Supporting Information

Mbua et al. 10.1073/pnas.1221105110

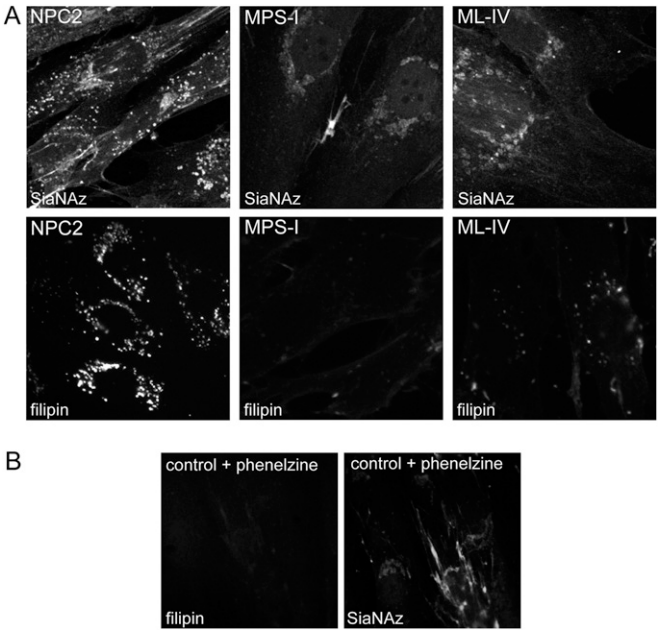

Fig. S1. (*A*) Vesicular *N*-azidoacetyl sialic acid (SiaNAz) staining is detected in Niemann–Pick type C (NPC) protein 2 (NPC2)-deficient fibroblasts, but not in mucopolysaccharidosis I (MPS-I) or mucolipidosis IV (ML-IV) fibroblasts. NPC2-deficient, MPS-I, and ML-IV fibroblasts were cultured in the presence of peracetylated *N*-α-azidoacetylmannosamine (Ac_4ManNAz) for 48 h before incubation with dibenzylcyclooctynol (DIBO) and filipin. The filipin-stained cells were imaged by fluorescence microscopy, and the SiaNAz-stained cells were analyzed by confocal microscopy. Note the lack of vesicular SiaNAz staining and cholesterol storage in MPS-I and ML-IV cells. n = 75–85 cells analyzed; three independent experiments. (*B*) Unlike treatment with imipramine, treatment of control fibroblasts with another antidepressant, phenelzine, did not result in cholesterol storage or vesicular accumulation of sialylated molecules.

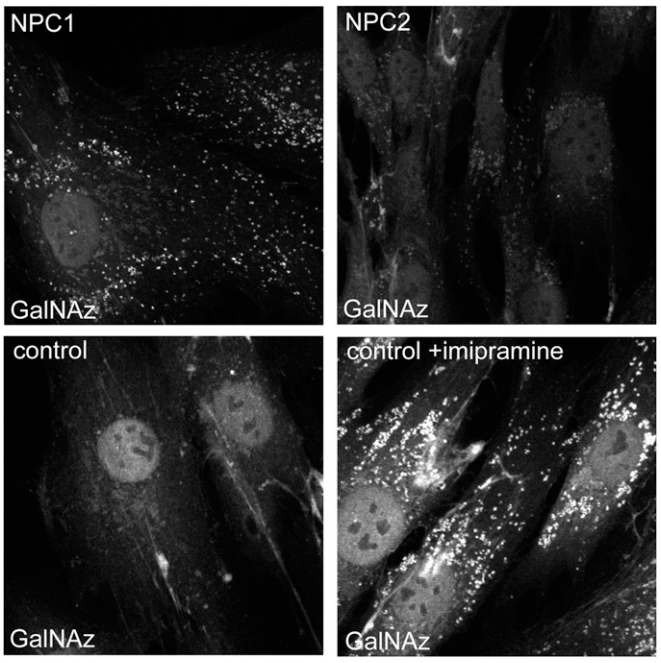

Fig. S2. Vesicular *N*-α-azidoacetylgalactosamine (GalNAz) staining is detected in NPC protein 1 (NPC1)-null, NPC2-deficient, and imipramine-treated control fibroblasts. Fibroblast cultures were labeled in the presence of peracetylated GalNAz (Ac_4GalNAz) for 48 h before incubation with DIBO. Stained cells were imaged by confocal microscopy. n = 20–27 cells analyzed; three independent experiments.

