**Regular Article**

**Sacl Phosphoinositide Phosphatase Regulates Foam Cell Formation by Modulating SR-A Expression in Macrophages**

Kiyomi Nigorikawa,*a Takuma Matsumura,a Hiromi Sakamoto,a Shin Morioka,a Satoshi Kofuji,a Shunsuke Takasuga,b and Kaoru Hazekib

a Graduate School of Biomedical & Health Sciences, Hiroshima University; 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8553, Japan; and b Department of Pathology and Immunology, Akita University School of Medicine; 1–1–1 Honjo, Akita 010–8543, Japan.

Received November 21, 2018; accepted March 11, 2019

Macrophages endocytose modified low-density lipoproteins (LDL) vigorously via scavenger receptor A (SR-A) to become foam cells. In the present study, we found that Sacl, a member of the Sac family of phosphoinositide phosphatases, increases the protein level of SR-A and upregulates foam cell formation. Mouse macrophages (RAW264.7) were transfected with short hairpin RNAs (shRNAs) against Sacl. Sacl knockdown decreased cell surface SR-A levels and impaired acetylated LDL-induced foam cell formation. Transfection of Sacl-knockdown cells with shRNA-resistant flag-Sacl effectively rescued the expression of SR-A. Glycosylation of SR-A was largely attenuated by Sacl knockdown, but neither mRNA expression nor protein degradation of SR-A were affected. These results suggest that Sacl maintains SR-A protein levels by modulating SR-A glycosylation.

**Key words** Sacl; phosphoinositide phosphatase; scavenger receptor A (SR-A); lipid droplet; foam cell formation; macrophage

**INTRODUCTION**

Mammalian Sacl is a phosphoinositide phosphatase and displays catalytic activity toward the D-4 and D-3 position of the inositol ring of phosphoinositides.1–3 Sacl localizes at the endoplasmic reticulum (ER) and Golgi apparatus and its physiological substrate is thought to be phosphatidylinositol 4-phosphate (PtdIns(4)P).1–3 In quiescent cells, Sacl associates with coat protein complex-II (COP-II) and accumulates in the Golgi to suppress secretion processes, whereas under standard growth conditions Sacl is associated with COP-I and is transported back to the ER, resulting in constitutive secretion.4 It has also been shown that Sacl partially colocalizes with the medial Golgi residents mannosidase II and N-acetylglucosamine transferase-I (GnT-I), and RNA interference (RNAi) knockdown of Sacl catalyzes mislocalization of these glycoprotein-processing enzymes.5 In the ER, Sacl catalyzes the dephosphorylation of PtdIns(4)P, which is exchanged for cholesterol by oxysterol-binding protein 1 to accelerate cholesterol transport from the ER to the trans-Golgi network (TGN).6,7

Macrophages endocytose modified low-density lipoproteins (LDLs) mainly through two scavenger receptors, scavenger receptor A (SR-A, also known as CD204 and Msr-I) and CD36.8 Endocytosed LDL is digested in the lysosome and the released free cholesterol is re-esterified in the ER to form lipid droplets.9 Accumulation of lipid droplets in the cytoplasm triggers formation of macrophage foam cells, which are a hallmark of early atherosclerotic lesions.

Because Sacl facilitates transport of cholesterol from the ER to the TGN, we investigated whether Sacl antagonizes foam cell formation in macrophages. For this purpose, we prepared RAW264.7 macrophages that displayed reduced Sacl expression. Unexpectedly, acetylated LDL (acLDL)-induced foam cell formation was attenuated in these cells, suggesting that Sacl upregulates the cell surface level of SR-A by modulating posttranslational processes.

