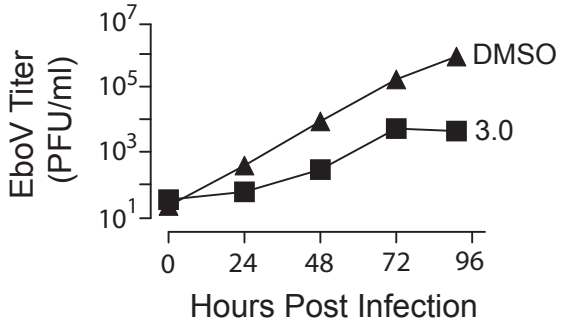
a



b

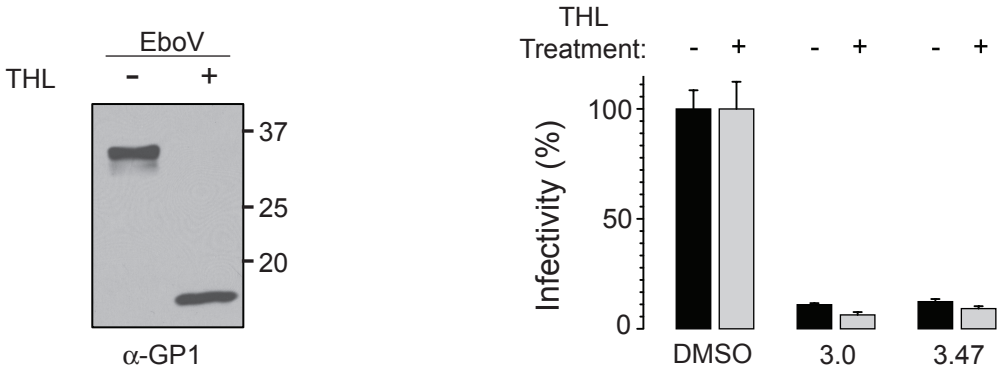


Figure S1.

a, EboV infection is inhibited by 3.0. Vero cells were cultured in media containing 3.0 (20 μM) or vehicle for one hour and then challenged with native ebolavirus Zaire-1995 (moi=0.1). Data for 3.0 is mean ± s.d. (n=4) and mean of duplicate DMSO-treated samples. (Note: Error bars are smaller than the bullets.)

b, (left) Compounds 3.0 and 3.47 inhibit infection by protease-cleaved virus particles.
EboV pseudotyped particles were incubated with thermolysin and cleavage of GP1 was analyzed by immunoblot following deglycosylation with PNGaseF (left panel). Vero cells treated with 3.0, 3.47 (5 μM) or vehicle and exposed to native and thermolysin-cleaved VSV EboV GP particles(right panel). Infection was measured as in Fig 1b. Data is mean ± s.d. (n=4) and is representative of 3 experiments.

