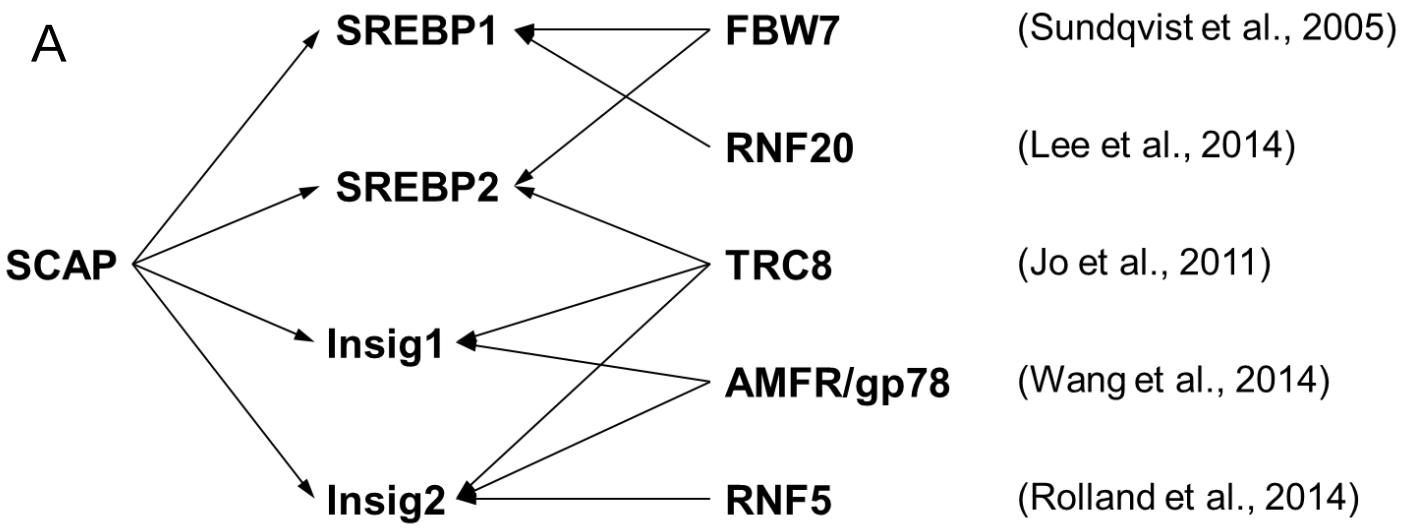
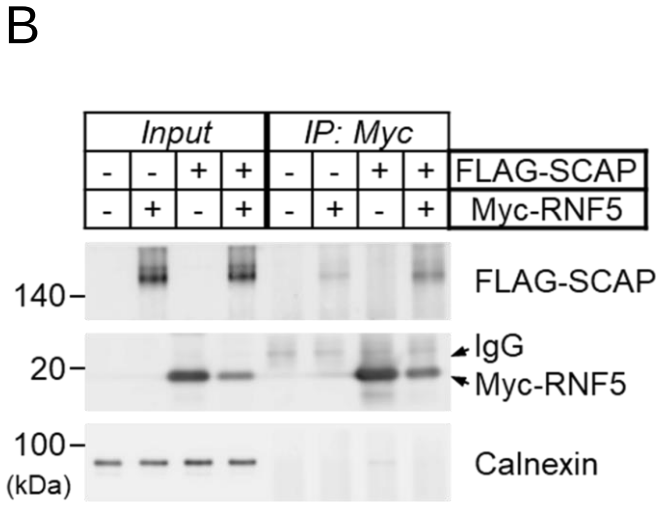
Supplementary Figure S1