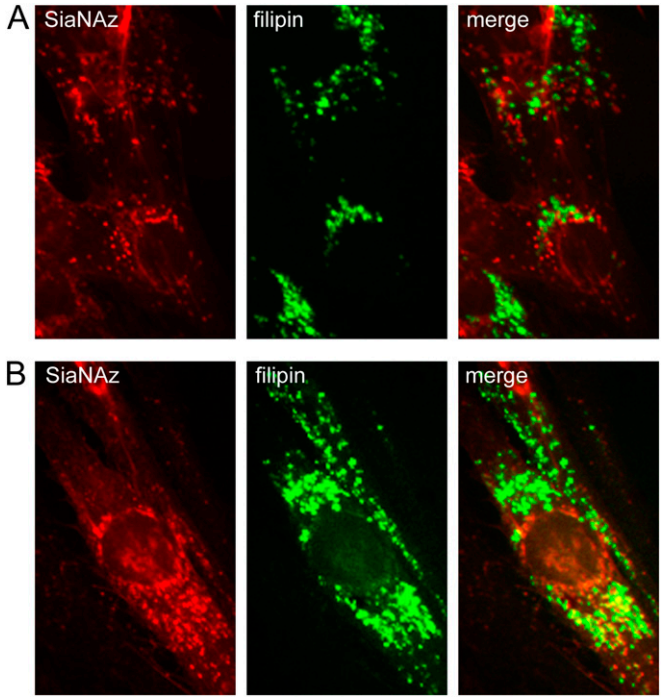

Fig. S3. Colocalization of cholesterol and SiaNAz in NPC2-deficient fibroblasts and imipramine-treated control fibroblasts. (*A*) NPC2-deficient fibroblasts and (*B*) imipramine-treated control fibroblasts were labeled with Ac_4ManNAz for 48 h, incubated with DIBO and Alexa Fluor 568 (to label sialylated molecules) and filipin (to label cholesterol), and prepared for imaging as described in *Materials and Methods* Staining was visualized by epifluorescence microscopy. $n = 30–35$ cells analyzed. Note the lack of colocalization in the NPC2-deficient fibroblasts between cholesterol and SiaNAz. In contrast, imipramine treatment of control cells results in accumulation of cholesterol and SiaNAz within common compartments.