**MATERIALS AND METHODS**

**Materials** acLDL and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-labeled acLDL (dil-acLDL) were obtained from Thermo Fisher Scientific (Waltham, MA, U.S.A.). Oil Red O was obtained from Waldeck (Munster, Germany). Brefeldin A (BFA) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Fluorescein-labeled Datura Stramonium lectin (DSL) was obtained from Vector Laboratories (Burlingame, CA, U.S.A.). Polyclonal antibodies against Sacl were purchased from Protein Tech (Chicago, IL, U.S.A.). The monoclonal antibody against SR-A (clone: 2F8) was obtained from Bio-Rad (Hercules, CA, U.S.A.). The monoclonal antibody against flag was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Monoclonal antibodies against CD11b, TLR2, and isotype controls were purchased from BioLegend (San Diego, CA, U.S.A.). Fluorescein isothiocyanate (FITC)-conjugated F (ab)2 fragment of goat anti-rat immunoglobulin G (IgG) was purchased from Santa Cruz Biotechnology (Dallas, TX, U.S.A.). DyLight 650-conjugated F (ab)2 fragments of donkey anti-rat IgG were purchased from Abcam (Cambridge, U.K.).

**Cells** RAW264.7 cells (ATCC) were cultured as adherent cells in RPMI 1640 medium (Nacalai Tesque, Kyoto, Japan) supplemented with 10% fetal bovine serum in a 37°C incubator in a humidified, 5% CO₂ atmosphere. Sacl-knockdown cells were produced by first cloning oligonucleotides targeting each gene into pH 1 vectors downstream of the H1 RNA promoter to express small interfering RNA (siRNA) hairpins, as previously described.10 The short hairpin RNA (shRNA)
sequences used were 5'-GAA ATG AGT TCT TTA GAA A-3' (Seq 1) and 5'-GTG GGA TGA TCA GAT A T-3' (Seq 2). Insert sequences were ascertained by DNA sequencing and the plasmids were transfected into RAW264.7 cells by electroporation at 250 V/950 µF (Gene Pulser II; Bio-Rad). Cells were treated with puromycin (3 µg/mL) 24 h after transfection, and further incubated to allow for selection of resistant cells. Control cells were produced as described above, by cloning a 400 bp stuffer sequence into the pH 1 vector instead of the target sequence.

**Plasmids** cDNA encoding the mouse Sac1 was synthesized from total RNA isolated from RAW264.7 cells and amplified by RT-PCR using primers containing further nucleotide sequences for subcloning. The cDNA construct was subcloned into the expression vector pcDNA3.1-hygro-flag. For the rescue experiments, silent mutations were introduced into the target sequence (seq 2) of Sac1 constructs (5'-cGGGeATG ATC AGA TAT AT-3') via PCR mutagenesis. The construct expressing myc-tagged SR-A has been previously described.13

**Foam Cell Formation** RAW264.7 cells were cultured with the indicated concentration of acLDL for 24 h then washed with phosphate buffered saline (PBS). Cells were fixed in 4% paraformaldehyde and stained with Oil Red O. Lipid droplet formation was quantified by measuring the area stained by the Oil Red O stained area.

**Dil-acLDL Uptake** RAW264.7 cells were cultured with the indicated concentration of acLDL, which consists of 25% dil-labeled and 75% non-labeled acLDL, for 6 h at 37°C. Cells were scraped and washed with PBS containing 0.5% bovine serum albumin (PBS/BSA buffer). Fluorescence signals of internalized dil-acLDL were analyzed by flow cytometry using the Guava easyCyte system (Merck Millipore, Darmstadt, Germany).

**Cell Surface Expression of Receptors and Glycoproteins** RAW264.7 cells were fixed in 4% paraformaldehyde and washed and resuspended in PBS/BSA buffer. Cells were then exposed to anti-SR-A, anti-CD11b, anti-TLR2, or each isotype control antibody for 1 h, followed by incubation with a FITC-labeled secondary antibody for 30 min. Mean fluorescence intensities obtained with the respective isotype controls were subtracted as the backgrounds. For analysis of DSL binding, fixed cells were incubated with fluorescein-DSL (10 µg/mL) for 1 h. After washing with PBS/BSA buffer, the fluorescence signals were analyzed by flow cytometry. For microscopic analysis, cells in glass-bottom dishes were fixed with PBS containing 4% paraformaldehyde and washed with PBS/BSA buffer. Cells were then exposed to anti-SR-A, anti-CD11b, anti-TLR2, or each isotype control antibody for 1 h, followed by incubation with a FITC-labeled secondary antibody for 30 min or with DSL for 1 h. After washing with PBS/BSA buffer, cells were stained with 4',6-diamidino-2-phenylindole (DAPI) for 20 min. Microscopic analysis was performed using a Keyence BZ-9000 equipped with a CFI Plan Apo VC60xH lens (Keyence, Osaka, Japan).

