**SUPPLEMENTARY MATERIALS**

# COPI-mediated retrieval of SCAP is critical for regulating lipogenesis under basal and sterol-deficient conditions

**Kouhei Takashima[1], Akina Saitoh[1], Teruki Funabashi[1], Shohei Hirose[1], Chikako Yagi[1], Shohei Nozaki[1], Ryuichiro Sato[2], Hye-Won Shin[1,3], and Kazuhisa Nakayama[1]**

[1]Graduate School of Pharmaceutical Sciences, and [3]Career-Path Promotion Unit for Young Life Scientists, Kyoto University, Kyoto 606-8501, Japan; and [2]Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo, 113-8657, Japan

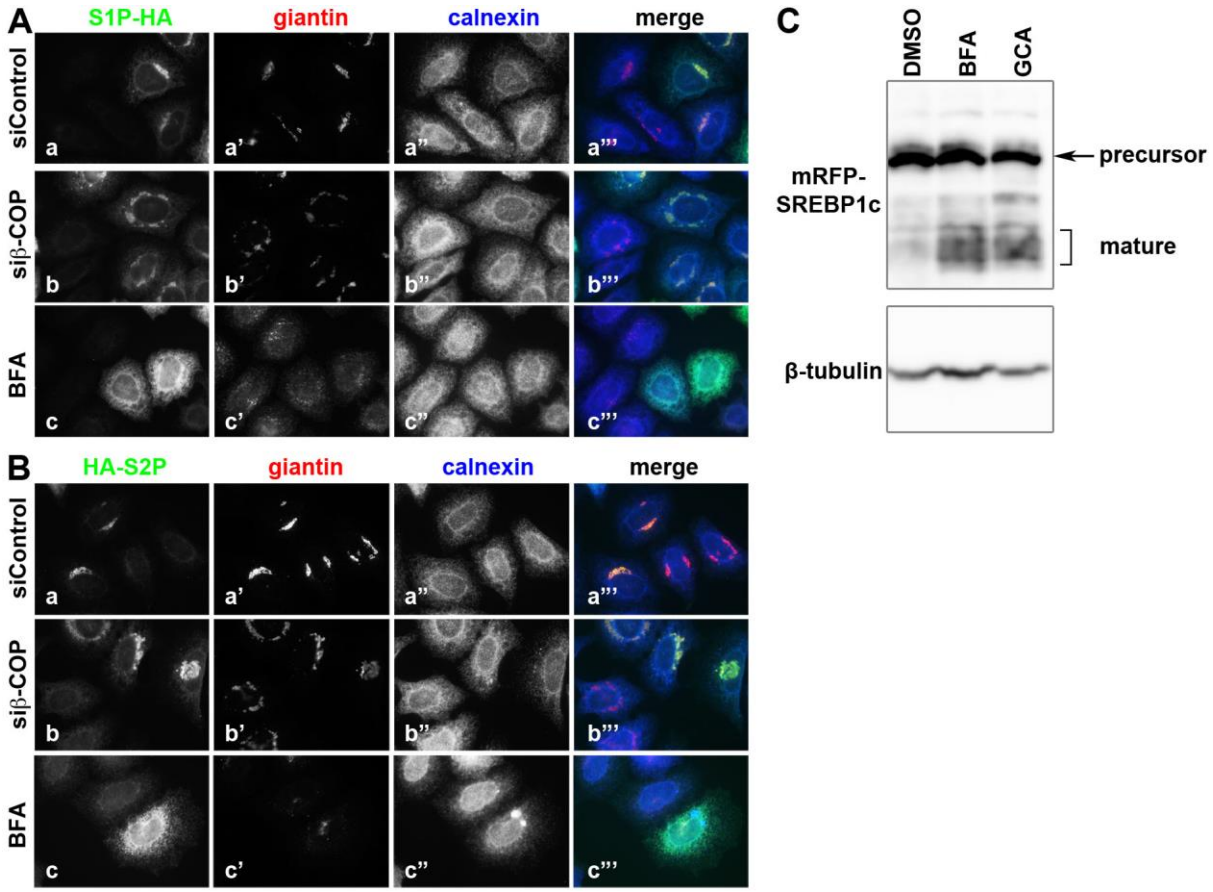



**Fig. S1.** ***Localization of S1P or S2P is not altered by a block in COPI-mediated trafficking***

(A and B) HeLa cells were transfected with an expression vector for C-terminally HA-tagged S1P (A) or N-terminally HA-tagged S2P (B), treated with control siRNAs or siRNAs for β-COP, and cultured in MEM supplemented with LPDS, OA, and 25-HC. In addition, cells not subjected to siRNA treatment were incubated in the presence of BFA. The cells were then subjected to triple staining with antibodies against HA, giantin (a Golgi marker), and calnexin (an ER marker). Bar = 10 µm. (C) Effects of brefeldin A and golgicide A on processing of SREBP1c. HeLa