SUPPLEMENTAL INFORMATION

RESULTS

Filipin staining of intact fixed cells. We performed intact cell staining with filipin, a naturally fluorescent small molecule that binds to cholesterol. After filipin staining, we viewed cells by using a spinning disc confocal fluorescence microscopy. The experiment was conducted as described in Method section. The results show that in WT and A1\(^+\) cells (Fig S1; 1\(^{st}\) and 2\(^{nd}\) columns), the cholesterol-rich domains, as represented by areas with strong filipin staining (1\(^{st}\) row), were mostly located in peripheral regions, away from the nucleus, whereas in mutant Npc1\(^{nmf}\) cells (Fig S1; 3\(^{rd}\) column), the cholesterol-rich domains (mostly LE/LYS; see Fig 4A) were mostly peri-nuclear located. When compared to WT cells, A1\(^+\) cells contained more cholesterol-rich domains that scattered throughout the cytoplasm (Fig S1; the 2\(^{nd}\) column), suggesting that A1\(^+\) causes significant alterations in cholesterol distribution in internal membrane organelle(s) in cells. In mutant Npc1\(^{nmf};A1\(^+\) cells, the cholesterol-rich particles exhibit a more complex distribution pattern (Fig S1; the 4\(^{th}\) column). We quantitated the total fluorescence intensity per cell and found that among these four cell types, the values were comparable (Fig S1, Bottom panel).

Test the pharmacological inhibition of ACAT1 in human cells with NPC mutations.

To test whether the effect of A1\(^+\) on increasing the ABCA1 protein levels in mutant NPC MEFs (Fig 8A) can also be observed in mutant NPC Hfs, we treated Hfs with a small-molecule ACAT inhibitor, K604. This inhibitor, specific for the isoenzyme ACAT1 but not ACAT2 (63) has been clinically tested as safe for anti-atherosclerosis purposes. Therefore, we treated Hfs with K604 at 0.5 µM for 24 h, a concentration at which K604 inhibits ACAT1 by 70-80 % without significantly inhibiting the ACAT2 isozyme. We found that in mutant NPC1 Hf cell line GM03123, K604 treatment caused a significant increase in ABCA1 protein content (Fig S2). Line GM03123 contains a mutant allele, I1061T, that occurs frequently among NPC1 patients of Western European descent (64). In the other four tested Hf cell lines, one healthy Hf (GM05659), three with mutant NPC1 (GM18453, NIH94.71, NIH2A), one with mutant NPA (GM00112), and one with mutant NPC2 (GM1910). K604 treatment tended to increase ABCA1 protein content, but not statistically significance (Fig S2). The results of single dose, single time point experiments show that the enhancing effect of A1B on ABCA1 protein content can be recapitulated in at least one of the four mutant NPC1 Hf lines we examined, with weaker findings for other Hf cell lines.
lacking mutant NPA and NPC2, supporting the rationales to use ACAT1 inhibitor to treat NPCD. The significance of these findings requires further investigation.

**Gene expression levels of Abca1, Abcg1, Cathepsin B, and Cathepsin D in MEFs.**

ABCG1 is a protein closely related to ABCA1 in protein sequence; it also functions as an important cellular lipid efflux protein especially in neurons and astrocytes (1, 2). Cathepsin B is a lysosomal cystine protease, different from cathepsin D, that encodes an aspartyl protease. We monitored the gene expression levels of Abca1, Abcg1, cathepsin B, and cathepsin D in MEFs of four genotypes. The results (Fig S3, top left panel) show that Npc1

| nmf |
|-----|
| A1-/ |

| cells | mRNA than that of WT cells, but the difference did not reach statistical significance. The lower mRNA expression of Abca1 was restored in the Npc1