**Transfection** Plasmids were transfected using the Neon transfection system (Life Technologies, Carlsbad, CA, U.S.A.) according to the manufacturer’s instructions. Briefly, RAW264.7 cells (10⁶ cells) were suspended in 100 µL of T buffer and mixed with 5 µg of plasmid DNA. The suspended cells were then transferred onto a gold tip and electroporated by a single pulse at 1800 V for 20 ms. Electroporated cells were cultured in growth media without antibiotics for 16 h.

**Western Blotting** RAW264.7 cells were cultured in the presence or absence of indicated inhibitors. After washing with PBS, cells were lysed in lysis buffer consisting of 25 mM Tris–HCl (pH 7.6), 100 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1% Nonidet P-40, 30 mM sodium fluoride, 1 mM sodium orthovanadate, 200 µM phenylmethylsulfonyl fluoride, protease inhibitor cocktail (Sigma-Aldrich). After centrifugation (20000 × g for 10 min), aliquots of supernatant were mixed with 5 × sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (310 mM Tris (pH 6.8), 10% SDS, 38% glycerol, 600 mM dithiothreitol, and 0.03% bromophenol blue) and heated for 5 min. For detection of deglycosylated SR-A, aliquots of the supernatant were treated with PNGase F (New England Biolabs, Hertfordshire, U.K.) according to the manufacturer’s instructions before they were mixed with the 5 × SDS-PAGE sample buffer. Proteins were separated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Millipore, Bedford, MA, U.S.A.). After blocking, the membranes were incubated with the indicated antibody, washed, and then incubated with a horseradish peroxidase-conjugated secondary antibody. Antibody binding was detected using an enhanced chemiluminescence detection system (Perkin-Elmer, Waltham, MA, U.S.A.).

**RNA Isolation and Real-Time PCR** Total RNA was isolated with Sepasol-RNA I Super G (Nacalai Tesque, Kyoto, Japan) according to manufacturer’s instructions. First-strand cDNA was synthesized by reverse transcription using M-MLV reverse transcriptase (Promega, Madison, WI, U.S.A.) and random primers (TaKaRa Bio, Shiga, Japan). Quantitative PCR (qPCR) analysis of the resulting cDNA was performed using a FastStart Universal SYBR Green Master kit (Roche, Mannheim, Germany). qPCR was performed on a PikoReal Real-Time PCR System (Thermo Fisher Scientific) with the primers described previously.11 The data were normalized against β-actin, and the relative quantification of mRNA expression levels were calculated using the ΔΔCt method.

**Statistical Analysis** Statistical significance was assessed using the 2-tailed student’s t-test. Data indicated with one asterisk or 2 asterisks have values of *p < 0.05 or **p < 0.01, respectively.

**RESULTS**

To investigate whether Sac1 antagonizes foam cell formation in macrophages, we prepared RAW264.7 macrophages expressing shRNA against Sac1. Transfection with shRNAs against Sac1 (seq1 or seq2) effectively lowered the target protein levels (Fig. 1A), allowing for assessment of the effects of Sac1 knockdown on acLDL-induced foam cell formation in transfected RAW264.7 macrophages. We predicted that transfection with these shRNAs would enhance foam cell formation, since Sac1 facilitates cholesterol transport from the ER to TGN; however, our results largely showed that these shRNAs inhibited acLDL-induced formation of lipid droplets (Figs. 1B, C). To explore the mechanisms underlying this unexpected result, we compared the amount of internalized dil-labeled acLDL between shRNA control and Sac1-knockdown cells and found that the internalized dil-acLDL was significantly lower in Sac1-knockdown cells (Fig. 1D). This result indicates
a possibility that the levels of acLDL receptors are lowered in Sac1-knockdown cells.

