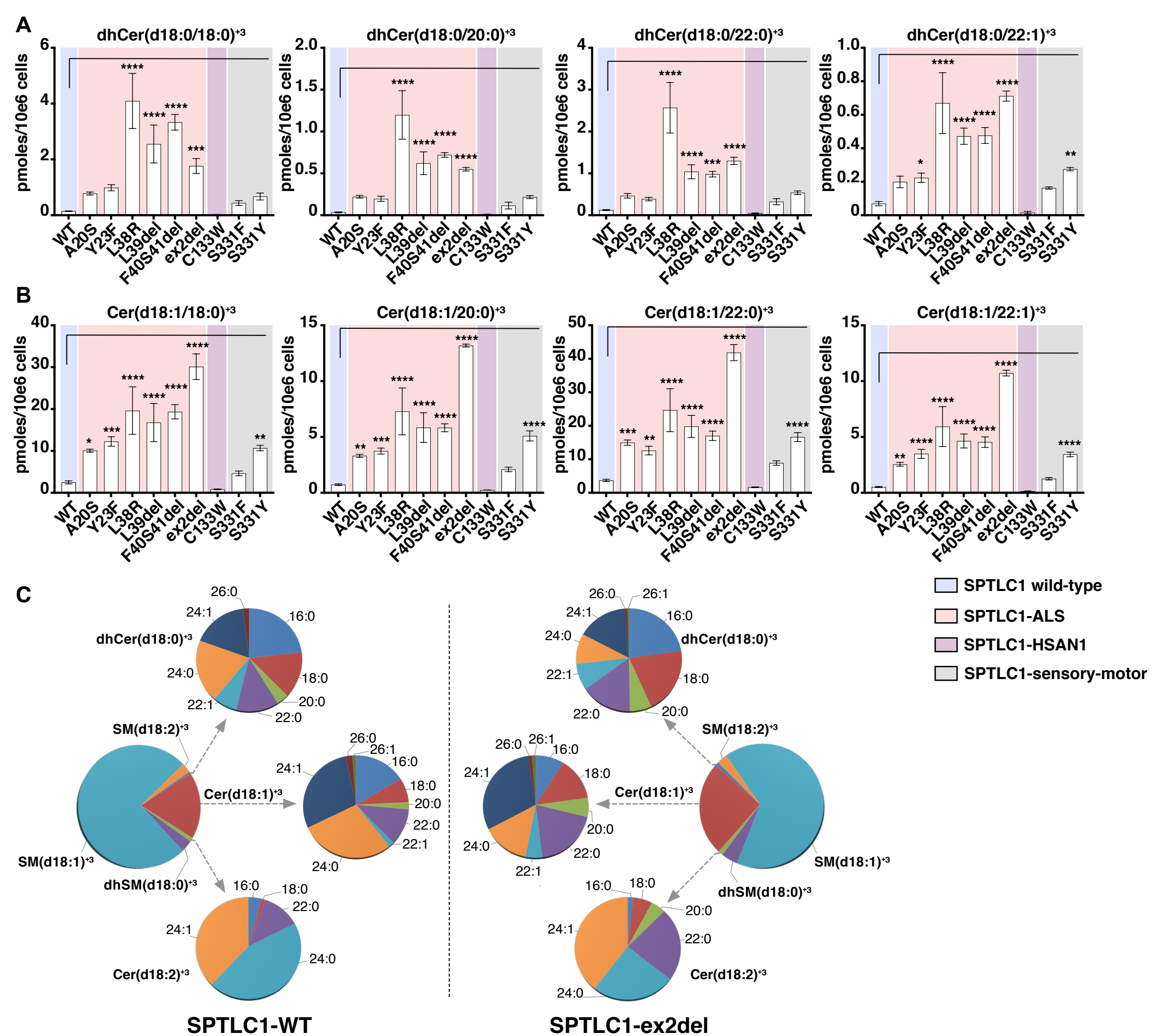