| nmf |
|-----|
| A1-/ |

| cells. This result suggests that the beneficial effects of A1-/- in restoring the ABCA1 protein content observed in Fig 8A, is a combination of increasing ABCA1 protein expression at the post-translational level and increasing Abca1 gene expression. Mutant NPC cells produce less oxysterols than the normal cells (3). Oxysterols bind to LXRs (4) (5) to control the transcriptions of multiple genes in sterol homeostasis, including ABCA1. It is possible that A1-/- causes mutant NPC1 cells to produce more oxysterol(s) to increase the transcription of Abca1. For Abcg1 (Fig S3, top right panel), no difference in gene expression can be found among these four MEF cells. For cathepsin D (Fig S3, bottom right panel), the mRNA expression level in mutant Npc1

| nmf |
|-----|
| MEFs is 20% less than that of WT MEFs. This difference cannot account for the lower cathepsin D protein content level (64% lower than that of WT MEFs) observed in the Npc1

| nmf |
|-----|
| MEFs (Fig 7B); it also cannot account for the robust increase (by 3.9-fold) in cathepsin D protein content observed in the Npc1

| nmf |
|-----|
| A1-/- MEFs (Fig 7B). For cathepsin B gene expression (Fig S3, bottom left panel), compare to WT MEF, A1-/- caused a moderate increase (by 2-fold), Npc1

| nmf |
|-----|
| mutation caused 47% decrease. While A1-/- in mutant Npc1

| nmf |
|-----|
| cells caused a relatively 7.4-fold increase. The significance of these results is not clear at present. Overall, these results indicate that in mutant Npc1

| nmf |
|-----|
| cells, A1-/- increases the ABCA1 protein content through both transcriptional and posttranslational mechanisms, while it increases the cathepsin D protein content mainly through a posttranslational mechanism. The RT-PCR experiment was conducted as described in Methods.

The ability of mutant Npc1

| nmf |
|-----|
| MEF cells to degrade long-lived proteins is not impaired. We asked if the diminished cathepsin D protein content observed in Npc1

| nmf |
|-----|
| cells may affect these cells’ ability to degrade long-lived proteins. To address this issue, we
monitored the degradation of long-lived proteins by using the protocol described by Auteri et al. (6). The result (Fig S4) shows the bulk turnover of long-lived proteins in WT, A1<sup>−/−</sup>, Npc<sub>1</sub><sup>nmf</sup>, and Npc<sub>1</sub><sup>nmf :A1<sup>−/−</sup></sup> MEF cells were comparable. This finding is consistent with the work of Pacheco et al. (7), who showed that in healthy and NPC1-deficient human fibroblast cells, the degradation of long-lived proteins was comparable. The experiment was conducted as described in Method section. The result was reported as relative proteolysis (%).
MATERIALS AND METHODS

MATERIALS

Fetal bovine serum was purchased from Sigma. Iron-supplemented calf serum was from Atlanta Biologicals. OptiPrep was from Axis-Shield. $^3$H-labeled oleate and $^3$H leucine were from PerkinElmer. Filipin was purchased from Sigma. 27-alkyne cholesterol was purchased from Click Chemistry Tools. Alex 594/488-azide and Lipofectamine 3000 were from Invitrogen. FuGENE 4K was purchased from FuGENE. All other chemicals, of analytical grade, were from Sigma-Aldrich or Fisher.

Animal use and maintenance. Mice were fed *ad libitum* with standard chow diet, maintained in a pathogen-free environment in single-ventilated cages, and kept on a 12 h light/dark schedule, using Dartmouth Animal Research Center Institutional Animal Care and Use Committee–approved protocol number 00002125. Animals were checked regularly for their entire lifespan. When *Npc1*<sup>nmf</sup> mice began to have trouble reaching food, wet food pellets were placed on the bottom of their cage for the remainder of their life. Death is marked as the point where the mice could no longer ingest food or water.

