Supplemental data and figures:

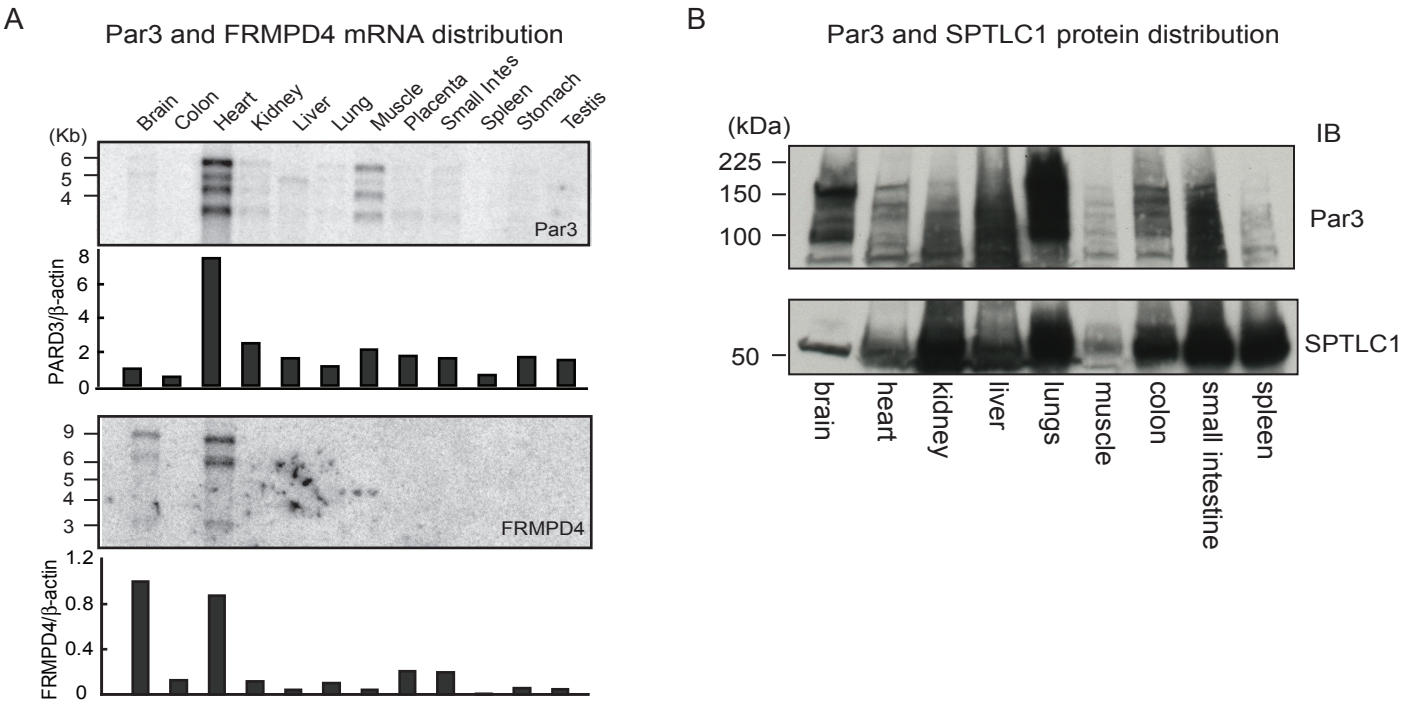
Supplemental Figure 1. **SPTLC1 and Par3 have a wide tissue distribution**. ***A,*** Par3 mRNA distribution was determined by probing a human mRNA tissue blot with a radiolabeled Par3 DNA probe (top panel). The blot was stripped and probed for the mRNA expression of the poorly described FRMPD4 protein (lower panel), which showed a more restricted distribution. ***B,*** Par3 and SPTLC1 protein expression are widespread and largely coincident as determined by immunoblots of the indicated mouse tissues.

Supplemental Figure 2. **N-terminal FLAG and HA tagged SPTLC1 stabilize SPTLC2 and stimulate SPT activity**. ***A,*** Immunoblots show the FL and HA tagged SPTLC1 constructs stabilize SPTLC1 protein expression, as does untagged SPTLC1. ***B,*** Expression of FL and HA tagged SPTLC1 stimulate SPT activity in LY-B cells that lack endogenous SPTLC1, and this activity is further stimulated by the co-expression of either untagged SPTLC2 or myc tagged SPTLC2 (n=2, results representative of two or more independent experiments).