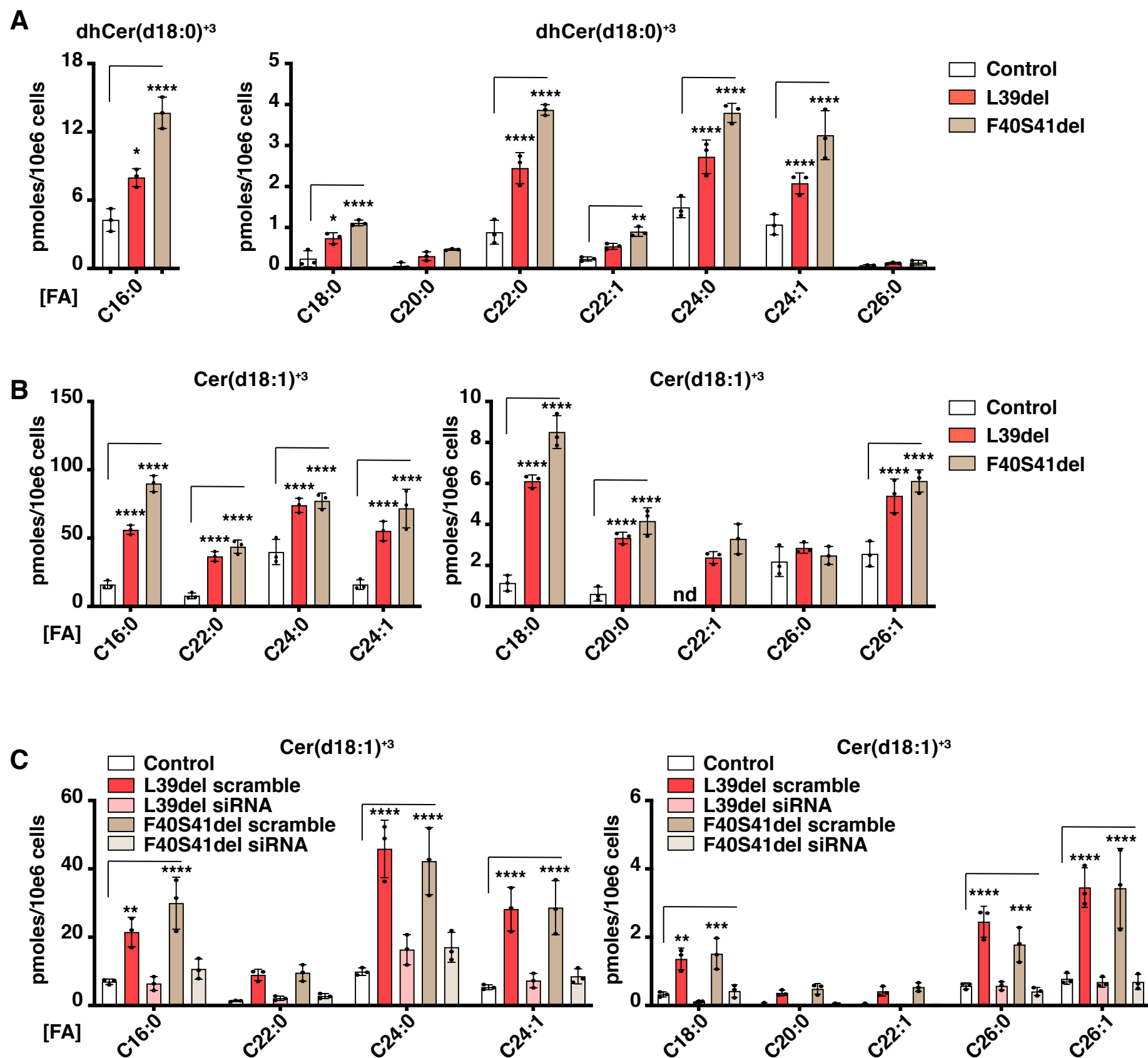