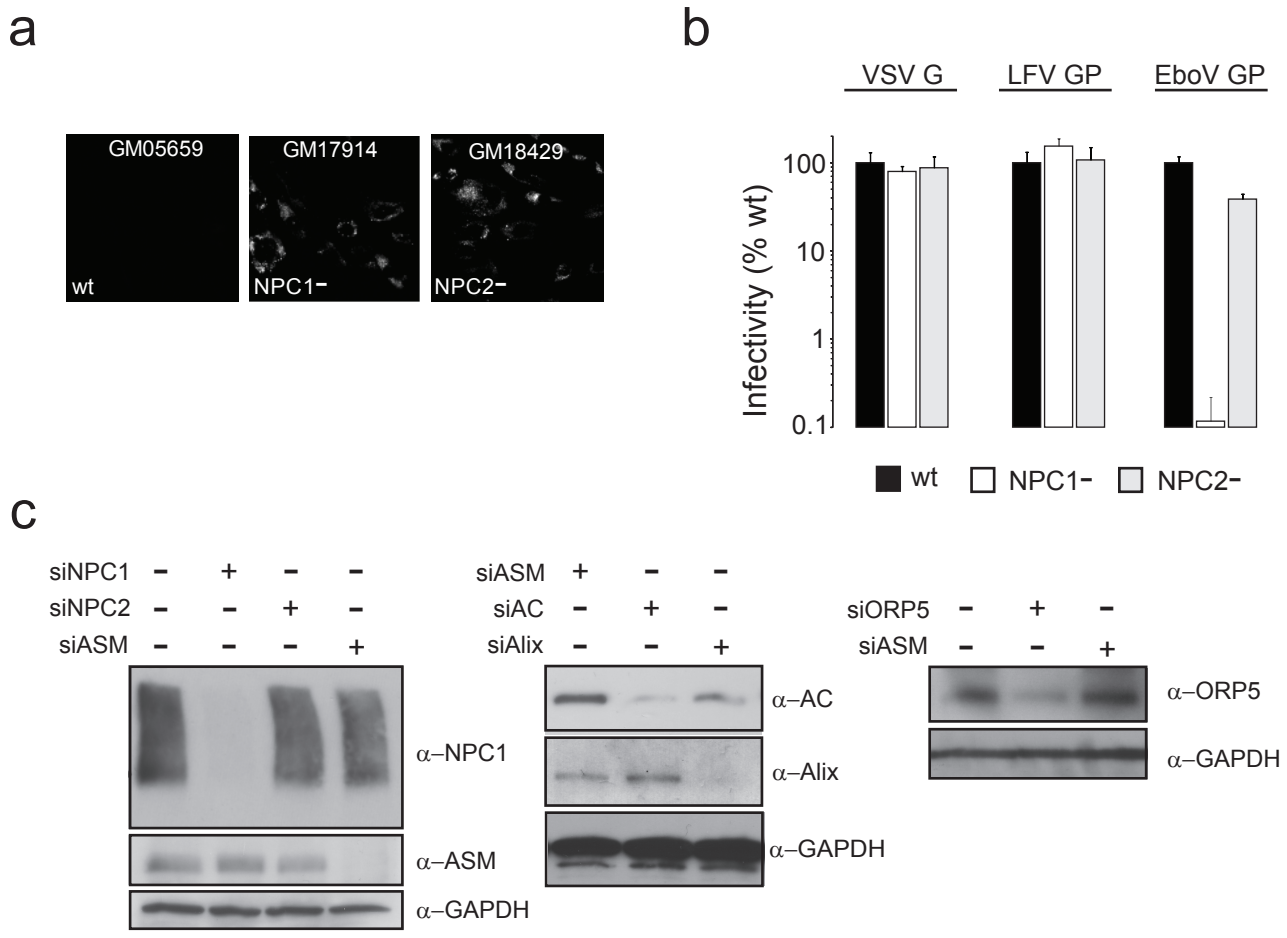


Figure S2. Analysis of proteins involved in cholesterol uptake in EboV GP infection

a, Niemann-Pick C1 and C2 cells contain cholesterol-rich vacuoles. Human fibroblast cell lines derived from patients with Niemann-Pick type C1 or C2 disease were analyzed for cytoplasmic cholesterol deposits using filipin staining. GM17914 (NPC1-) is a compound heterozygote with a frameshift and a missense mutation (I106T) that results in a misfolding[26]; GM18429 (NPC2-) is homozygous for a substitution that results in defective splicing of NPC2 RNA; and GM05659 (wt) fibroblasts are from a healthy human donor. Representative images are shown.

b, Ebolavirus infection of Niemann-Pick C1 and C2 cells. Wt, NPC1-, and NPC2- fibroblasts were exposed to VSV particles pseudotyped with VSV G, LFV GP or EboV GP. Infection was measured as in Fig 1c. Data is mean ± s.d. (n=3) and is representative of 3 experiments.

c, Protein expression in siRNA treated HeLa cells from Fig 2b. Expression of NPC1, NPC2, ASM, Alix, acid ceramidase (AC) and ORP5 was knocked-down in HeLa cells using SMARTpool siRNA (20 nM, Dharmacon). After 72 hours, cells were assessed for infection (Fig 2b) or protein expression. Protein expression was measured by immunoblot of cell lysates using anti-ASM 1H7 (Genzyme), anti-NPC1 (Abcam), anti-AC (BD Biosciences), anti-Alix (Biolegend), and anti-ORP5 (Abcam).

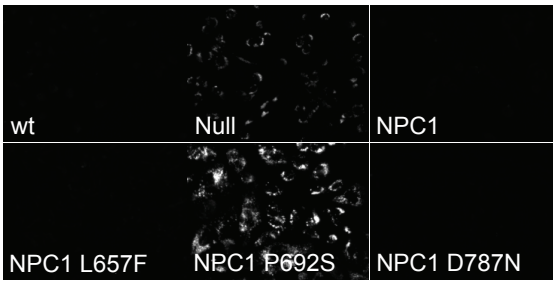


Figure S3. Phenotype of cells expressing NPC1 mutant proteins.

