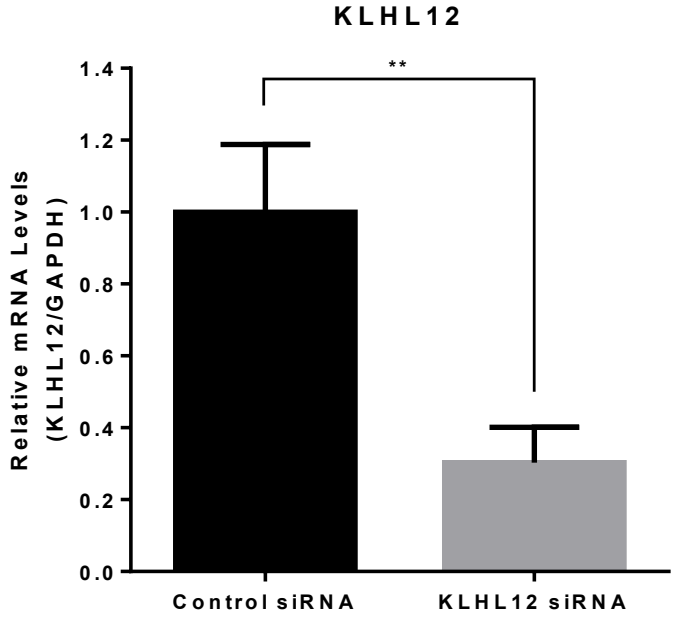
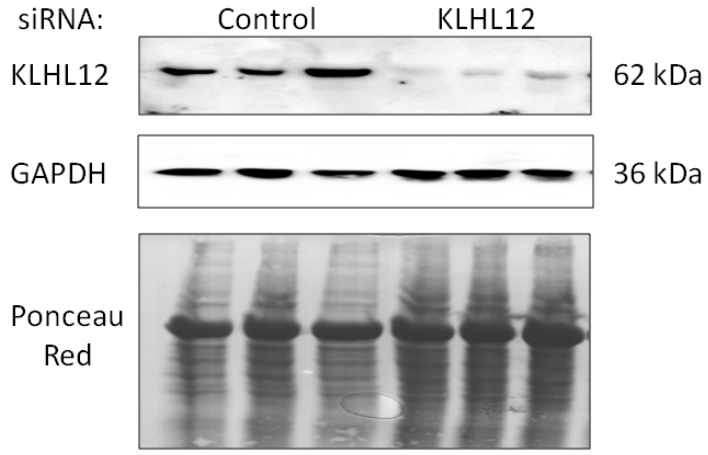
Online-only Data Supplement

Supplemental Figure I



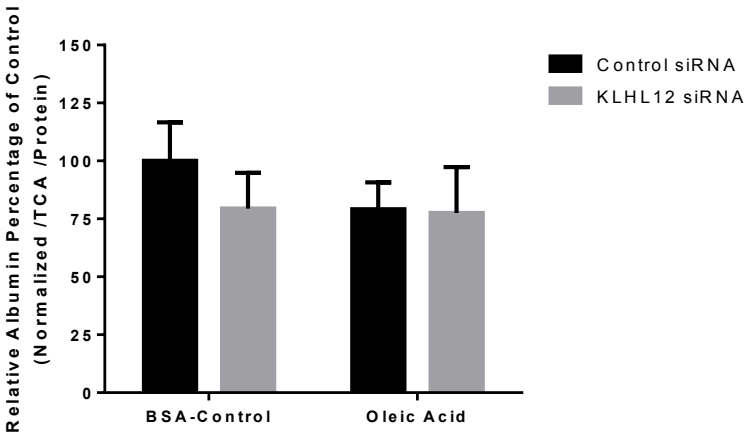
Supplemental Figure I. KLHL12 mRNA abundance in McA cells transfected with control or KLHL12 siRNA. McA cells were transfected with 20 nM control or KLHL12 siRNA. 48 h post transfection, total RNA was extracted and mRNA levels of KLHL12 were measured by qPCR, and normalized to the levels of GAPDH. $^{**}p<0.01$.