Macrophages are reported to uptake modified LDLs mainly through two scavenger receptors, SR-A and CD36. We have previously reported that RAW264.7 cells display undetectable levels of CD36, therefore we attempted to analyze the cell surface expression of SR-A in this study. SR-A cell surface expression was significantly reduced in Sac1-knockdown cells (Figs. 2A, B and Fig. S1A). Mouse SR-A is reported to be a complex-type \(N\)-glycan glycosylated at five asparagine (Asn) residues. When the lysate was subjected to deglycosylation with PNGase F, the molecular mass of the SR-A protein is reduced from 80 to less than 46 kDa (Fig. 2C). In addition to less cell surface SR-A, levels of fully glycosylated, mature SR-A protein (ca. 80 kDa) in the whole cell lysate were lower in Sac1-knockdown cells (Figs. 2C, D). This effect of Sac1-knockdown was also observed after acLDL treatment (Fig. S2). The deglycosylated SR-A protein (less than 46kDa) level was also decreased by Sac1 knockdown, but the difference in deglycosylated SR-A protein between control and knockdown cells (seq1) was less than that in mature protein level (Figs. 2C, D). The glycosylated bands around 80 kDa in the absence of PNGase F was a cluster of multiple lines regardless of normal or shSac1 cells, suggesting that SR-A may exist with various glycosylation states. It is likely that seq1 cells include immature forms of SR-A, which have various molecular weights and are hardly detectable with the antibody used. Next, a rescue assay was carried out to confirm the results from the knockdown experiments. For this purpose, we examined the effects of shRNA-resistant cDNA encoding Sac1 on the expression of co-transfected myc-SR-A. Similar to the expression of endogenous SR-A, myc-SR-A expression was lower in Sac1-knockdown cells than in shRNA control cells (Figs. 2E, F). Transfection of Sac1-knockdown cells with shRNA-resistant flag-Sac1 effectively increased the myc-SR-A level (Figs. 2E, F). Flag-Sac1 exhibited a similar effect on myc-SR-A levels in control cells (Figs. 2E, F).

Sac1 knockdown did not change the mRNA levels of SR-A (Fig. 3A), indicating that the observed decrease in SR-A protein levels was not due to suppression of transcription. Brefeldin A (BFA) is a potent inhibitor of membrane traffic from the ER to the Golgi complex that attenuates the synthesis of mature SR-A. Treatment with BFA facilitated monitoring of degradation of preexisting mature SR-A in BFA-treated cells. The degradation rates of mature SR-A were comparable between the control and knockdown cells (Figs. 3B, C). We also monitored BFA-induced accumulation of immature SR-A (ca. 60 kDa), which is thought to be decorated with high-mannose N-glycan, finding less accumulation of immature SR-A induced by BFA treatment in Sac1-knockdown cells (Figs. 3D, E). Together, these results suggest that Sac1 regulates early glycosylation processes and/or translation of SR-A.

It has been reported that human Sac1 regulates glycosylation and cell surface expression of \(N\)-glycans. We examined...
Fig. 2. Knockdown of Sac1 by shRNA Decreased SR-A Protein Level

(A) Cells were fixed and immunostained with anti-SR-A antibody (or isotype control) and FITC-labeled secondary antibodies. The mean fluorescence intensities on cell surface were analyzed by flow cytometry. Data are shown as the means ± S.E.M. (n = 3). (B) Cells were fixed and immunostained with anti-SR-A antibody and DyLight 650-labeled secondary antibody and counterstained with 4',6-diamidino-2-phenylindole (DAPI). Exemplary images from three independent experiments are shown. Scale bar = 10 µm. (C, D) Cells lysates were treated with PNGase F (+) or vehicle (−) and subjected to Western blot analysis. (C) Exemplary blots from three independent experiments are shown. (D) The protein expression levels quantified from (C) were normalized against actin, and values relative to those of control cells are shown as the means ± S.E.M. (n = 3). (E, F) Cells were transiently transfected with empty vector (pcDNA3) or shRNA-resistant flag-Sac1 (flag-Sac1'') expression vectors along with myc-SR-A. (E) Exemplary blots from three independent experiments are shown. (F) The protein expression levels of myc-SR-A quantified from (E) were normalized against actin, and values relative to those of control cells are shown as the means ± S.E.M. (n = 3).