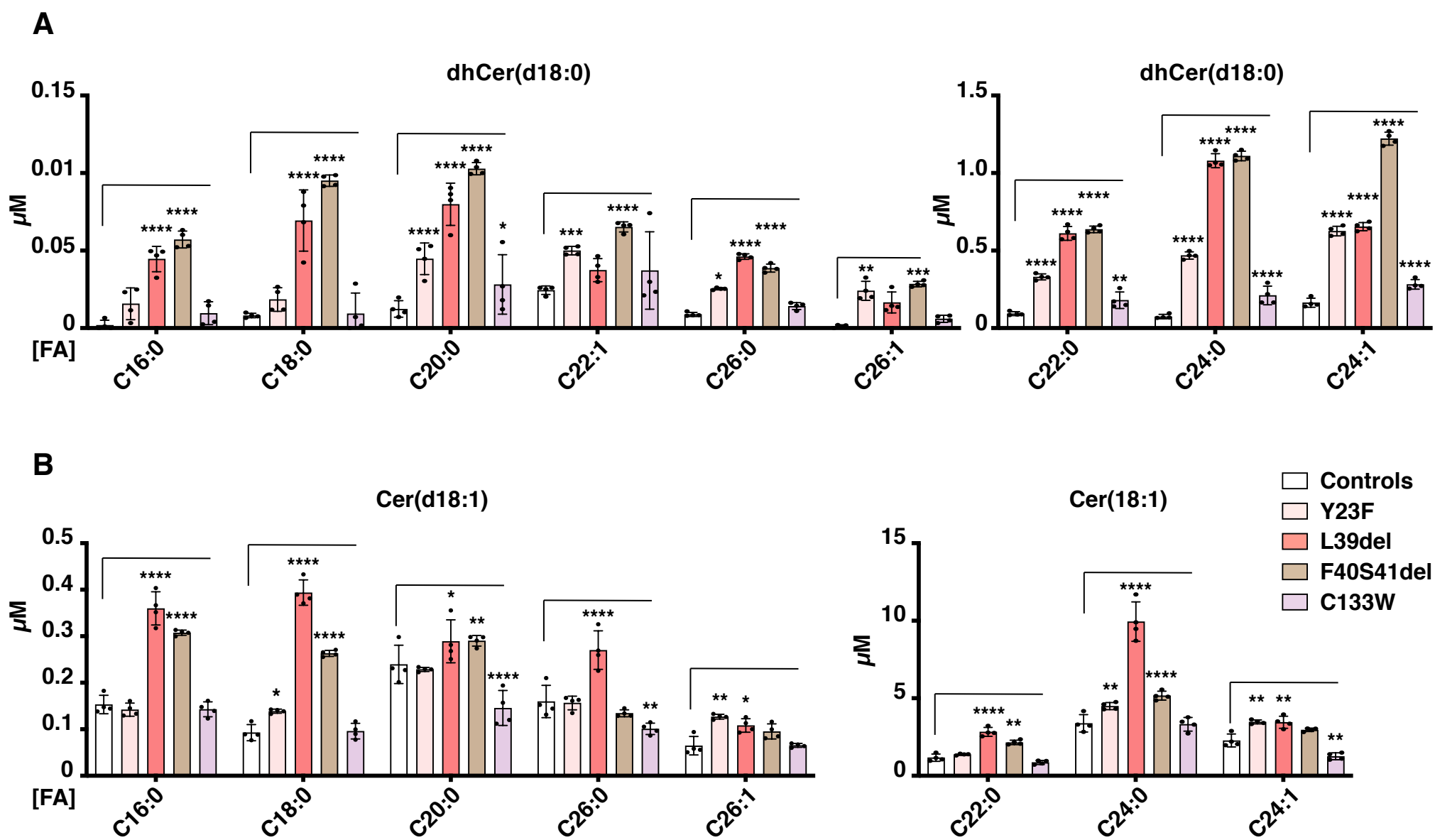