Mouse breeding. The heterozygous mutant NPC1 mouse in C57BL/6 background (*Npc1*<sup>nmf/wt</sup>:*Acat1*<sup>+/+</sup> mouse; from Jackson laboratories) was crossed with the *Acat1*<sup>−/−</sup> mouse (the global ACAT1 KO mouse) received from Dr. Sergio Fazio in C57BL/6 background to produce the *Npc1*<sup>nmf/wt</sup>:*Acat1*<sup>−/−</sup> mice and the *Npc1*<sup>nmf/wt</sup>:*Acat1*<sup>+/+</sup> mice. After two rounds of breeding, the resultant *Npc1*<sup>nmf/wt</sup>:*Acat1*<sup>−/−</sup> mice were set up as breeding pairs to generate the *Npc1*<sup>nmf/nmf</sup>:*Acat1*<sup>−/−</sup> mice (Designated as the *A1*<sup>−/−</sup> mice). The *Npc1*<sup>nmf/wt</sup>:*A1*<sup>−/−</sup> mice were set up as breeding pairs to generate the *Npc1*<sup>nmf/nmf</sup>:*A1*<sup>+/+</sup> mice (Designated as the WT mice).

*Npc1*<sup>nmf</sup> mouse genotyping. We followed the protocol described by (8), with minor modification: 10 µL of reaction buffer that contains 10 ng of mouse-tail genomic DNA, 1x TaqMan genotyping master mix (#4371353, from Life Technologies), and 1x SNP Custom TaqMan SNP assay mixture (Assay ID: AHGJW83, from ThermoFisher). The reaction was carried out by amplifying at 95 °C for 5 min, followed by 45 cycles of 92 °C for 15 s, and 60 °C for 1 min.
**Npc1<sup>nih</sup> mouse genotyping.** PCR conditions: 94 °C for 1.5 min, followed by 35 rounds of: 94 °C for 30 s, 62 °C for 60 s, 72 °C for 60 s. Lastly, 72 °C for 2 min.

**WT:**
- Forward primer: 5'-'GGTGTTCACATGGTGCA\text{GAGACAGATAC}-3'
- Reverse primer: 5'-'GACTTTTCAATGAGTTGTCACA-3'
- PCR product: 445 bp

**Mutant:**
- Forward primer: 5'-GGTGTTCACATGGTGCA\text{GAGACAGATAC}-3'
- Reverse primer: 5'-AGGATCTCCTGTCAT\text{TC ACC TTG CTC CTG}
- PCR product: 1052 bp

**Human Fibroblasts.**
- Healthy HF: **GM05659:** 1 year-old male, from Coriell Institute
- NPC1 HF: **GM03123:** A 9 years-old female, I1061T/P237S, from Coriell Institute
- NPC1 HF: **GM18453:** A male, I1061T/I1061T, from Coriell Institute
- NPC1 HF: **NIH 94.71:** provided by Dr. P. Pentchev
- NPC1 HF: **NIH 2A:** provided by Dr. P. Pentchev
- NPA HF: **GM00112:** a 10-month-old male, L302P/L302P, from Coriell Institute
- NPC2 HF: **GM17910:** A male, C93F/R1266Q, from Coriell Institute