Fig. 3. SR-A mRNA Level and Protein Degradation Were Not Altered, but Early Glycosylation Processes Were Impaired in Sac1-Knockdown Cells

(A) Total RNA prepared from the cells was subjected to real-time PCR analysis. The mRNA levels were normalized against actin, and values relative to those of control cells are shown as the means ± S.E.M (n = 3). (B, C) Cells were treated with 300 ng/mL BFA for the indicated times. The cell lysates were subjected to Western blotting analysis. (B) Exemplary blots from three independent experiments are shown. (C) The mature 80 kDa SR-A protein levels were normalized against actin, and values relative to those of control cells are shown as the means ± S.E.M. (n = 3). (D, E) Cells were treated with 300 ng/mL BFA (+) or vehicle (−) for 6 h and subjected to Western blot analysis. (D) Exemplary blots from three independent experiments are shown. (E) The immature 60 kDa SR-A protein levels were normalized against actin, and values relative to those of control cells are shown as the means ± S.E.M. (n = 3).
whether Sac1 knockdown influenced cell surface expression of N-glycans other than SR-A in RAW264.7 macrophages. Consistent with a previous report,\textsuperscript{11} binding of Datura stramonium lectin (DSL), which prefers N-acetyllactosamine repeats, on the cell surface was significantly lowered in Sac1-knockdown cells (Figs. 4A, B). On the other hand, cell surface levels of two N-glycans, CD11b and Toll-like receptor 2 (TLR2) were not largely lowered in Sac1-knockdown cells (Figs. 4C and Fig. S1B). Furthermore, protein levels of TLR2 in the whole cell lysate were not affected by knockdown of Sac1 (Fig. S3). These results imply that Sac1 influences the protein level of a certain type of N-glycans.

**DISCUSSION**

Macrophages uptake modified LDLs via scavenger receptors and degrade them in the lysosome. The liberated free cholesterol is esterified in the ER to form lipid droplets or trafficked to TGN to be released from the cell.\textsuperscript{16} Since Sac1 promotes cholesterol transport from the ER to TGN,\textsuperscript{6} we expected that knockdown of Sac1 would induce cholesterol accumulation in the ER, thereby increasing the accumulation of lipid droplets in macrophages. However, acLDL-induced lipid droplet formation was impaired by Sac1 knockdown in RAW264.7 macrophages (Figs. IB, C).

In the present study, we found that repression of Sac1 expression resulted in a decreased level of the acLDL receptor, SR-A (Figs. 2C, D). Furthermore, the expression of exogenous myc-SR-A also decreased in Sac1-knockdown cells, a phenotype that could be rescued by treatment with shRNA-resistant flag-Sac1 (Figs. 2E, F). In Sac1-knockdown cells, both the mRNA levels of SR-A and degradation rate of mature SR-A were comparable with those of control cells (Figs. 3A–C). Therefore, Sac1 is thought to regulate translation and/or post-translational processes.

Mouse SR-A is a complex-type N-glycan.\textsuperscript{12} A previous report has shown that Sac1 knockdown in HeLa cells results in lowered expression of cell surface N-glycans.\textsuperscript{5} This effect was due to the mislocalization of Golgi enzymes responsible for glycan processing, such as GnT-I.\textsuperscript{5} However, green fluorescent protein-tagged GnT-I localized at the perinuclear region neighboring endogenous TGN38, a TGN marker protein, in both shRNA control and Sac1-knockdown RAW264.7 cells (data not shown). It has been reported that BFA inhibits ADP-Ribosylation Factor-Guanine Nucleotide Exchange Factors to abrogate membrane traffic from the ER to Golgi, resulting in the formation of BFA compartments.\textsuperscript{14–16} The BFA compartments contain residents of both ER and Golgi, including early oligosaccharide-processing enzymes.\textsuperscript{14} In BFA-treated cells, the early glycosylation processes occurred, but the late processes and glycoprotein secretion were inhibited.\textsuperscript{14,17} In the present study, we detected immature, low-molecular weight SR-A in BFA-treated cells, the levels of which were decreased following Sac1 knockdown (Figs. 3D, E). This observation indicates that the activities of early oligosaccharide-processing enzymes were impaired in Sac1-knockdown cells. Thus, we speculate that Sac1 maintains proper glycosylation in macrophages.