CHO_{wt}, CHO_{null}, and CHO_{null} cells stably expressing wild type mouse NPC1, NPC1 L657F, NPC1 P692S, and NPC1 D787N were fixed and stained with filipin as in Figure S2a. Representative images are shown.

CHO Cell Line	Retroviral Titer (FFU/ml)							
	ZEboV GP	CIEboV GP	BEboV GP	SEboV GP	REboV GP	MARV GP	LFV GP	VSV G
wt	$(4.3 \pm 0.4) \times 10^6$	$(1.1 \pm 0.1) \times 10^6$	$(1.1 \pm 0.8) \times 10^7$	$(4.2 \pm 0.1) \times 10^6$	$(3.0 \pm 0.4) \times 10^5$	$(1.2 \pm 0.4) \times 10^6$	$(2.6 \pm 0.6) \times 10^5$	$(8.0 \pm 0.8) \times 10^6$
Null	< 4.0	< 4.0	4 ± 0	< 4.0	< 4.0	2.7 ± 2.3	$(2.2 \pm 0.9) \times 10^6$	$(2.7 \pm 1.5) \times 10^7$
NPC1	$(1.6 \pm 0.6) \times 10^6$	$(7.3 \pm 0.2) \times 10^5$	$(2.7 \pm 0.3) \times 10^6$	$(2.5 \pm 0.6) \times 10^6$	$(5.0 \pm 0.9) \times 10^4$	$(2.1 \pm 0.3) \times 10^5$	$(1.2 \pm 0.3) \times 10^6$	$(7.1 \pm 0.2) \times 10^6$

Figure S4. Infection of CHO cells by filovirus GP pseudotyped retrovirus particles.

CHOwt, CHO cells lacking NPC1 (CHO_{null}) and CHO_{null} cells stably expressing mouse NPC1 (CHO_{NPC1}) were exposed to MLV particles encoding LacZ and pseudotyped with GPs from ebolavirus Zaire (ZEboV), Côte d'Ivoire (CIEboV), Bundibungyo (BEboV), Sudan (SEboV), or Reston (REboV), marburgvirus (MARV), Lassa fever virus (LFV) or VSV G. Results are the mean ± s.d. (n=12).

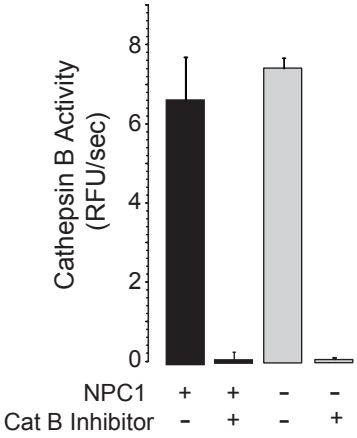


Figure S5. The relationship between NPC1 expression and cathepsin B activity in CHO cells.

CHO_{wt} and CHO_{null} cells were incubated in medium containing the Cat B inhibitor CA074 (80 μM) or vehicle (1% DMSO) for 4 hours, and Cat B protease activity was measured in cell lysates using a fluorogenic substrate[3]. Cat B activity (V0, relative fluorescence units (RFU)/sec) is plotted. Results are mean ± s.d. (n=9).