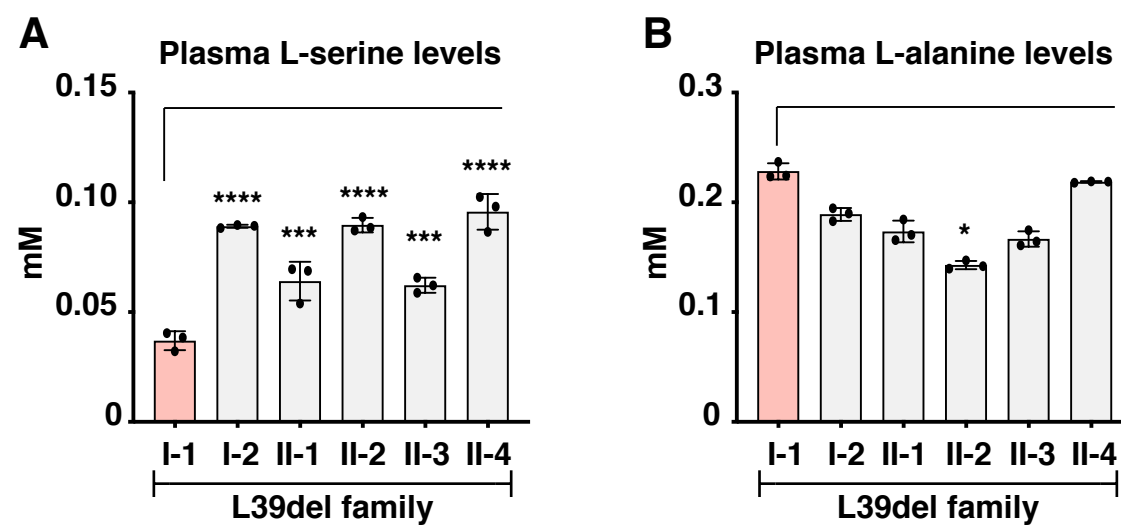
Figure S4



**Figure S5**

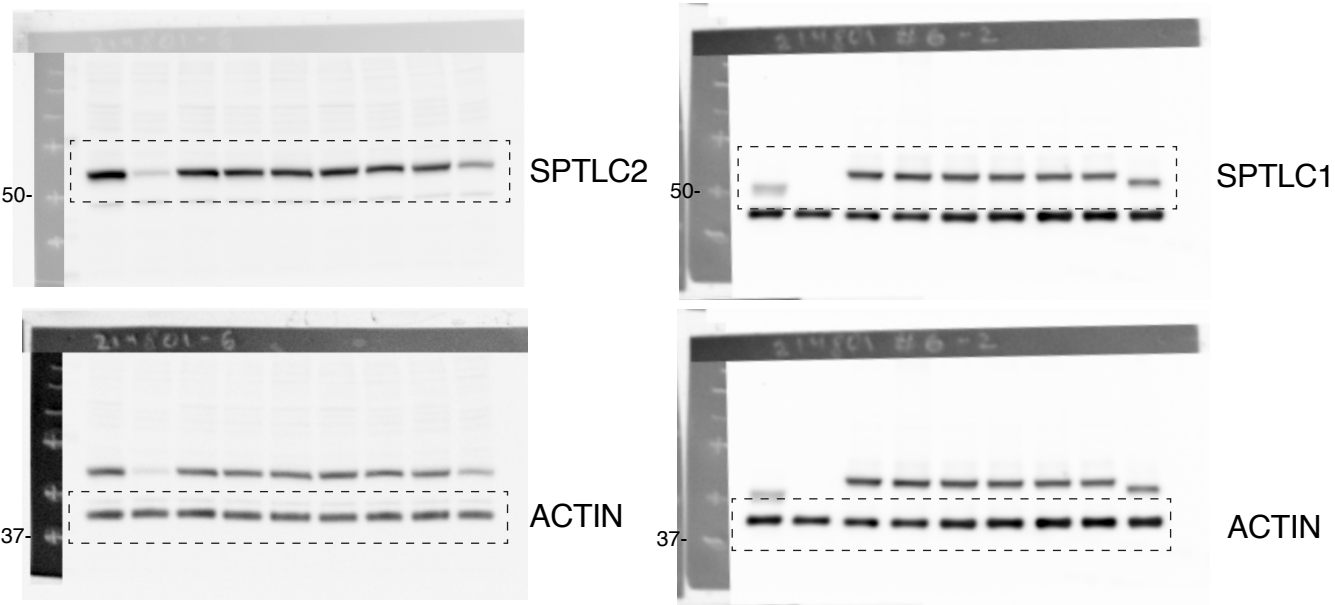


**Figure S6**

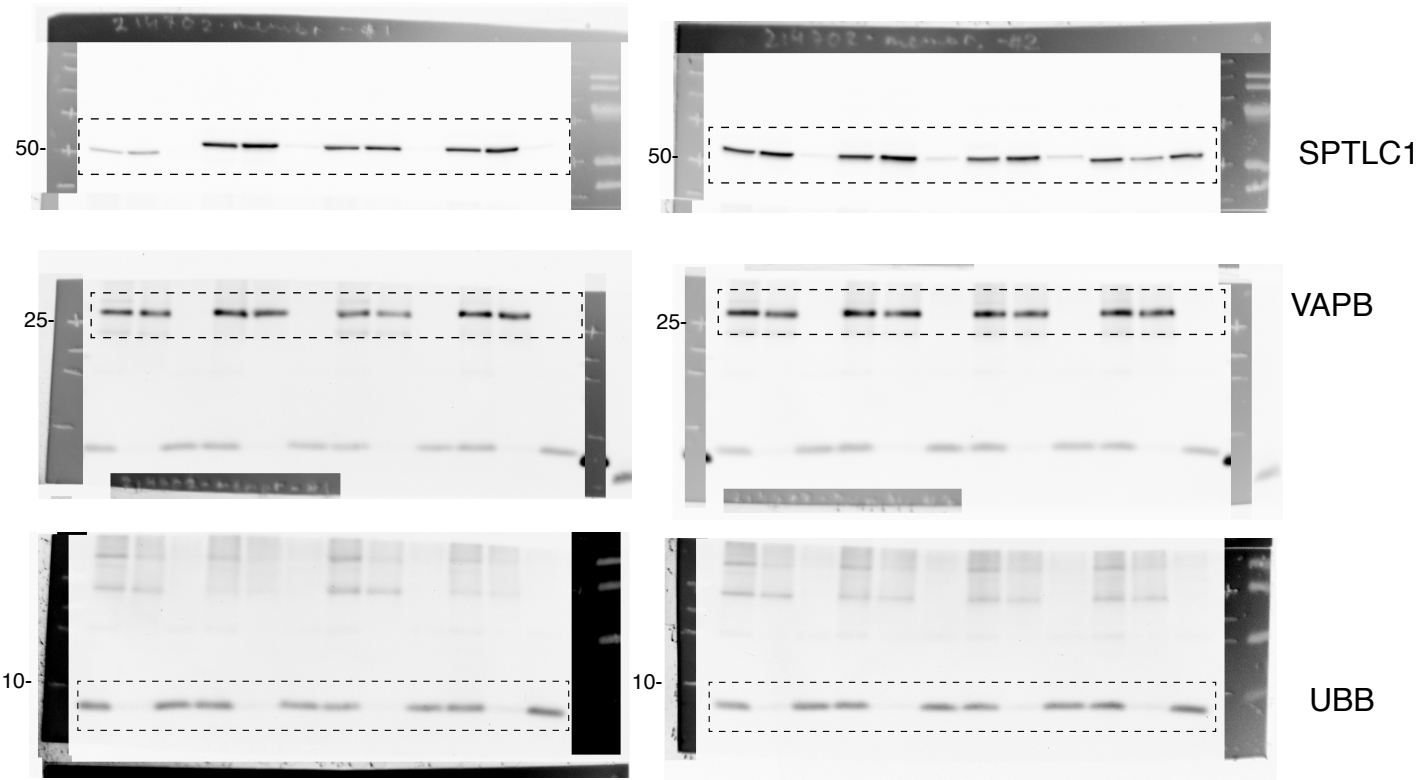