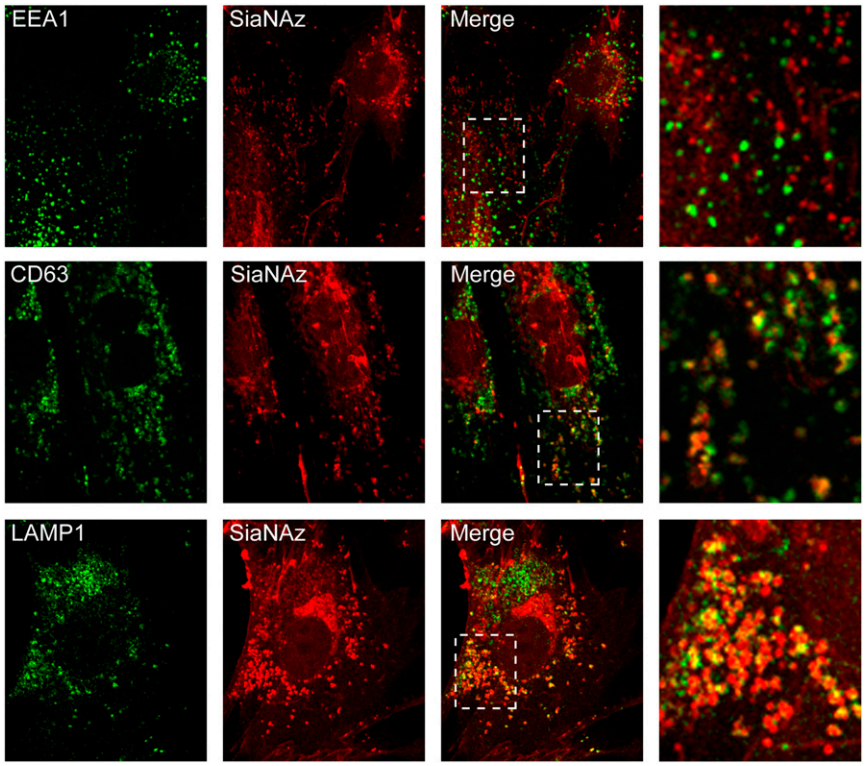

Fig. S4. Colocalization of SiaNAz with endosomal and lysosomal markers in NPC2-deficient fibroblasts. NPC2-deficient cells were cultured in the presence of Ac_4ManNAz for 48 h before incubation with DIBO. After fixation, coverslips were incubated with mouse monoclonal antibodies against early endosome antigen protein 1 (EEA1; to mark early endosomes), CD63 (to mark late endosomes and multivesicular bodies), and lysosomal-associated membrane protein 1 (LAMP1; to mark lysosomes). Cells were then incubated with appropriate secondary antibodies and visualized by confocal microscopy. n = 20–23 cells analyzed per costain; two independent experiments. Maximum intensity z-projections are shown. Whereas the vesicular SiaNAz staining in NPC1-null fibroblasts exhibits a high degree of colocalization with the endosomal marker CD63, the SiaNAz staining in NPC2-deficient cells has the most apparent overlap with the lysosomal marker LAMP1.