We previously reported that another Sac family phosphatase, Sac3, also functions in maintaining SR-A protein levels.\textsuperscript{11} Sac3 plays a role in PtdIns(3,5)P\textsubscript{2} turnover in the endosomes, and has been suggested to have catalytic activity towards PtdIns(4)P.\textsuperscript{18,19} Furthermore, we previously reported that Sac3 colocalizes with an ER resident protein, calnexin.\textsuperscript{11} Thus, ER-localized PtdIns(4)P phosphatases (Sac1 and Sac3) possibly play key roles in maintaining SR-A protein levels.

Previous studies showed that CD11b and TLR2, N-glycans on the cell surface of macrophages, were expressed even when cells were treated with N-glycosylation inhibitor, tunicamycin.\textsuperscript{20,21} By contrast, cell surface and total level of SR-A were decreased in tunicamycin-treated RAW264.7 cells (11). These results imply that non-glycosylated SR-A is labile. Thus, we conjecture that early glycosylation of SR-A is attenuated in Sac1-knockdown cells, resulting in its degradation. At present, we cannot exclude the possibility that translational regulation specific for SR-A is present and controlled by Sac1. Further investigations are required to uncover the mechanism of the decreased expression of certain types of N-glycans in Sac1-deficient macrophages.

**Acknowledgments** SK was supported, in part, by Home for Innovative Researchers and Academic Knowledge Users (HIRAKU).

**Conflict of Interest** The authors declare no conflict of interest.
interest.

**Supplementary Materials** The online version of this article contains supplementary materials.

**REFERENCES**

1. Nemoto Y, Kearns BG, Wenk MR, Chen H, Mori K, Alb JG Jr, De Camilli P, Bankaitis VA. Functional characterization of a mammalian Sac1 and mutants exhibiting substrate-specific defects in phosphoinositide phosphatase activity. *J. Biol. Chem.*, 275, 34293–34305 (2000).
2. Rohde HM, Cheong FY, Konrad G, Paiha K, Mayinger P, Boehmelt G. The human phosphatidylinositol phosphatase SAC1 interacts with the coatomer I complex. *J. Biol. Chem.*, 278, 52689–52699 (2003).
3. Liu, Boukhelifa M, Tribble E, Morin-Kensicki E, Uetrecht A, Bear JE, Bankaitis VA. The Sac1 phosphoinositide phosphatase regulates Golgi membrane morphology and mitotic spindle organization in mammals. *Mol. Biol. Cell.*, 19, 3080–3096 (2008).
4. Blagoveshchenskaya A, Cheong FY, Rohde HM, Glover G, Knödler A, Nicolson T, Boehmelt G, Mayinger P. Integration of Golgi trafficking and growth factor signaling by the lipid phosphatase SAC1. *J. Cell Biol.*, 180, 803–812 (2008).
5. Cheong FY, Sharma V, Blagoveshchenskaya A, Oorschot VM, Brankatschk B, Klumperman J, Freeze HH, Mayinger P. Spatial regulation of Golgi phosphatidylinositol-4-phosphate is required for enzyme localization and glycosylation fidelity. *Traffic*, 11, 1180–1190 (2010).
6. Mesmin B, Bigay J, Moser von Filseck J, Lacas-Gervais S, Drin G, Antony B. A four-step cycle driven by Ptd(4)P hydrolysis directs sterol/Ptd(4)P exchange by the ER-Golgi tether OSBP. *Cell.*, 155, 830–843 (2013).