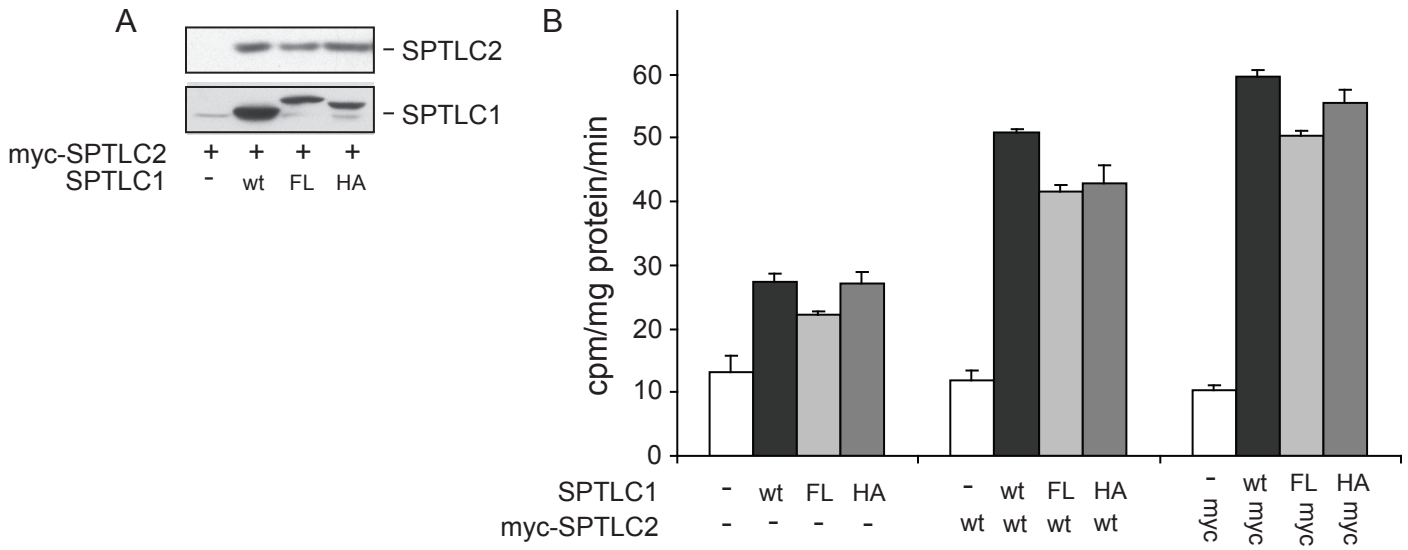
Supplemental Figure 3. **Mutation of the SPTLC1 C-terminal PDZ motif can destabilize SPTLC1 protein expression but does not prevent the SPTLC1 stabilization of SPTLC2**. ***A,*** Deletion (Δ3) or substitution to alanines (AAA) of the SPTLC1 C-terminal PDZ motif (VLL) disrupts SPTLC1 protein expression. However, more subtle mutations of the motif (ALA, VLA and ALL), which disrupt Par3 binding, do not strongly affect SPTLC1 protein expression (shown immunoblots for the HA-SPTLC1 constructs and for β-actin). ***B,*** The SPTLC1-AAA mutation disrupts SPTLC1 protein, but not mRNA expression as determined by immunoblots and RT-QPCR assay run in parallel using protein and mRNA from 293 HEK cells expressing HA tagged SPTLC1 and SPTLC1-AAA (n=2, results representative of two or more independent experiments). ***C,*** The SPTLC1-AAA and ALA mutants like wt SPLTC1 are able to stabilized SPTLC2 protein expression indicating these mutations do not disrupt SPTLC1 protein folding. Immunoblots show expression levels of SPTLC1, SPTLC2 and β-actin and the graph shows the SPTLC2/SPTLC1 protein ratio as determined by densitometric quantification of the immunoblots using a BioRad ChemiDoc XRS+ ECL imager.