cells were triply infected with retroviruses for mRFP-SREBP1c, SCAP-HA and Insig2-FLAG, cultured in MEM supplemented with 10% LPDS, 1 µg/mL 25-HC, and 400 µM OA for 6 h, and mock-treated with DMSO or treated with 10 µM brefeldin A (BFA) or 30 µM golgicide A (GCA) for 6 h. Lysates prepared from the cells were processed for immunoblot analysis with antibody against RFP (upper) or β-tubulin (lower). The band for mature mRFP-SREBP1c was rather heterogeneous as reported for that of mature SREBP2 detected in cells treated with BFA (DeBose-Boyd et al., 1999).

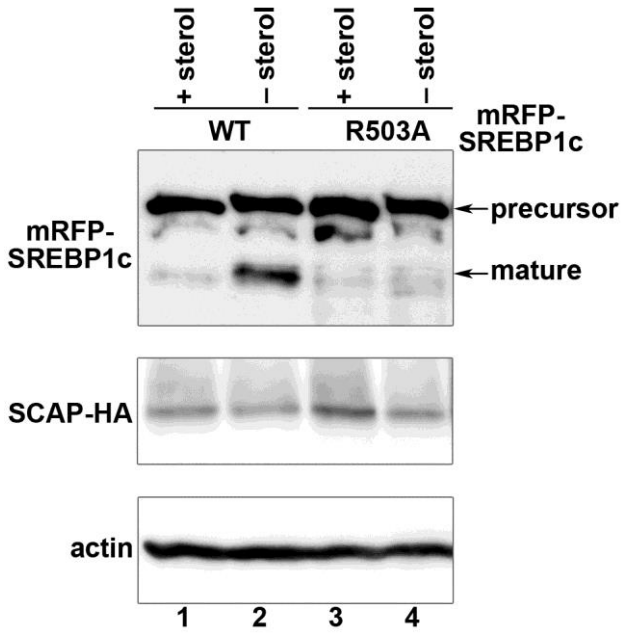



**Fig. S2.** ***Levels of the SREBP1c or SCAP protein are not different between cells expressing SREBP1c(WT) and SREBP1c(R503A)***

HeLa cells were triply infected with retroviruses for either mRFP-SREBP1c(WT) (lanes 1 and 2) or -SREBP1c(R503A) (lanes 3 and 4), SCAP-HA and Insig2-FLAG; cultured in MEM supplemented with 10% LPDS and 400 μM OA, with (lanes 1 and 3) or without (lanes 2 and 4) 1 μg/mL 25-HC for 13 h. After addition of CHX (a final concentration, 50 μg/mL), the cells were further incubated for 3 h and the cell lysates were processed for immunoblot analysis with antibody against RFP (top), HA (middle) or β-actin (bottom).