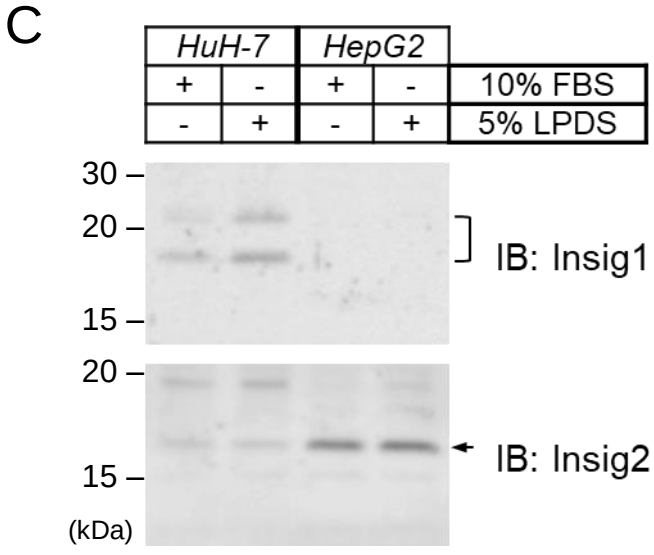


Figure S1. ***A***, Known E3 ligases reported to form protein complex with Insig and SREBP. ***B***, HEK293 cells were transfected with indicated plasmids for anti-Myc-RNF5 IP as described in Figure 1C and 1D. ***C***, HuH-7 and HepG2 cells were seeded in 6-well plates and cultured until cell density reached 70% confluent. The cells were then switched into DMEM supplemented with either 10% (v/v) FBS or 5% (v/v) LPDS and cultured for 16 h and then harvested for immunoblot analysis.