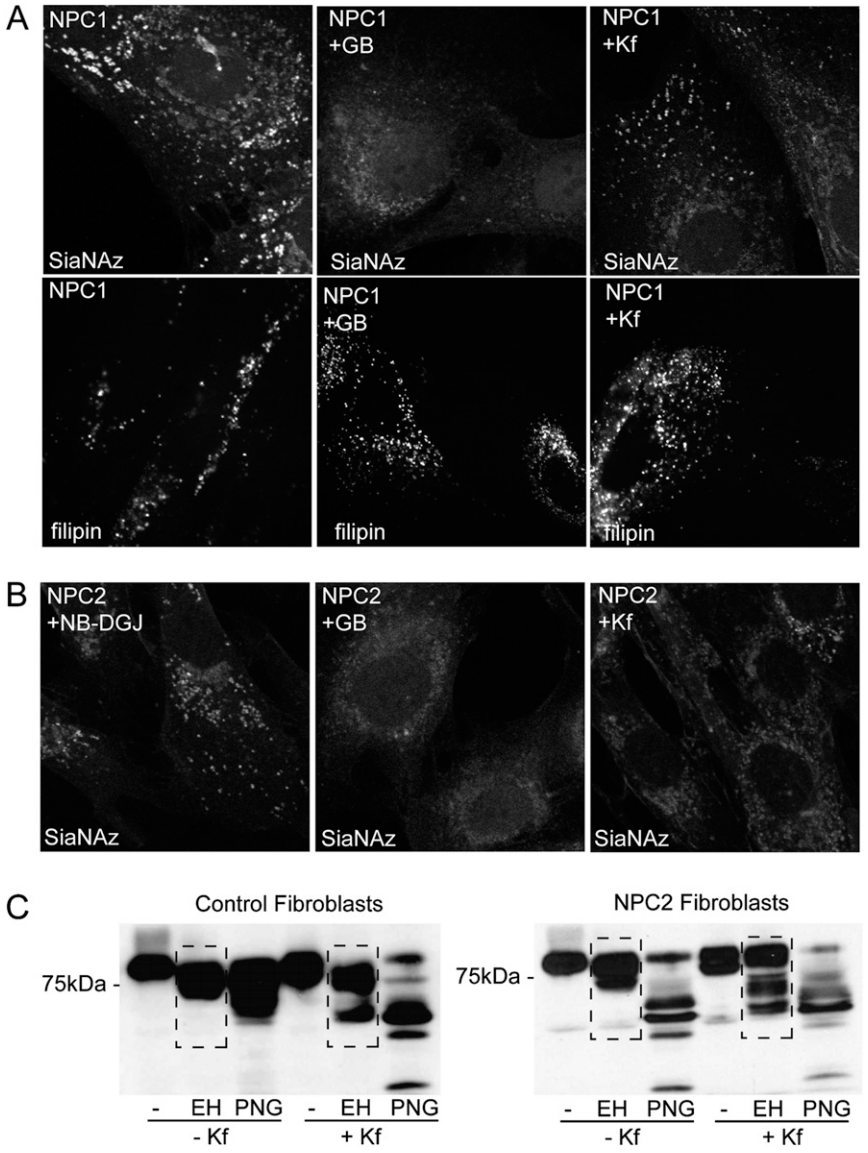

Fig. S5. Inhibitors of glycoprotein glycosylation reduce the SiaNAz phenotype in NPC fibroblasts. (*A* and *B*) NPC1-null (*A*) and NPC2-deficient (*B*) cells were labeled with Ac_4ManNAz for 2 d in the absence or presence of the inhibitors benzyl-α-GalNAc (GB; 2 mM) and kifunensine (Kf; 10 μM). (*Upper*) Treated and nontreated NPC fibroblasts were incubated with DIBO (30 μM) for 1 h at room temperature, followed by streptavidin-conjugated fluorophore (SiaNAz). (*Lower*) Filipin staining of the same cells under the same conditions. Treatment of NPC fibroblasts with inhibitors of glycoprotein biosynthesis led to a reduction of accumulated sialylated molecules. n = 20–25 cells analyzed; three independent experiments. No reduction in filipin staining was observed with either treatment. (*C*) Control and NPC2-deficient fibroblasts were treated with and without Kf (10 μM) for 2 d. Equivalent amounts of detergent cell lysate were subjected to digestion with either Endo H (EH; which removes unprocessed, high mannose-type *N*-glycans) or PNGaseF (PNG; which removes all *N*-glycans), and then resolved by SDS/PAGE. After transfer, blots were probed with a polyclonal antibody against the lysosomal glycoprotein lysosomal integral membrane protein 2 (LIMP2). Boxes highlight the loss of *N*-glycan processing in both control and NPC2-deficient fibroblasts treated with Kf.