| Reagent type or resource | Designation | Source or reference | Identifiers | Additional information |
|--------------------------|-------------|---------------------|-------------|------------------------|
| 2° Antibody              | Alexa fluor 568 | Molecular Probe     |             | 1:500                  |
| 2° Antibody              | Alexa fluor 488 | Molecular Probe     |             | 1:500                  |
| Antibody                | ABCA1       | Novus               | NB-400-105  | 1:500                  |
| Antibody                | B-Tubulin   | GenScript           | A01717      | 0.16 µg/mL             |
| Antibody                | Calnexin    | GenScript           | A01234      | 1 µg/ml                |
| Antibody                | Cathepsin D | Santa Cruz          | SC-6486     | 1:200                  |
| Antibody                | Caveolin 1  | Santa Cruz          | SC-894      | 1:500                  |
| Antibody                | CD-M6PR     | Novus               | NBP-1-20167 | 1:500                  |
| Antibody                | Cyto C Oxidase I | Santa Cruz        | SC-58347    | 1:200                  |
| Antibody                | Golgin 97   | Invitrogen          | A-21270     | 1:500                  |
| Antibody         | LAMP1   | Cell Signaling | D401S | 1:1000 |
|------------------|---------|----------------|-------|--------|
| Antibody         | NPC1    | William Garver’s lab |       |        |
| Antibody         | Syntaxin 6 | Andrew Peden’s lab |       |        |
| Antibody         | Vinculin | Millipore      | 05-386| 1:2500 |
| Antibody         | GM130   | BD Transduction | 610822| 1:250  |
| Fluorescent Probe| DAPI    | CalBiochem     | 26898 | 0.2 µg/mL |
| Fluorescent Probe| Filipin | Sigma          | F-9765| 0.05 mg/mL |
| Plasmid          | BFP-ER  | AddGene        | 49150 | Gia Voeltz Lab (KDEL sequence) |
| Plasmid          | Mito-BFP| AddGene        | 49151 | Gia Voeltz Lab (COX4 sequence) |
| Plasmid          | GFP-syntaxin 6 | Jeffrey Pessin Lab |       | (Full length Syntaxin 6) |
| Plasmid          | LAMP1-mEmerald | Addgene      | 54149 | Michael Davidson Lab (Lysosomes-20 Sequences) |

**METHODS**

**Mouse motor performance.** Mouse motor performance was assessed by Rotarod test using an instrument purchased from Med Associates Inc. (Fairfax, VT), in a manner similar to that previously reported (9) with modifications. Briefly, after an initial training, mouse motor skills were monitored from six weeks postnatal age until failure. Each week mice were given three consecutive trials on a constant speed rotarod at 24 rotations per min for up to 90 s for each of the three trials. WT and A1<sup>−/−</sup> mice passed all trials running for at least 10 s on any of the three consecutive trials during every week assessed. Age at rotarod test failure in Npc1<sup>−/−</sup> and Npc1<sup>−/−</sup>;A1<sup>−/−</sup> mice was measured as the age at which mice failed to run on the rotarod for at least 10 s during at least one of the three consecutive trials. Rotarod trial failure included falling off the rod before 10 s or freezing and clasping to the rotarod and not running or moving.
Histological analyses. Hematoxylin and Eosin staining of mouse liver, spleen and lung tissues and Purkinje neurons in cerebellum at postnatal day 80 were performed by the Histology Service at the Jackson Laboratory, using standard protocols in a Leica Autostainer XL automated processor.

Free cholesterol content in tissues. Tissues were homogenized in 3 mL 2:1 chloroform: methanol twice, filtered by Whatman filter paper, dried under nitrogen then resuspended in methanol. Free cholesterol content was determined in triplicate by using Wako’s Free Cholesterol E kit (#993-02501).

Cell culture. MEFs were isolated according the procedure described (10). MEF cells were grown as monolayers at 37 °C with 5 % CO₂ in DMEM supplemented with 10 % serum, MEM non-essential amino acids (Gibco), and penicillin/streptomycin; or with 5 % delipidated fetal bovine serum and 35 μM oleic acid. Each experiment was performed by using cells grown in triplicate dishes. Both healthy and mutant Hfs were from Coriell Institute, except the 2 mutant NPC1 lines indicated as NIH94.71 and NIH2A, which were gifts from Dr. Peter Pentchev. Hfs were grown in 60 mm dishes until confluency in the same conditions as MEFs.

Cholesterol distribution analyses in various subcellular organelles. After OptiPrep density gradient ultracentrifugation, for each sample, 14 fractions were collected, and subject to cholesterol measurement in triplicate using a Wako Free Cholesterol E kit.