**Figure S7**

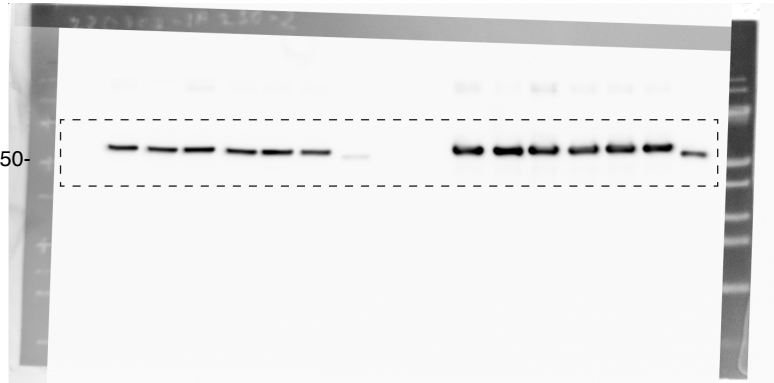
Full unedited blots for Figure 1C



Full unedited blots for Figure 1E



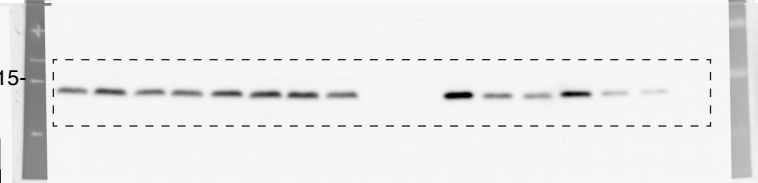
Full unedited blots for Figure 2A