Supplemental Figure 4. **Par3 is expressed human THP-1 monocytes and binds the SPT holoenzyme in primary mouse bone marrow derived macropages.** ***A,*** Lysates from human THP-1 monocytes (-, untreated with the phorbol ester PMA) and macrophages (+, 100 nM PMA treatment for 72h) were immunoblotted for Par3 and SPTLC1 expression. ***B,*** Lysates from primary mouse bone marrow derived macrphages were immunoblotted for the indicated proteins (left panels) and were immuno-precipitated with normal IgG or equivalent amounts of the anti-SPTLC2 antibody (top left panel) or the anti-SPTLC1 antibody (bottom left panel) and the amount of Par3 in the precipitates was determined by immuno-blotting. ***C,*** Lentiviral mediated expression of shRNAs targeting Par3 significantly reduces Par3, but not SPTLC1 or 2 protein expression. Conversely, shRNAs targeting SPTLC1 significantly reduces SPTLC1 and 2, put not Par3, protein expression (β-actin normalized values expressed as a present of the GFP control cells, n=3, ± SD, * $p < 0.05$, shSPTLC1 vs. shPar3). ***D,*** Lentiviral mediated expression of shRNAs targeting Par3 or SPTLC1 does not alter cell viability as assessed by luminescent quantitation of cellular ATP levels (left graph, n=3, ± SD), or by MTT assays of mitochondrial activity (right graph, n=3, ± SD). Results representative of two or more independent experiments.

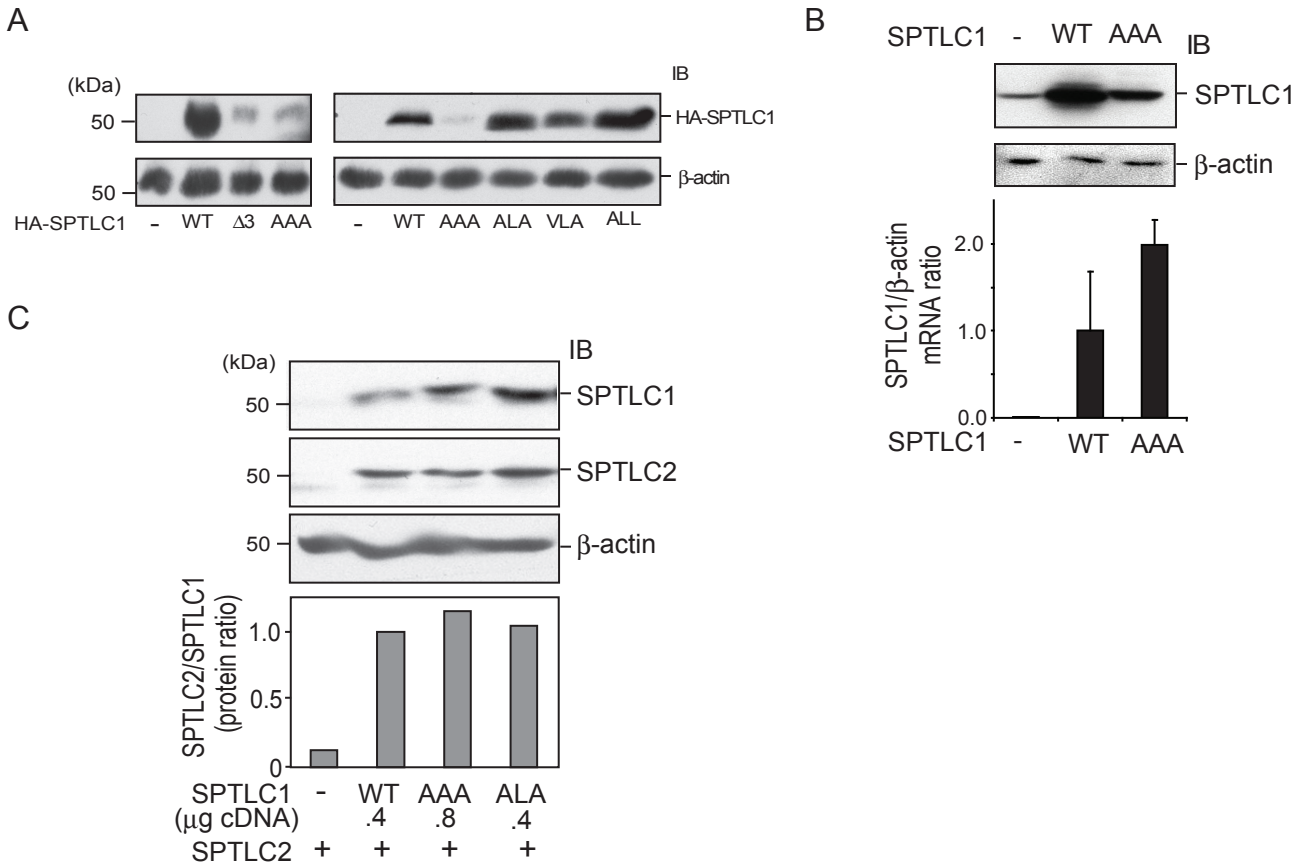
Supplemental Figure 5. **THP-1 cells expressing Par3 shRNAs have a significant deficit in the de novo synthesis of ceramide and sphingomyelin.** ***A,*** STP activity is still significantly reduced in Par3 shRNA expressing cells after normalization of SPT activity to the microsomal membrane levels of SPTLC1 protein (n=3, ± SD, * $p < 0.05$ vs. shGFP). ***B,*** Quantitation of de novo synthesis of ceramide (Cer), sphingomyelin (SM) and phosphatidylserine (PS) in THP-1 expressing Par3 shRNAs compared to cells expressing a GFP shRNA (n=3, ± SD, * $p < 0.05$ vs. PS). ***C,*** Lentiviral mediated expression of shRNAs targeting Par3 and SPTLC1 does not significantly affect the random migration THP-1 monocytes as determined in Boyden chamber assays (n=3, ± SD). Results representative of two or more independent assays.