Western analyses. Cells or mouse tissues were homogenized in a stainless-bed Bullet Blender twice for 3 min at 4 °C in either 10 % SDS (for syntaxin 6, CD-M6PR, NPC1, or cathepsin D), or RIPA buffer (for ABCA1), plus protease inhibitor (Sigma). After protein concentrations were determined, 150 µg of homogenized lysates were run onto 6 % gels for ABCA1, 10 % gels for NPC1, LAMP1, or 12 % gels for syntaxin 6, CD-M6PR, cathepsin D, caveolin 1, and cytochrome C oxidase, and transferred to PVDF membranes in Towbin buffer. The intensities were normalized to Vinculin (117 kDa) or beta tubulin (50 kDa) expression, quantitated by NIH Imaging software.

RNA isolation and real-time PCR experiments. Total RNA was isolated from TRIzol reagent (Invitrogen) followed the manufacturer’s instruction. The RNA was dissolved in sterile water treated with Dnase1 (Ambion). 1 µg of RNA was used to synthesize cDNA, according to the
instruction from BioRad iScript cDNA Synthesis Kit. Real-time PCR was carried out using the iTaq Universal SYBR Green Supermix (from Bio-Rad) with Applied Biosystems Step One RT-PCR system. Relative quantification was determined by using the delta CT method. The primer sequences used are listed below.

**Primer sequences used for RT-PCR.**

| Gene   | Sense/Antisense | Amplicon size (bp) |
|--------|-----------------|--------------------|
| GAPDH  | 5'-ATGGTGAAGGTCGGTGTG-3' 5'-CATTCTCGGCCTTGACTG-3' | 186 |
| ABCA1  | 5'-GGTTTGGAGATGGTTATACAATAGTTGT-3' 5'-TTCCCGGAAACGCAAGTC-3' | 96 |
| CTSD   | 5'-CATGCAGTCATCGCTAAGA-3' 5'-AGGGGACCCACAGGTTAGAG-3' | 152 |
| ABCG1  | 5'-AGGTCTCAGCTTCATCGCTAAGA-3' 5'-TCTCTCGAAGTGAAATTTGACTTG-3' | 96 |
| CTSB   | 5'-GAAGCCATTTCGACCGTCCCTTCTAAAGTTTCCTC-3' 5'-CACCTGAAACCAGGCTTT-3' | 169 |
| Calbindin | 5'-GAGCTATCACGGAAATGAAA-3' 5'-ATTTCTCGCAGGACTTCAG | 152 |

**Subcellular fractionation.** Cells grown in one 150-mm dish to near confluence, cells were washed twice with phosphate-buffered saline, once with homogenization buffer (HB, 250 mM sucrose, 20 mM Tris-HCl, pH 7.4, 1 mM EDTA), harvested to 1 mL HB with protease inhibitors, and homogenized by using a stainless-steel homogenizer with 40 strokes. The post-nuclear supernatants were placed onto the top of an 11 mL 5-25 % OptiPrep discontinuous gradient in HB. The gradient was centrifuged at 200,000 x g (40,000 rpm) for 3 h in a Beckman SW41 rotor; 15 fractions (800 µL each) were collected from the top.
**Lipid syntheses in intact cells.** Measurement of cholesterol esterification in intact cells was performed by exposing cells to $^{3}$H-labeled oleate/BSA for 1 h followed by lipid extraction and analysis as described in (11).

For filipin staining, MEFs were seeded on poly-D-lysine treated glass coverslips (MatTek) in 12-well plates. Filipin staining was carried out as previously employed without using detergent, viewed under confocal microscopy using Texas Red filter.