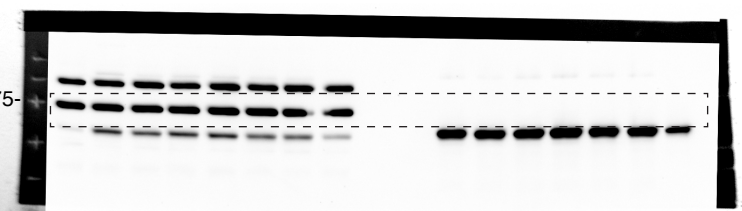
FLAG



SPTLC2



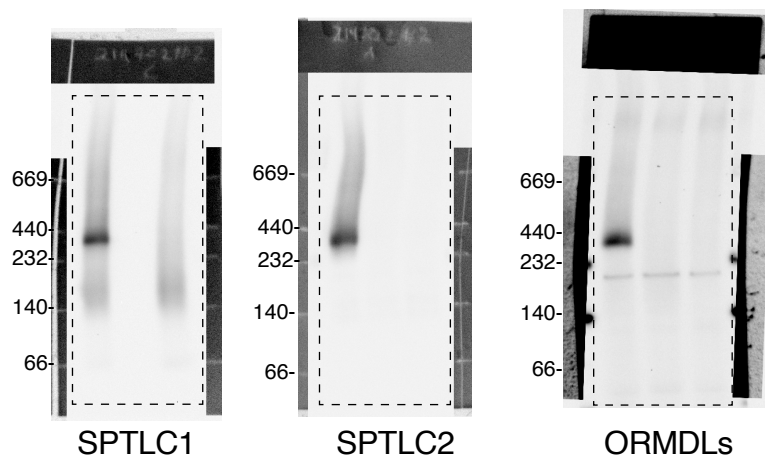
ORMDLs



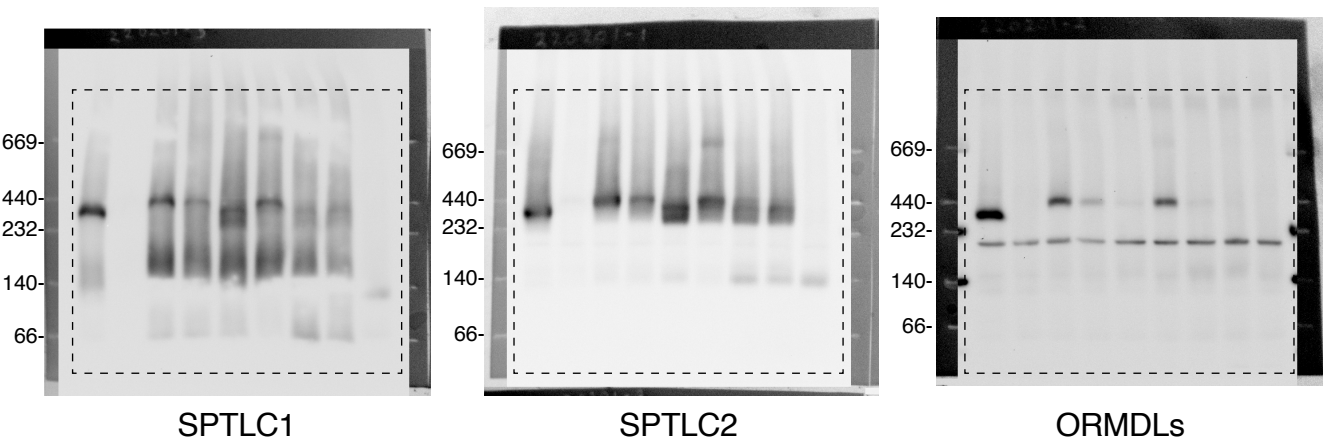
HSPA5



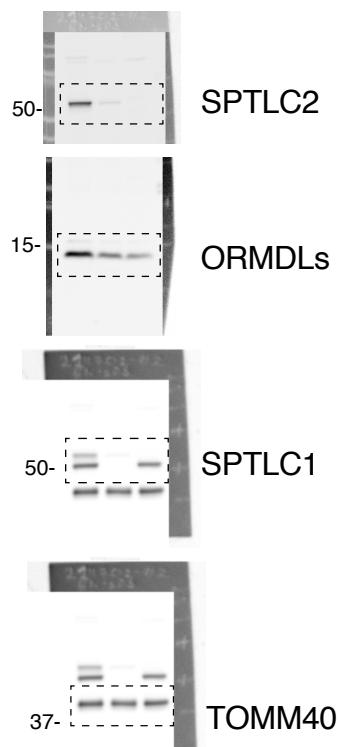
