



Supplementary Figure 1

Supplementary Figure 1. Characterization of LPCAT1/ β geo fusion protein. (A) HeLa cells transfected with LPCAT1-HA plasmid (WT) or LPCAT1/ β geo-HA plasmid (LPCAT1/ β geo) were co-stained with α -HA and α -calreticulin. Both the WT and LPCAT1/ β geo proteins colocalize to the ER. Scale bar = 50 μ m. (B) Colorimetric β -galactosidase assay from lysates of HeLa cells transiently transfected with a LPCAT1/ β geo plasmid, CMV bgal plasmid or cells only. Detectable product indicates that the LPCAT1/ β geo transcript generates a functional protein. Assay was performed in triplicate for each construct. (C&D) FACS analysis of HeLa cells transfected with WT LPCAT1, H135A mutant, LPCAT1/ β geo plasmid or empty vector (pcDNA3.1+). Cells were transfected for 24hrs, fixed, permeabilized and stained with an anti-HA primary antibody followed by an Alexa488 secondary antibody and analyzed by flow cytometry. The gate shown on the graph represents stained cells (HA+). Note the lower percentage of expressing cells (7.86 \pm 0.24% vs 41.7 \pm 0.87%) and the lower Mean Fluorescence Intensity (MFI=7450 \pm 58.07 and 20045 \pm 664.6) in LPCAT1/ β geo expressing cells vs. WT expressing cells, indicating increased turnover of the LPCAT1/ β geo fusion protein compared to WT or H135A mutant. Each construct was transfected in triplicate and the experiment was repeated twice. Data represent mean \pm SEM. (E) Acyltransferase activity assays of lysates from cells transfected with wild-type LPCAT1-HA (WT), LPCAT1 H135A, LPCAT1/ β geo or cells only. Data are normalized to MFI values as measured by FACS analysis due to unequal levels of expression of the LPCAT1/ β geo fusion to the WT protein. Data represent activities from two independent experiments with each group performed in triplicate. *p<0.001 vs. cells only. (F) Viability assay of HeLa cells transfected for 24hrs. No differences were detected within transfected groups. Values in graph represent percent of live cells normalized to cells only group. Data represent two independent experiments with each group performed in triplicate. *p<0.001 vs. all transfected groups. (G) qPCR analysis of *Hspa5(BiP)* levels in lung tissue of *Lpcat*^{GT/GT}, *Lpcat*^{GT/+}, *Lpcat*^{+/+} mice at E18.5. Data represent an n=5 per group.

Supplementary Methods

Cloning of LPCAT1/βgeo cDNA. Total RNA isolated from primary alveolar epithelial cells of adult *Lpcat1*^{GT/+} mice was reverse transcribed into cDNA using the iScript kit (Promega). The 4.8kb *LPCAT1/βgeo* cDNA was amplified from the cDNA using the following primers - forward primer: GCACGAGCTGCGACTGAG, reverse primer: **GCGGCCGCCTATGCATAGTCCGGGACGTCATAGGGATAGGCGATACCGTA** AAGCACGA) and cloned into the pGEM-T easy vector (Promega). The reverse primer contained a NotI site (bold) and an in-frame, C-terminal HA tag (underlined) for cloning and tracking expression of the construct in vitro. The amplified insert was cloned into the pGEM-T easy vector (Promega) and fully sequenced to ensure integrity of the amplicon. The insert was then subcloned into the PstI/NotI sites of pcDNA3.1+ (Invitrogen) to generate the LPCAT1/βgeo+pcDNA3.1+ construct.

Colorimetric βgal assay.

Transfected HeLa cells were lysed in RIPA buffer and β-galactosidase activity was determined by incubating 20ul of cell lysate with 80ul of β-gal assay buffer (0.2M Na₂PO₄ buffer (pH7.3), 2mM MgCl₂, 5mM 2-Nitrophenyl b-D-galactopyranoside (ONPG), 50mM β-mercaptoethanol) for 1hour at 37°C.

FACS analysis.

Cells were fixed in 4% paraformaldehyde for 15min, permeabilized with 0.01% saponin for 10min and blocked with 4% donkey serum for 30min. Cells were then incubated with anti-HA antibody (Cell Signaling Technologies) followed by anti-mouse Alexa 488 (Invitrogen). Data were acquired on a LSRII flow cytometer and analyzed with FloJo software (Becton Dickinson). 50,000 events were acquired for each data point.

Cell viability assay.

HeLa cells were transfected for 24hrs and cell viability was determined by MTS assay (Promega).