Supplemental Figure II



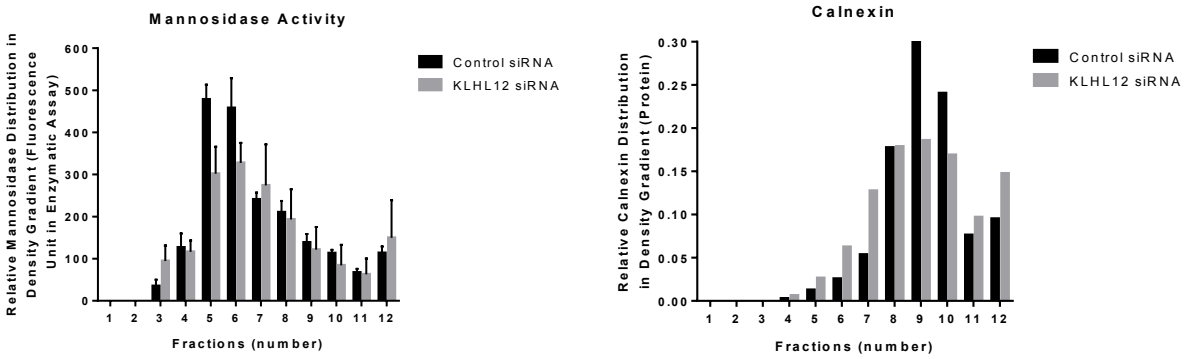
Supplemental Figure II. KLHL12 protein levels in McA cells transfected with control or KLHL12 siRNA. McA cells were transfected with 20 nM control or KLHL12 siRNA. 48 h post transfection, total cell lysate was harvested, and KLHL12 and GAPDH protein levels were measured by Western blotting. Ponceau Red staining was performed after protein transfer to indicate the total protein in each lane.

Supplemental Figure III



Supplemental Figure III. Effects of KLHL12 knockdown on albumin recovery from McA cells. McA cells were transfected with either scrambled (control) or KLHL12-specific siRNA or pcDNA3.1 vector (control) or pcDNA3.1-KLHL12. 48 h after transfection, cells were incubated with 0.6 mM OA/BSA complexes (or BSA as a control) for 1 h, followed by an additional 3 h incubation in which ^{35}S-met/cys was added to the conditioned media. Total levels (sum of secreted and intracellular recoveries) of radiolabeled albumin in the conditioned media and cell lysates were normalized against total TCA-precipitable counts and total protein mass.

Supplemental Figure IV

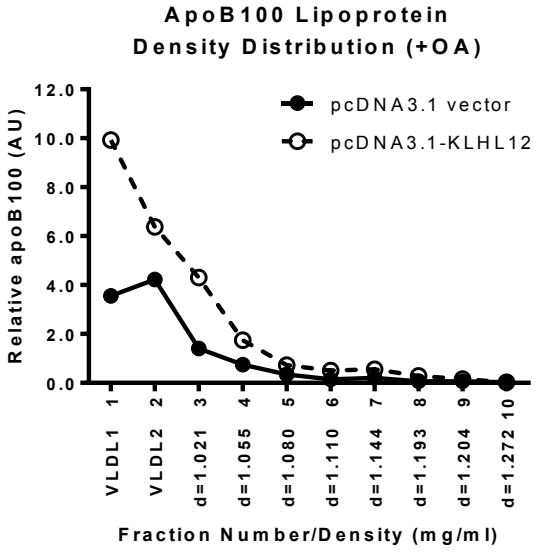


Supplemental Figure IV. Mannosidase (Golgi-specific) and Calnexin (ER-specific) distributions in sucrose density gradient fractions of cell lysates. McA cells were transfected with 20 nM control or KLHL12 siRNA, and 48 h after transfection cells were harvested and ER microsomes and Golgi membranes were isolated and separated by sucrose gradient centrifugation. The fractional contents of mannosidase activity and calnexin were measured by enzymatic assay and Western blotting, respectively.