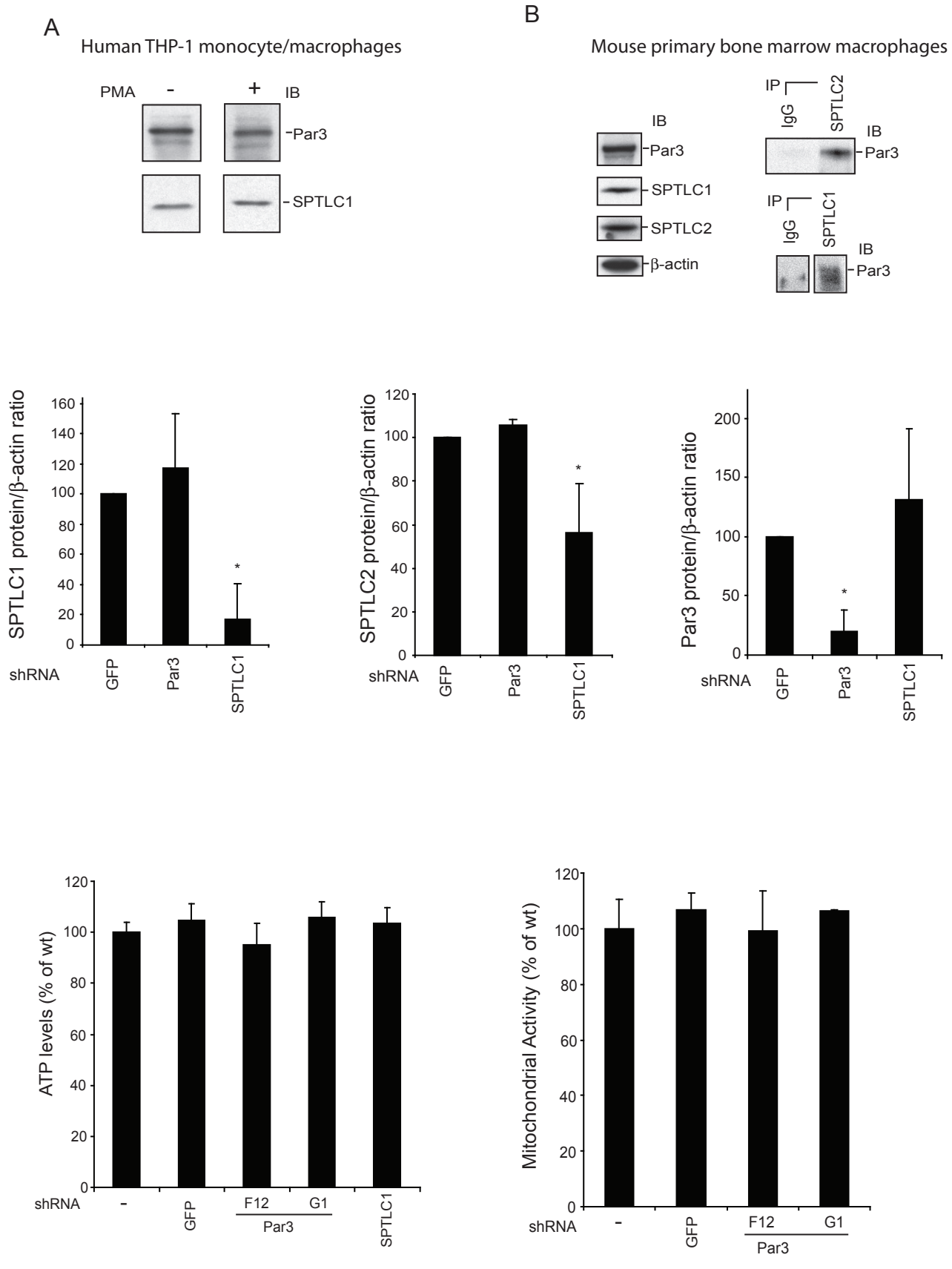
Supplementary Figure 1



