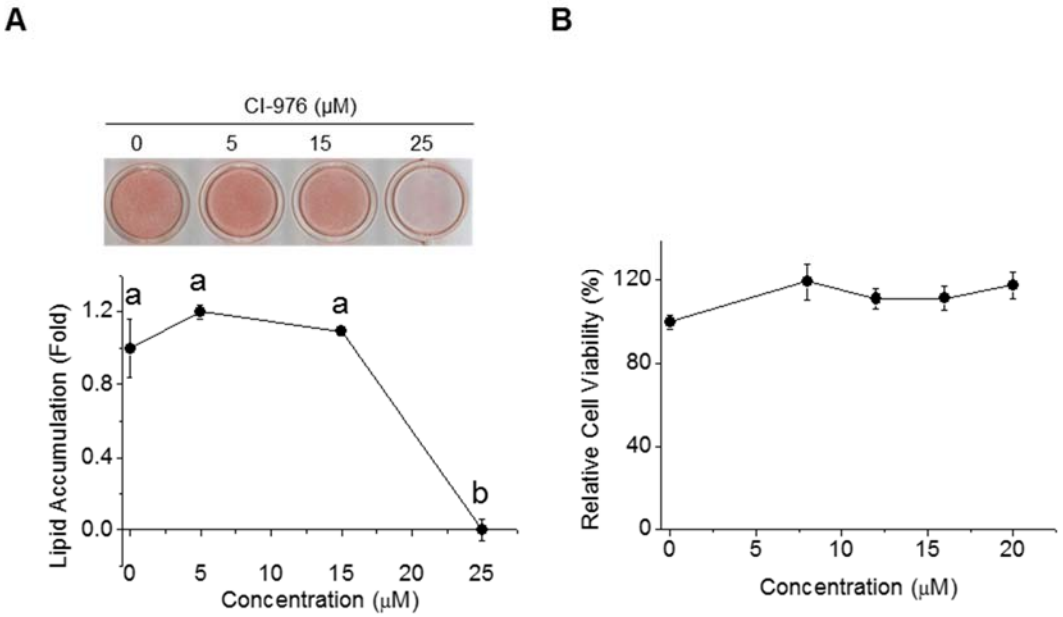
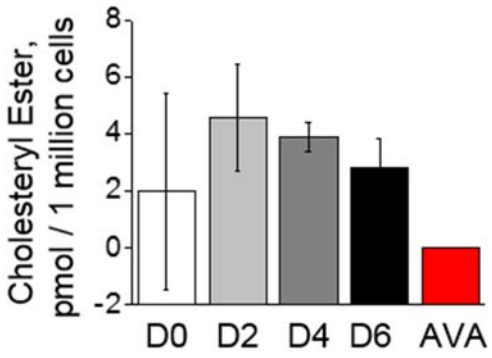


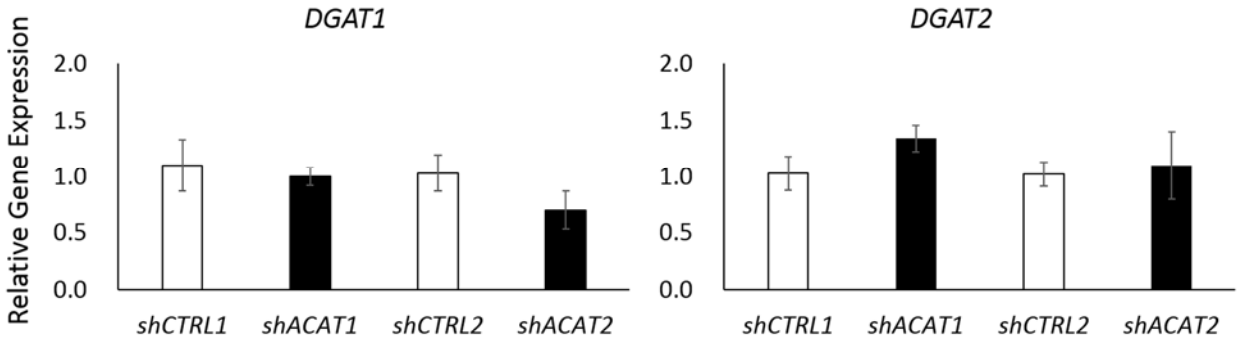
Supplemental Figure S1. ACATs blockage by CI-976 inhibits lipid accumulation

(A) Oil red O (ORO) staining analysis for measuring the accumulated lipids was performed in adipocytes (Day 6) that were differentiated with or without CI-976 (an known ACAT inhibitor) at varies concentrations (1–25 μM) for 6 days (n = 3). One-way ANOVA with Bonferroni post hoc test was applied to panel A, and different letters indicate significant different ($p < 0.05$). (B) Adipocyte viability upon CI-976 treatment (0 – 20 μM) for 48hr was determined by MTT assay (n = 3).



Supplemental Figure S2. Avasimibe inhibits intracellular level of CE in adipocytes

3T3-L1 preadipocytes were differentiated with the standard adipogenic cocktail as described in the Method section. These cells treated without or with AVA (20 μM, during day 0-6) were harvested at various stages of adipogenesis as indicated D0, D2, D4 and D6. It should be noted that this figure shows large error bars because the intracellular levels of CE in undifferentiated 3T3-L1 preadipocytes and adipocytes treated with AVA were near or below the detection limit of the enzymatic assay kit used in this study. CE level was determined as described in the Method section (n=3). Data presented were means ± S.E.M.



Supplemental Figure S3. *ACAT1* and *ACAT2* knockdown show no effect on *DGAT1* and *DGAT2* expression in preadipocytes

3T3-L1 preadipocytes infected with lentiviral particles carrying either an ACAT1 target shRNA (shACAT1), ACAT2 target shRNA (shACAT2) or control shRNA were subjected to qPCR assay to determine the mRNA levels of *DGAT1* and *DGAT2*. Data were normalized to β-action (biological repeat: n=3, technical repeat: 2 per sample). Data presented were means ± S.E.M. The gene knockdown efficacy of these shRNAs in these preadipocytes was assessed in Figure 4D and E.