Supplementary Figure S2-R

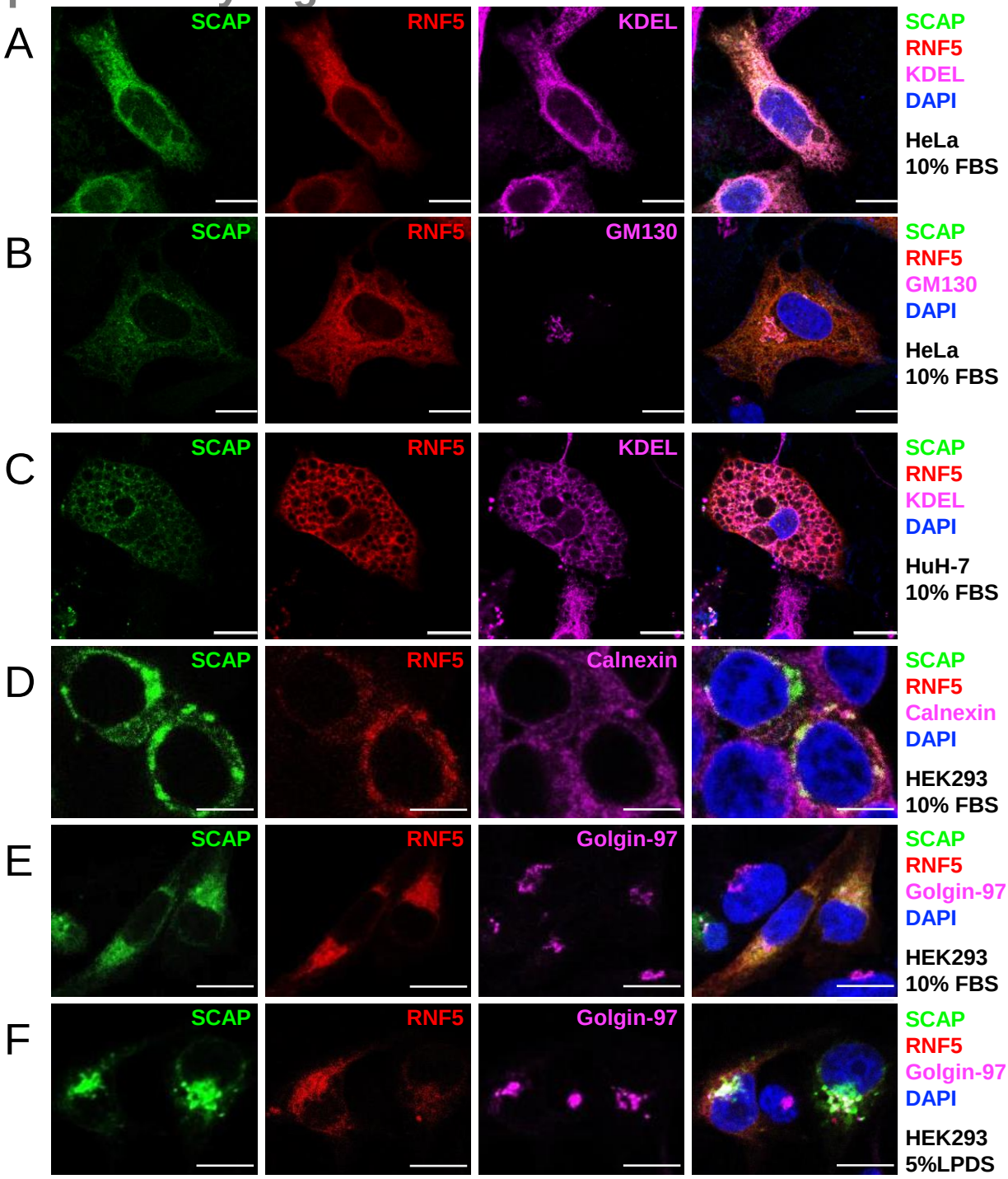


Figure S2. The localization of RNF5 and SCAP was determined by confocal microscopy. EGFP-SCAP and mCherry-RNF5 expression plasmids were co-transfected in HeLa (***A***, ***B***), Huh7 (***C***), and HEK293 (***D*** – ***F***) cells, and cultured in complete medium (10%FBS) (A – E) or sterol-depleted medium (5%LPDS) (F). The cells were fixed, permeabilized, and stained with anti-KDEL (A, C), anti-GM130 (B), anti-calnexin (D), or anti-Gogin97 (E, F) antibodies followed by AlexaFluor647-conjugated secondary antibodies. Nuclei were stained with DAPI. (Scale bar, 10 nm)

Supplementary Figure S3

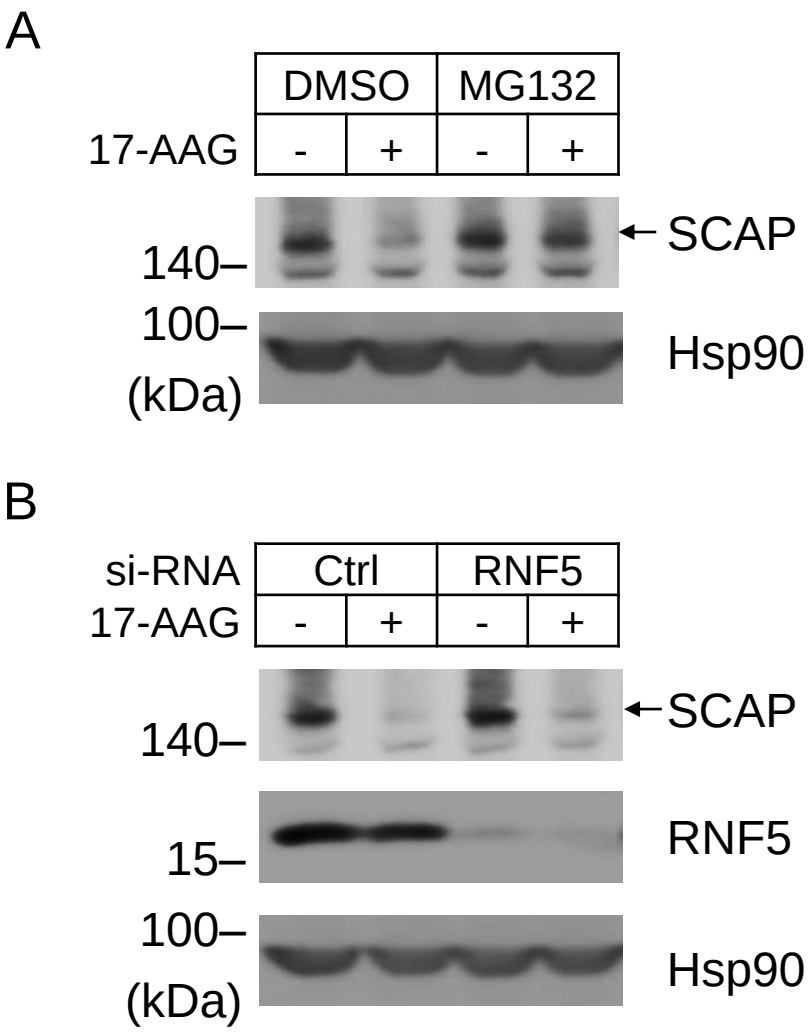


Figure S3. ***A***, HuH-7 cells were seeded in 6-well plates and cultured until cell density reached to 80% confluent. The cells were then switched to fresh medium containing 2.5 μM MG132 or 0.025% DMSO and cultured for 30 min. After preincubation with MG132, 1 μM of 17-AAG or equal volume of 0.001% DMSO were applied to the cells. The cells were then cultured for 12 h and then harvested for immunoblot analysis. ***B*****,** HuH-7 cells were seeded in 6-well plates and cultured until cell density reached to 50% confluent. The cells were then transfected with control or RNF5 siRNA and cultured for 36 h. The cells were then switched into fresh medium containing 1 μM of 17-AAG or equal volume of 0.001% DMSO and cultured for 12 h. The cells were then harvested for immunoblot analysis.