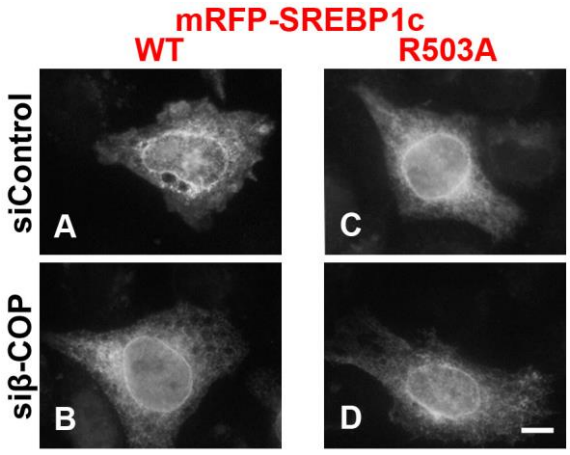



**Fig. S3.** ***Exogenously expressed SREBP cannot exit the ER***

HeLa cells were transfected with an expression vector for mRFP-SREBP1c(WT) (A and B) or -SREBP1c(R503A) (C and D) and control or β-COP siRNAs, cultured in MEM supplemented with 10% LPDS and 400 μM OA in the absence of 25-HC, and fixed for observation of mRFP fluorescence. Bar = 10 μm.

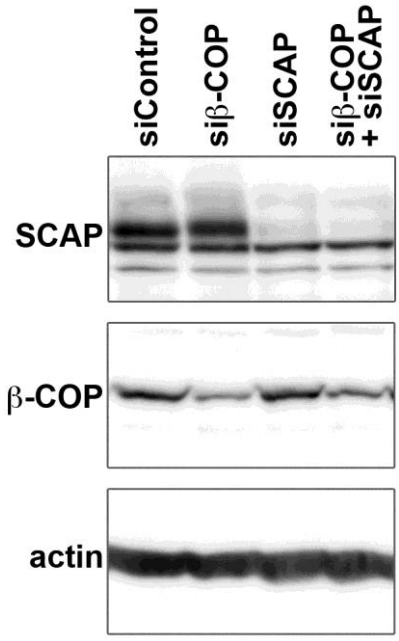
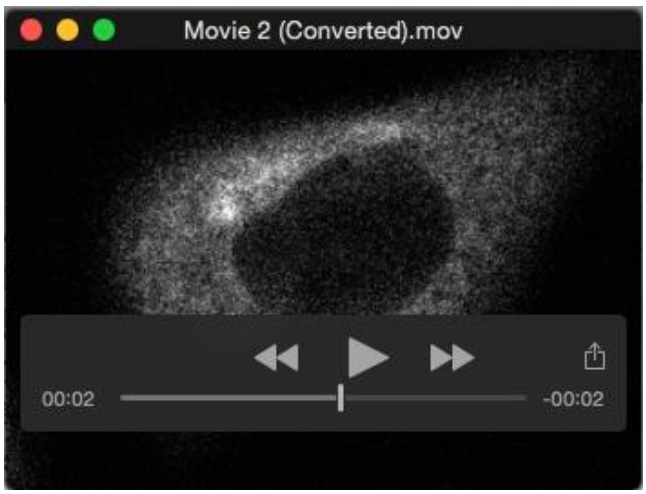



**Fig. S4.** ***Examination of knockdown efficiencies of β-COP and SCAP***

HeLa cells were treated with control siRNAs, siRNAs targeting β-COP or SCAP, or a combination of β-COP and SCAP siRNAs. Lysates prepared from the cells were subjected to immunoblot analysis for SCAP (top panel), β-COP (middle panel) or β-actin (bottom panel). In the SCAP blot, the lower two bands are likely to be non-specific, because the SCAP siRNA treatment did not affect the intensity of these bands.


**Movie 1. Retention of SCAP-EGFP at the Golgi in the continual presence of sterol**

Cells were triply infected with retroviruses for mRFP-SREBP1c, SCAP-EGFP and Insig2-FLAG, and cultured in MEM supplemented with 10% FBS. The cells were then incubated in MEM containing 10% LPDS, 1% HPCD and 50 µg/mL CHX for 90 min to deplete cellular sterols and inhibit *de novo* protein synthesis. After washing twice with MEM containing LPDS and CHX, the cells were incubated in the washing medium and subjected to time-lapse recording for 30 min using an A1-RMP confocal microscope. Images were acquired every 5 min.




**Movie 2. Retrieval of SCAP-EGFP from the Golgi to the ER upon sterol replenishment**

After washing as described in the legend for Video S1, the medium was changed to MEM containing FBS, 25-HC and CHX and the cells were subjected to time-lapse recording for 30 min. Images were acquired every 5 min.