Supplemental Figure V

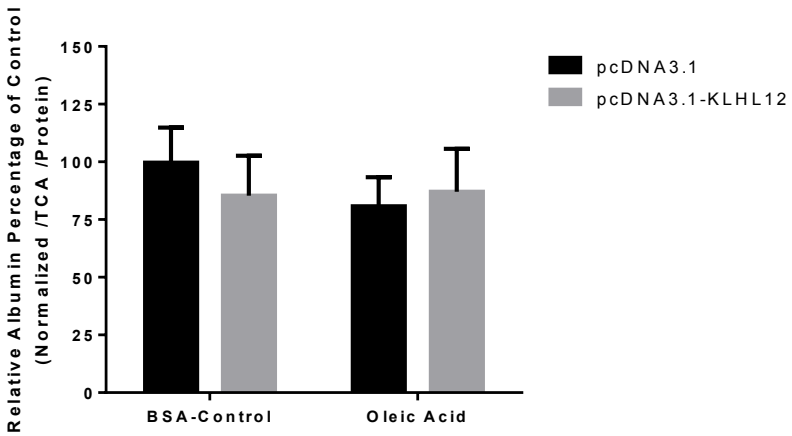
Supplemental Figure V. KLHL12 protein levels in McA cells transfected with a vector control, pcDNA3.1, or a FLAG-tagged pcDNA3.1-KLHL12 plasmid. McA cells were transfected with a pcDNA3.1 or FLAG-tagged pcDNA3.1-KLHL12 plasmid. 48 h post transfection, proteins were extracted from the cells, and KLHL12 and GAPDH protein levels were measured by Western blotting using anti-FLAG or anti-GAPDH antibodies.

Supplemental Figure VI



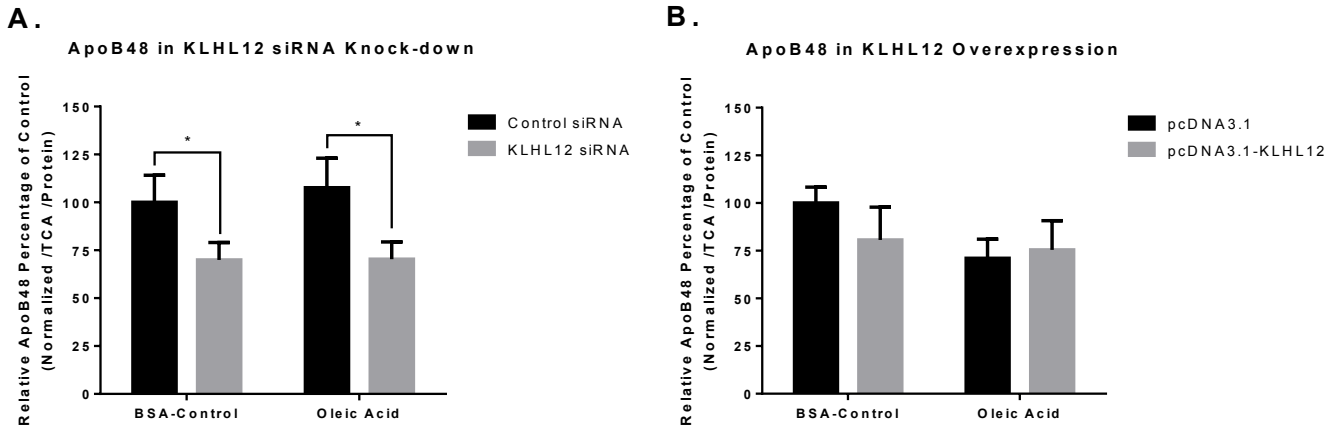
Supplemental Figure VI. Effects of KLHL12 over-expression on the density distribution of apoB100-lipoproteins secreted from McA cells. McA cells treated and analyzed as in Figure 1C, except that instead of being knocked down, KLHL12 expression was increased by transfection with pcDNA3.1-KLHL12 (see Supplemental Figure V). Transfection with pcDNA3.1 was used as a control. The graph shows results representative of 3 independent