Full unedited blots for Figure 2B



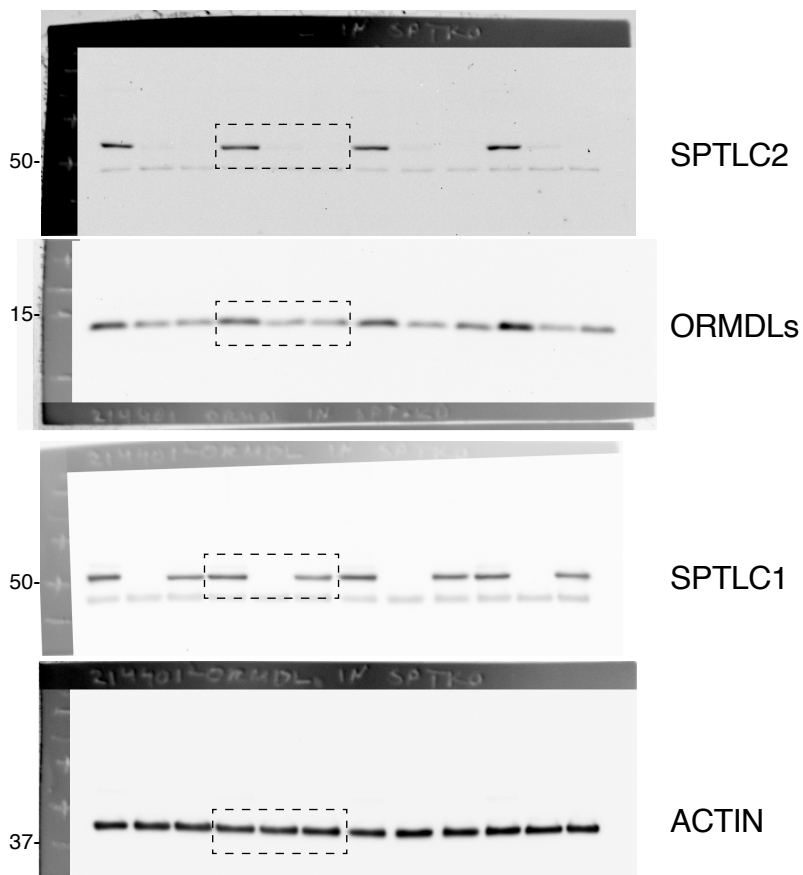
Full unedited blots for Figure 2C



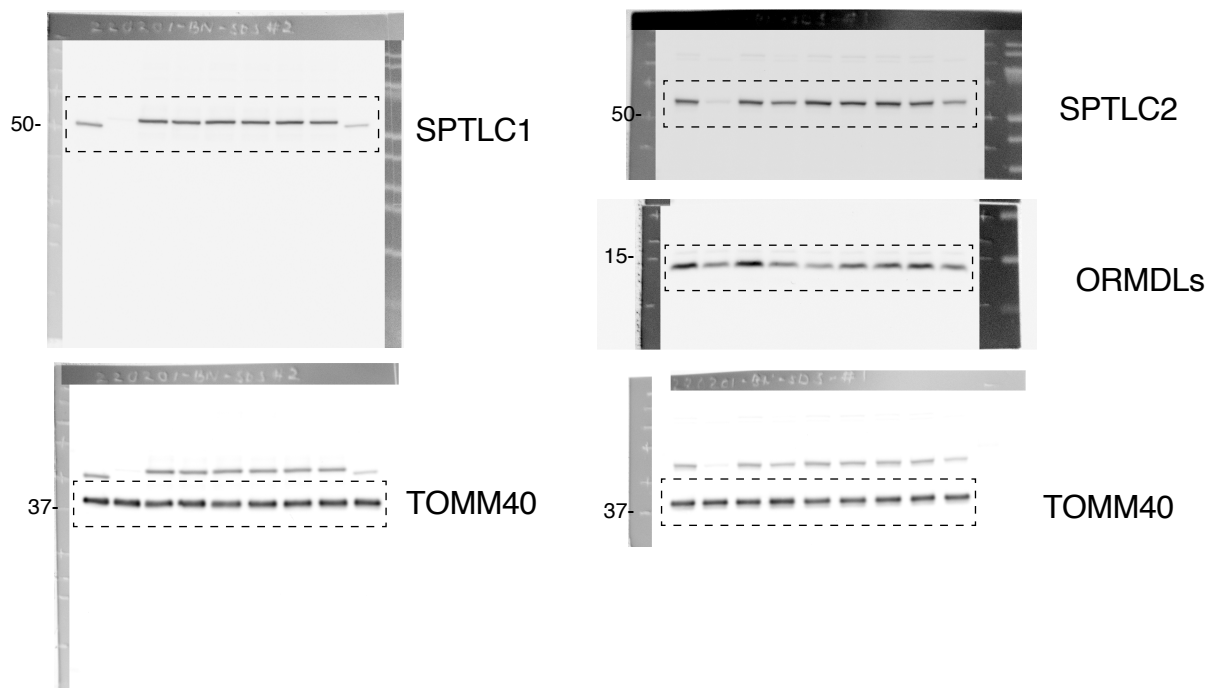
Full unedited blots for Figure S2A