Supplementary Figure S3

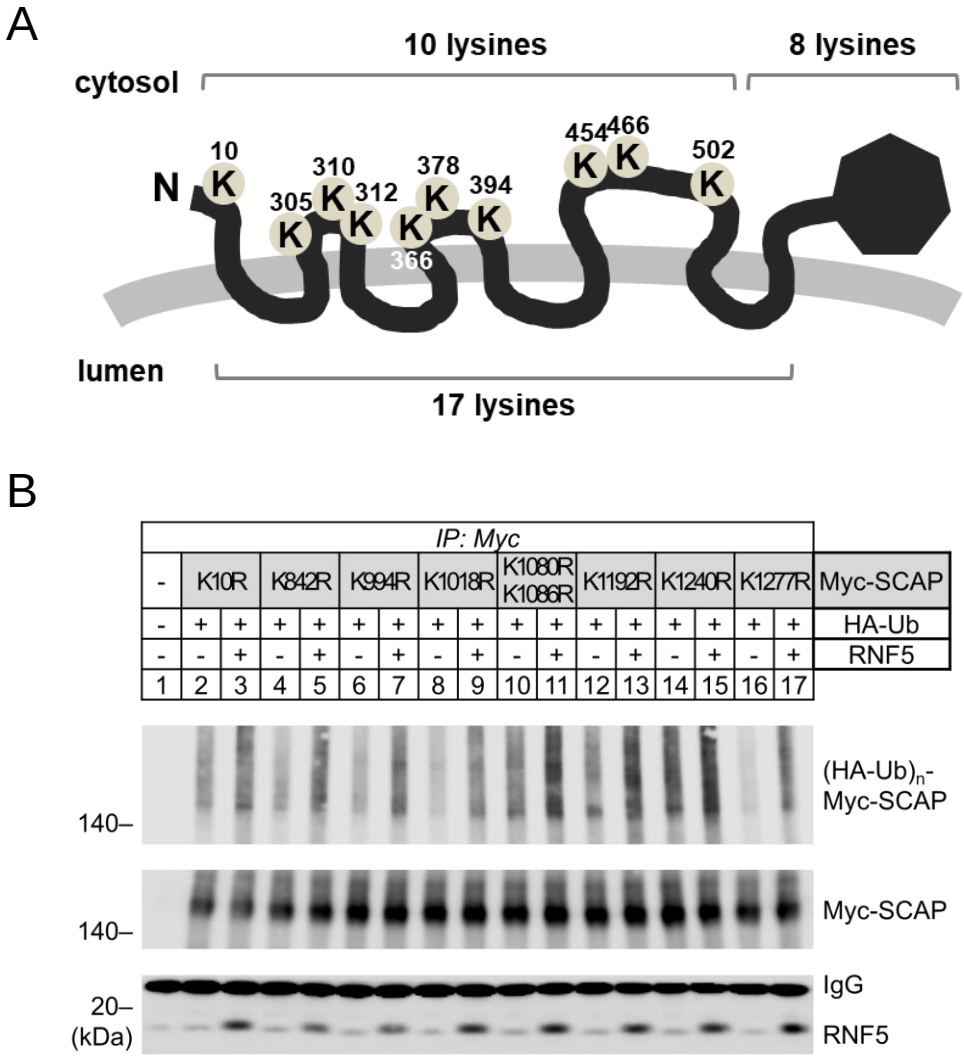


Figure S4. ***A***, Schematic illustration of lysine residues on the cytosolic loops of SCAP. ***B*****,** SRD-13A cells were treated as described in Figure 4A for anti-Myc-SCAP IP.