**Immunofluorescent staining of MEF cells.** MEF cells were cultured on glass coverslips in 12-well plates for 24 h, and fixed in 4 % paraformaldehyde (EMS) at RT for 10 min. After washing with PBS, cells were permeabilized with 0.3 % Triton X-100 for 20 min, washed with PBS prior to blocking with 5 % goat serum in PBS for 1 h, followed by staining with rabbit anti-syntaxin 6, mouse anti-golgin 97, or mouse GM130 for 1 h at RT. Cells were washed with PBS before and after incubation with Alexa Fluor 568 or Alexa Fluor 488 secondary antibodies at 1:500 dilution for 1 h, and DNA was counter stained with DAPI (0.2 µg/ml) was incubated at RT for 10 min. The "Reflex angle" is defined by Mitchel et al. (12) as the angle subtended by the edges of the syntaxin 6-positive signals, using the center of the nucleus (DAPI positive signal) as the vertex. For each cell type, 50 to 70 individual cells were measured for statistical analyses.

**Fluorescence microscopy.** Images were acquired by using the Andor W1 Spinning Disk Confocal system with a Nikon Eclipse Ti inverted microscope equipped with a 60x oil-immersion lens, three laser lines (403-nm laser for DAPI, 488-nm and 561-nm filters for FITC and Texas red respectively), and an Andor Zyla camera. Selected Z-stacked fluorescent images consisted of 11 optical slices collected at 0.2 µm intervals to enhance the spatial signal allocation. Images were acquired using Fiji Software and processed using Nikon Elements to create the Maximum Intensity Projection used to calculate the Pearson's Coefficient.

**Transfection.** MEF cells were seeded in 6-well plates at density of $5 \times 10^4$ cells per well, in medium containing 10 % serum, and incubated for 48 h. When cell density reached ~ 80 % confluence, medium was refreshed the night before the transfection. For transfection, cells were rinsed twice with PBS before 1 mL of Opti-MEM with 10 % serum without antibiotics was added. Then 2 µg of plasmid DNA and 5 µL FuGene 4K (or Lipofectamine 3000) in final volume of 100 µL Opti-MEM without serum were incubated together for 15-45 min at RT. The mixture was added in each well of cells, and incubated overnight. On the next day the medium was removed
and replaced with fresh medium containing 10% serum. When cells became confluent, cells were trypsinized and seeded on glass coverslips pretreated with either 70-150 kDa poly-d-lysine, or with fibronectin. At least three days or longer were needed for the transfected proteins to be fully expressed in the MEF cells.

**Click Chemistry:** Cells were incubated with 0.5 µM of 27-alkyne cholesterol for at least 18 h and then with or without 30 µg/mL cholesterol were added for 90 min at 37°C. Cells were fixed with 4% paraformaldehyde for 10 min at RT, followed by 2 TBS buffer rinses. The click reaction was performed by incubating the cells in 1 µM Alex 594/488-azide (Invitrogen) in 100 mM Tris at pH 8.8, 1 mM CuSO₄, and 100 mM ascorbic acid for 30 min in the dark. Cells were rinsed with TBS buffer containing 0.1% BSA and 0.5 M NaCl for 5 min with shaking, followed by 2 TBS buffer rinse and 2 PBS rinses. Cells were incubated with DAPI in PBS if needed, followed by a brief rinse in H₂O and mounted onto glass slides with Antifade reagent.

**Quantification of cholesterol content in various cellular compartments.** Images were acquired by using the Andor W1 Spinning Disk Confocal system, as described in in the **Method**, under **Fluorescence microscopy** section. Fluorescence intensities from the Images were obtained by using the Fiji Software, with Max-Intensity from the Z stacks. To quantitate the cholesterol content associated with each cellular compartment, first the fluorescent images of each organelle marker were acquired, and the “regions of interest (ROI)” were selected by using the freehand selection tool, provided by Fiji Software. Second, the fluorescent intensities of 27-alkyne cholesterol in the corresponding area were determined by using the ROI manager. The relative cholesterol content associated in various cellular compartment was determined as the fluorescence intensity of alkyne cholesterol divided by the fluorescence intensity from each cellular compartment.

**Statistical analysis:** Statistical comparisons were made by using a two-tailed, unpaired Student’s t test using GraphPad Prism 9. The difference was considered statistically significant when the P value was less than 0.05. p < 0.0001****, p < 0.001 ***, p < 0.01 **, p < 0.05 *, n.s., not significant.