7. Zewe JP, Willis RC, Sangappa S, Goulden BD, Hammond GR. SAC1 degrades its lipid substrate PtdIns4. *PLoSLife*, 7, e55588 (2018).
8. Kunjathoor VV, Febbraio M, Podrez EA, Moore KJ, Andersson L, Koehn S, Rhee JS, Silverstein R, Hoff HF, Freeman MW. Scavenger receptors class A-I/II and CD36 are the principal receptors responsible for the uptake of modified low density lipoprotein leading to lipid loading in macrophages. *J. Biol. Chem.*, 277, 49982–49988 (2002).
9. Maxfield FR, Tabas I. Role of cholesterol and lipid organization in disease. *Nature*, 438, 612–621 (2005).
10. Hazeki K, Nigerikawa K, Takaba Y, Segawa T, Nukuda A, Masuda A, Ishikawa Y, Kobata K, Takasuga S, Hazeki O. Essential roles of PIKfyve and PTEN on phagosomal phosphatidylinositol 3-phosphate dynamics. *FEBS Lett.*, 586, 4010–4015 (2012).
11. Moriooka S, Nigerikawa K, Hazeki K, Ohnura M, Sakamoto H, Matsumura T, Takasuga S, Hazeki O. Phosphoinositide phosphatase Sac3 regulates the cell surface expression of scavenger receptor A and formation of lipid droplets in macrophages. *Exp. Cell Res.*, 357, 252–259 (2017).
12. Tian G, Wilcockson D, Perry VH, Rudd PM, Dwek RA, Platt FM, Platt N. Inhibition of alpha-glucosidases I and II increases the cell surface expression of functional class A macrophage scavenger receptor (SR-A) by extending its half-life. *J. Biol. Chem.*, 279, 39303–39309 (2004).
13. Penman M, Lux A, Freedman NJ, Rohrer L, Ekkel Y, McKinstry H, Resnick D, Krieger M. The type I and type II bovine scavenger receptors expressed in Chinese hamster ovary cells are trimeric proteins with collagenous triple helical domains comprising noncovalently associated monomers and Cys83-disulfide-linked dimers. *J. Biol. Chem.*, 266, 23985–23993 (1991).
14. Sampa D, Varki A, Freeze HH. The spectrum of incomplete N-linked oligosaccharides synthesized by endothelial cells in the presence of brefeldin A. *J. Biol. Chem.*, 267, 4440–4455 (1992).
15. Donaldson JG, Finazzi D, Klausner RD. Brefeldin A inhibits Golgi membrane-catalysed exchange of guanine nucleotide onto ARF protein. *Nature*, 360, 350–352 (1992).
16. Roth MG. Lipid regulators of membrane traffic through the Golgi complex. *Trends Cell Biol.*, 9, 174–179 (1999).
17. Misumi Y, Misumi Y, Miki K, Takatsuki A, Tamura G, Ikehara Y. Novel blockade by brefeldin A of intracellular transport of secretory proteins in cultured rat hepatocytes. *J. Biol. Chem.*, 261, 11398–11403 (1986).
18. Yuan Y, Gao X, Guo N, Zhang H, Xie Z, Jin M, Li B, Yu L, Jing N. rSac3, a novel Sac domain phosphoinositide phosphatase, promotes neurite outgrowth in PC12 cells. *Cell Res.*, 17, 919–932 (2007).
19. Balla T. Phosphoinositides: tiny lipids with giant impact on cell regulation. *Physiol. Rev.*, 93, 1019–1137 (2013).
20. Kataoka H, Yasuda M, Miyata M, Miyata K, Murata K, Nakata T, Shibata K. Roles of N-linked glycans in the recognition of microbial lipopeptides and lipoproteins by TLR2. *Cell. Microbiol.*, 8, 1199–1209 (2006).
21. Morova J, Osicka R, Masin J, Sebo P. RTX cytotoxins recognize beta2 integrin receptors through N-linked oligosaccharides. *Proc. Natl. Acad. Sci. U.S.A.*, 105, 5355–5360 (2008).