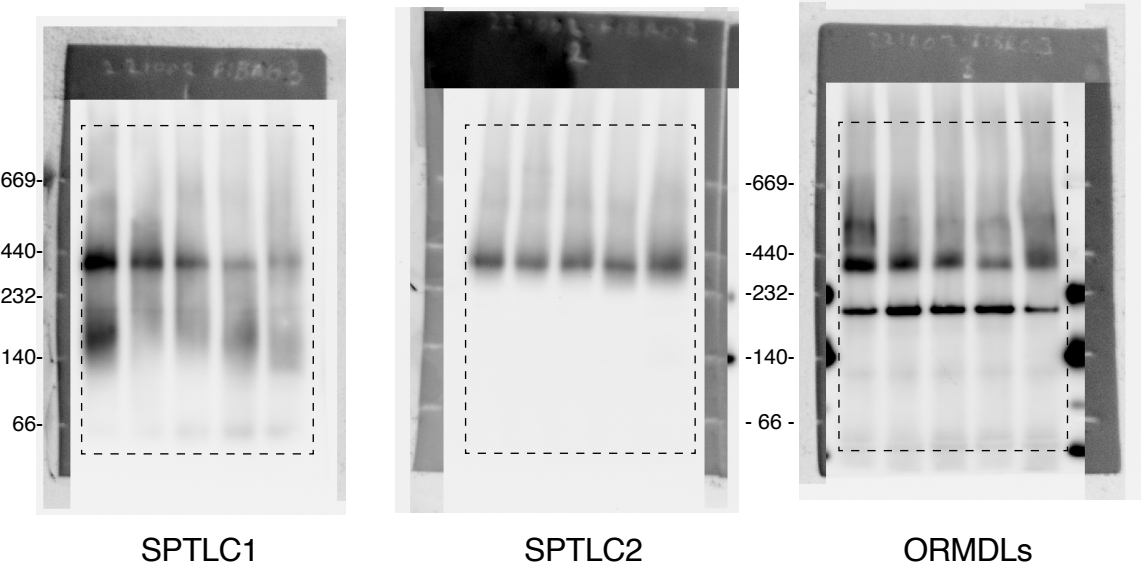
Full unedited blots for Figure S2B



Full unedited blots for Figure S2D



Full unedited blots for Figure S2E



Full unedited blots for Figure S2F