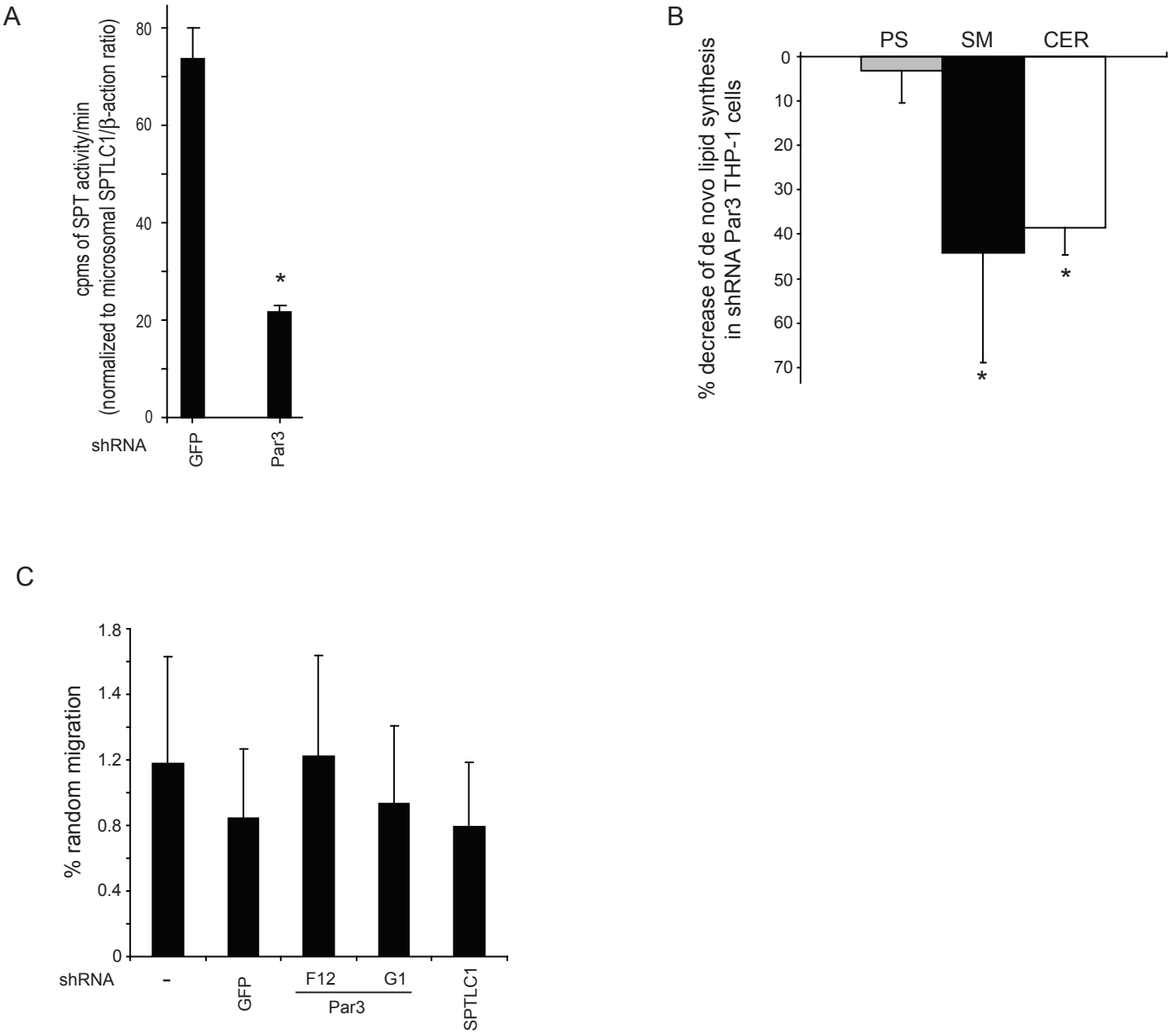
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5