**Measurement of long-lived proteins.** MEFs were seeded on 12-well plates at density of 0.012×10⁶, with triplicates for each time point. Fresh media were replaced the night before. Cells were rinsed with 2 mL of MEM; and labeled with 1 mL of 2 µCi/ml of ³H leucine in MEM+10%
serum. At zero time, cells were washed twice with HBSS, then chased by 1 mL MEM+2.8 mM leucine without serum. At each time point as indicated, media were transferred to a microcentrifuge tube, final concentration of 20 % TCA was added, samples were stored at 4 °C until all the samples were collected. When all the samples were collected, BSA at final concentration of 3 mg/mL was added, samples were incubated at 4 °C for 1 h, then tubes were centrifuged at 15,000 g at 4 °C for 5 min. Supernatant were collected, pellets were washed three times with cold 20 % TCA, pool all the washed together, Ecoscint H was added and count at scintillation counter. Cells were washed with PBS; and incubated in 0.1 M NaOH in 0.1 % DOC for 1 h, 40 µL in duplicates were aliquoted for protein determination. Samples were then neutralized by 60 µL 3 M HCl and 50 µL Na₂HPO₄ (pH 7.8). For each of 0.5 mL samples, add 5.15 mL of Ecoscint H for scintillation counting. Error bars indicate 1 SEM.
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FIGURE LEGENDS

S1. Syntaxin 6 localization and reflex angle measurement in MEFs. Cells were seeded and stained as described in the Fluorescence microscopy section in Methods. Reflex angles were measures as described in the same section.

S2. ABCA1 protein expression and quantitation in healthy and mutant Hfs. The growth condition and Western analyses were described in Methods. The Western blot as shown is representative of 7 independent experiments.

S3. Relative mRNA expressions of ABCA1, ABCG1, cathepsin B, and cathepsin D as indicated. RNA isolation and real-time PCR experiments were described in Methods section. GAPDH was used for normalization. For each MEF, 8 individual clones were employed. Error bars indicate 1 SEM.

S4. Degradation of long-lived proteins in MEFs. Cells were seeded, labeled by $^3$H leucine and chased at various time points. The procedures were described in Methods section. Error bars indicate 1 SEM.
**S1**

Filipin

DAPI

Merge

Cell fluorescence intensity of Filipin (a.u.)

| WT | A1<sup>-/-</sup> | Npc1<sup>nmf</sup> | Npc1<sup>nmf</sup>:A1<sup>-/-</sup> |
|----|------------------|---------------------|----------------------------------|
| ![Filipin Image](image1) | ![Filipin Image](image2) | ![Filipin Image](image3) | ![Filipin Image](image4) |
| ![DAPI Image](image5) | ![DAPI Image](image6) | ![DAPI Image](image7) | ![DAPI Image](image8) |
| ![Merge Image](image9) | ![Merge Image](image10) | ![Merge Image](image11) | ![Merge Image](image12) |

**S2**

ACAT inhibitor

| ACAT inhibitor | GM05659 | GM03123 | NIH94.71 | NIH2A | GM18453 | GM00112 | GM17910 |
|----------------|---------|---------|---------|------|---------|---------|---------|
| ![](image13) | ![](image14) | ![](image15) | ![](image16) | ![](image17) | ![](image18) | ![](image19) | ![](image20) |

Light exposure

| Light exposure | ABCA1 | Vinculin |
|----------------|-------|----------|
| ![Light Exposure Image](image21) | ![Light Exposure Image](image22) | ![Light Exposure Image](image23) |

Longer exposure

| Longer exposure | ABCA1 | Vinculin |
|-----------------|-------|----------|
| ![Longer Exposure Image](image24) | ![Longer Exposure Image](image25) | ![Longer Exposure Image](image26) |

Fold increase of ABCA1 protein expression