Supplementary Figure S4

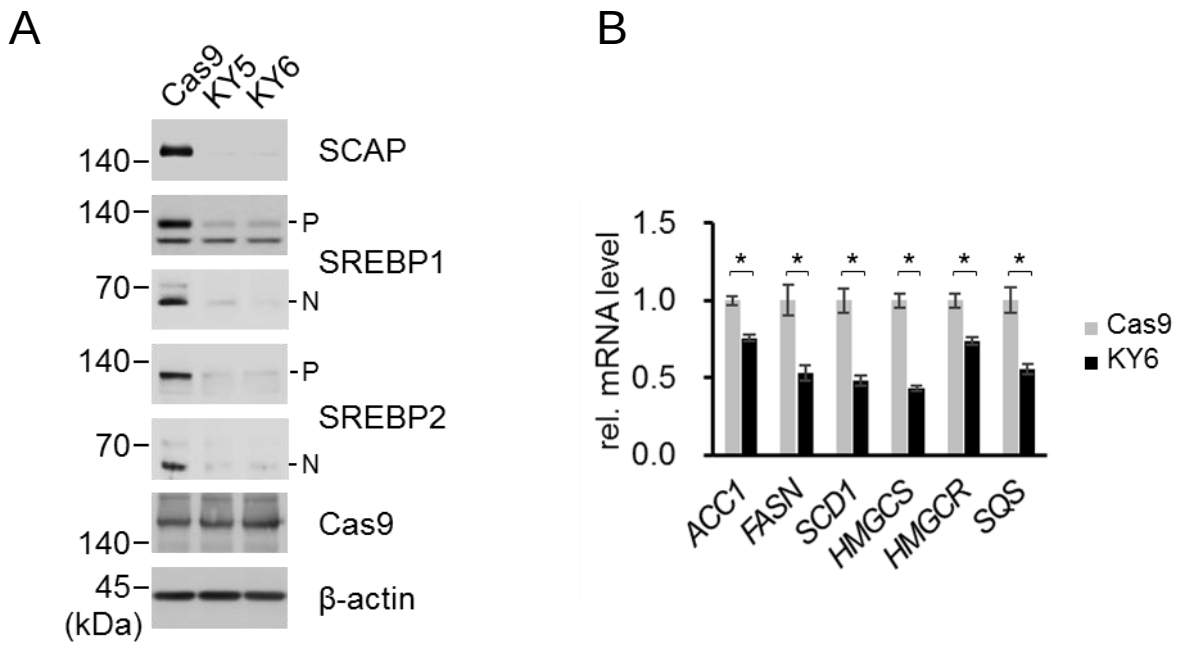


Figure S5. Establishing *SCAP*-deficient HEK293 cell lines. *A*, HEK293 cells were transfected with Cas9 expression plasmids in the presence or absence of a guide RNA targeting exon 2 of human *SCAP* Two lines of HEK293 cells were examined by immunoblot analysis and showed no detectable SCAP protein and drastic reduced level of SREBP proteins. These SCAP-deficient lines were named as KY5 and KY6. Another line derived from the cells transfected with Cas9 plasmid only was maintained as a control and named as Cas9.(26). ***B*,** Cas9 and KY6 cells were seeded in 12-well plates and cultured until cell density reached 90% confluent. The cells were then harvested for total RNA extraction followed by reverse transcription and quantitative PCR. Data are pooled from two independent experiments performed in triplicate and presented as mean ± S.D. (n = 6). Asterisks indicate difference between groups were significant as determined by two-tailed unpaired Student's t-tests ($p < 0.00005$).

Supplementary Figure S5

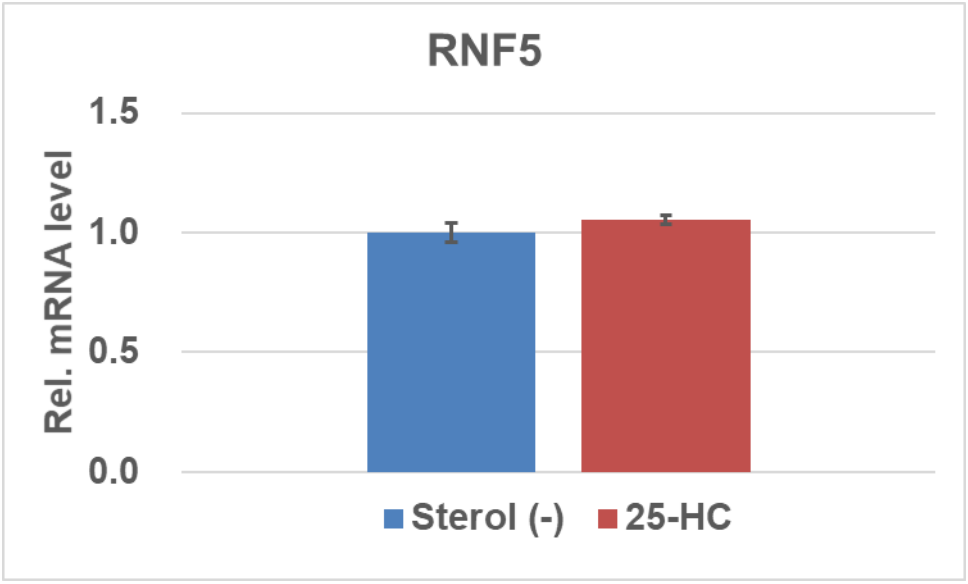


Figure S6. *RNF5* gene expression was not changed by 25-hydroxylcholesterol. HuH-7 cells were cultured until cell density reached to 70% confluent. The cells were then switched to DMEM supplemented with 5% LPDS, 50 μM sodium mevalonate, and 12.5 μM Fluvastatin in the presence of 1 μg/ml 25-hydroxylcholesterol or equal volume of ethanol. The cells were cultured for 16 h and then harvested for total RNA extraction followed by reverse transcription and quantitative PCR. Data are pooled from two independent experiments performed in triplicate and presented as mean ± S.D. (*n* = 6).