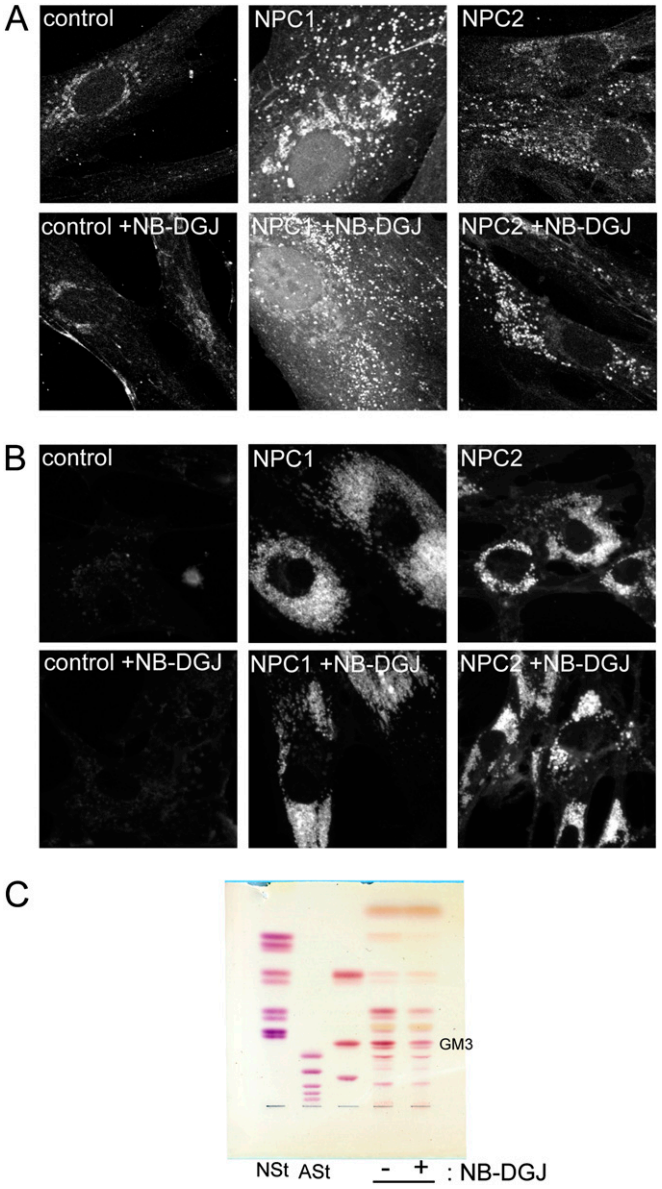

Fig. S6. Inhibition of glycolipid biosynthesis does not alter the SiaNAz phenotype in NPC fibroblasts. (*A*) Control, NPC1-null, and NPC2-deficient cells were cultured in the presence or absence of *N*-butyldeoxygalactonojirimycin (NB-DGJ; 100 μM) for 5 d. Cultures were labeled with Ac_4ManNAz for 2 d starting at day 3 of the NB-DGJ treatment. Treated and nontreated fibroblasts were incubated with DIBO (30 μM) for 1 h at room temperature, followed by streptavidin-conjugated fluorophore (SiaNAz). $n = 30–40$ cells analyzed; two independent experiments. (*B*) Filipin staining of the same cells under the same conditions. (*C*) Glycolipids from untreated and NB-DGJ–treated NPC2-deficient fibroblasts were extracted, subjected to saponification, and analyzed by high performance thin layer chromatography. The mobility of acidic glycolipid standards (ASt) and neutral glycolipid standards (NSt), as well as the migration of a mono-sialoganglioside standard, is shown. Five days of NB-DGJ treatment of NPC2-deficient fibroblasts was sufficient to reduce total glycolipid levels by roughly 50%.

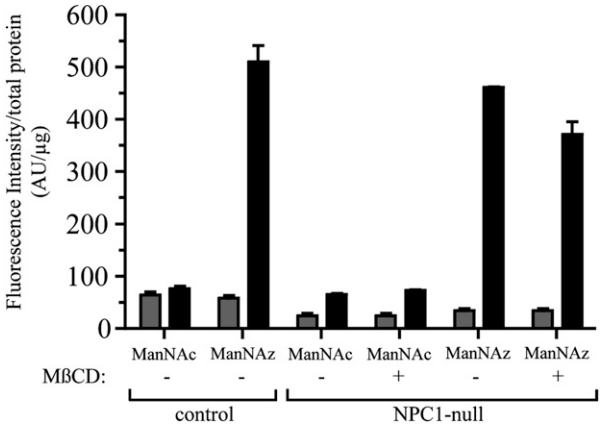

Fig. S7. Cyclodextrin treatment of control and NPC2-deficient fibroblasts does not significantly alter cell surface sialylation. Control and NPC2-deficient fibroblasts were labeled with Ac_4ManNAz, and cell surface fluorescence intensity was measured as described earlier. The results represent three independent experiments. Error bars indicate SDs.