Supplemental Figure VII



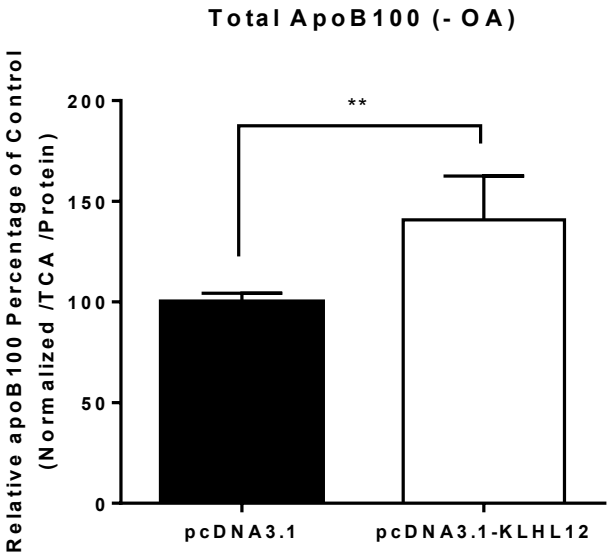
Supplemental Figure VII. Effects of KLHL12 over-expression on albumin recovery from McA cells. McA cells were transfected with either pcDNA3.1 vector (control) or pcDNA3.1-KLHL12. 48 h after transfection, cells were incubated with 0.6 mM OA/BSA complexes (or BSA as a control) for 1 h, followed by an additional 3 h incubation in which ^{35}S-met/cys was added to the conditioned media. Total levels (sum of secreted and intracellular recoveries) of radiolabeled albumin in the conditioned media and cell lysates were normalized against total TCA-precipitable counts and total protein mass.

Supplemental Figure VIII



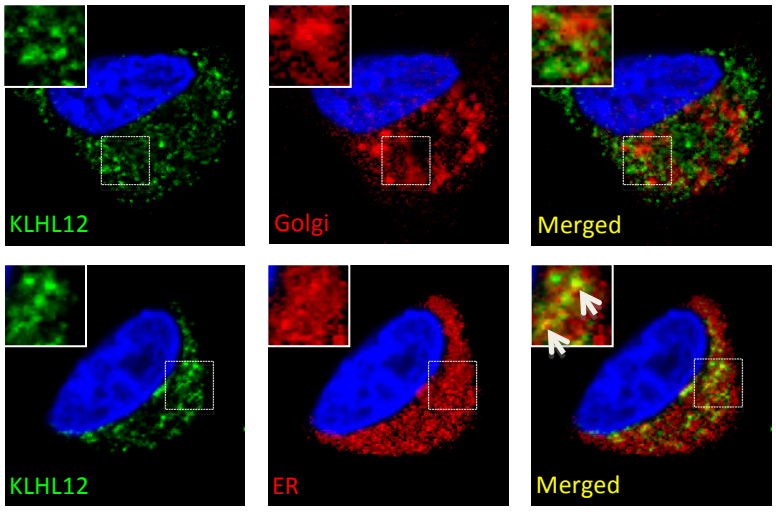
Supplemental Figure VIII. Effects of KLHL12 knockdown and overexpression on apoB48 in McA cells. McA cells were transfected with either a scrambled control or KLHL12-specific siRNA (**A)**, or pcDNA3.1 vector or pcDNA3.1-KLHL12 (**B)**. 48 h later, cells were incubated with 0.6 mM OA/BSA complexes (or BSA control) for 1 h, and then another 3 h after addition of ^{35}S-met/cys to radiolabel apoB48 to steady state. Total levels (sum of secreted and intracellular recoveries) of radiolabeled apoB48 in the conditioned media and cell lysates were normalized against total TCA-precipitable counts and total protein mass. $^{*}p<0.05$.

Supplemental Figure IX



Supplemental Figure IX. Effect of over-expression of KLHL12 on the recovery of apoB100 from McA cells in the absence of oleic acid. McA cells were transfected with a KLHL12 expression vector as in Supplemental Fig. IV. Recovery of apoB100 in the absence of exogenous oleic acid was determined as in Supplemental Fig. III for albumin, but now using an apoB antibody for the immunoprecipitation. $^{**}p<0.01$.

Supplemental Figure X



Supplemental Figure X. KLHL12 (green) co-localizes not with the Golgi-RFP (red) marker (top panel), but with the ER-RFP (red) marker (bottom panel). Nuclei were stained with DAPI. Magnification is 63X. The inset is a ×10 digital zoom of the area of interest, with co-localization reflected by yellow signals (arrowheads). Shown are the results representative of 3 independent experiments.