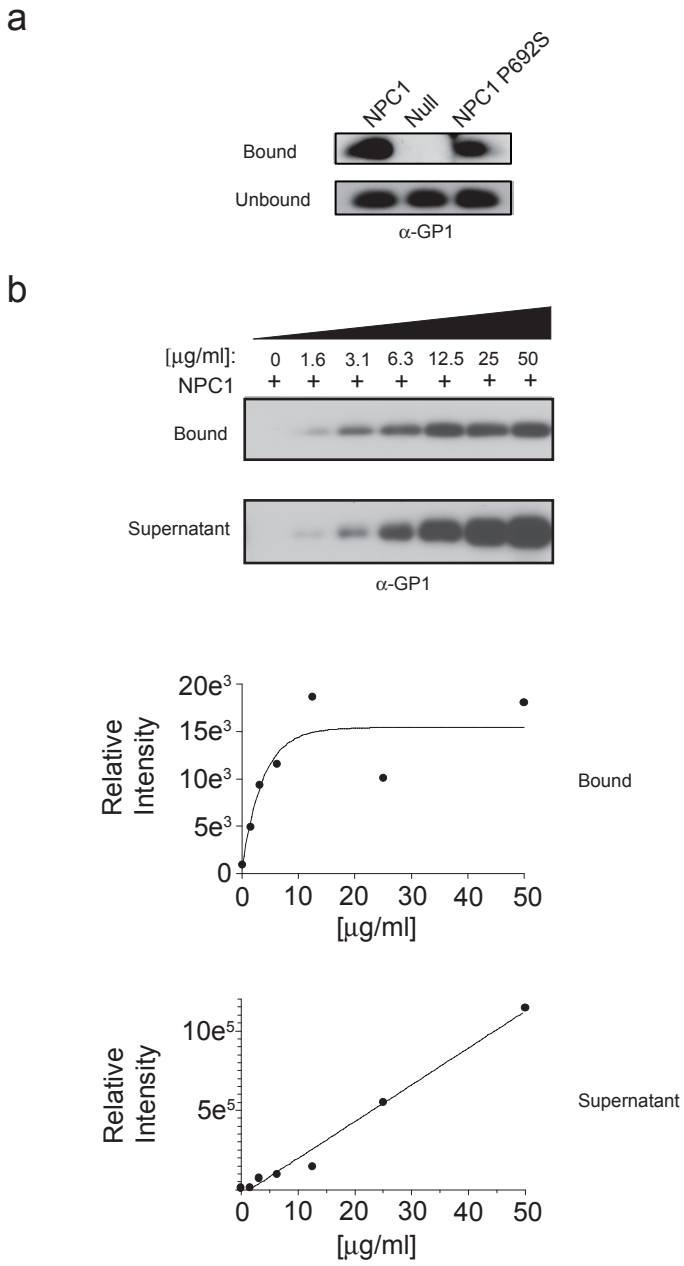


Figure S6.

a, EboV $GP_{\Delta TM}$ binds to membranes containing the NPC1 mutant P692S. Thermolysin-cleaved EboV $GP_{\Delta TM}$ protein (1 μg) was added to LE/LY membranes from CHO_{null}, CHO_{NPC1}, or CHO_{P692S} cells and analyzed as in Figure 3.

b, Saturable binding of thermolysin-cleaved EboV $GP_{\Delta TM}$ to NPC1 membranes.
LE/LY membranes from CHO_{NPC1} were incubated with increasing concentrations of thermolysin-cleaved EboV $GP_{\Delta TM}$ as in Figure 3a. GP1 was analyzed in membrane bound and supernatant fractions using immunoblot (top). Densitometry was performed and the relative intensity of each GP1 band was measured using Quantity One Software (Bio-Rad). The data was used to plot the amount of GP1 in the supernatant and the amount bound to LE/LY membranes as a function of the input concentration of EboV $GP_{\Delta TM}$.

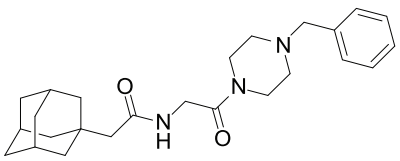
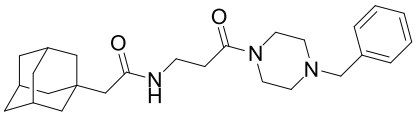
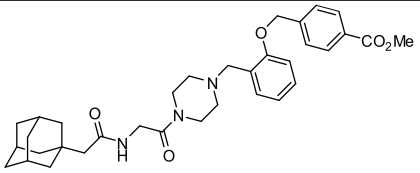
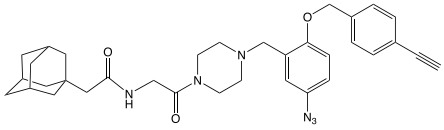
3.0		IC_{50}= 1.6 μM	IC_{90}= 5.8 μM
3.18		IC_{50}= 41 μM	IC_{90}= >50 μM
3.47		IC_{50}= 130 nM	IC_{90}= 400 nM
3.98		IC_{50}= 1 μM	IC_{90}= >40 μM

Figure S7. Structure and antiviral activity of 3.0 analogs 3.18, 3.47, and 3.98.

Inhibition of virus infection by 3.0 analogs 3.18, 3.47, and 3.98. Vero cells were incubated in the presence of 3.18, 3.47, 3.98 or vehicle for 90 minutes prior to the addition of VSV particles pseudotyped with EboV GP. Virus infection was calculated as in Fig. 1b. IC_{50} and IC_{90} values were determined from this data.

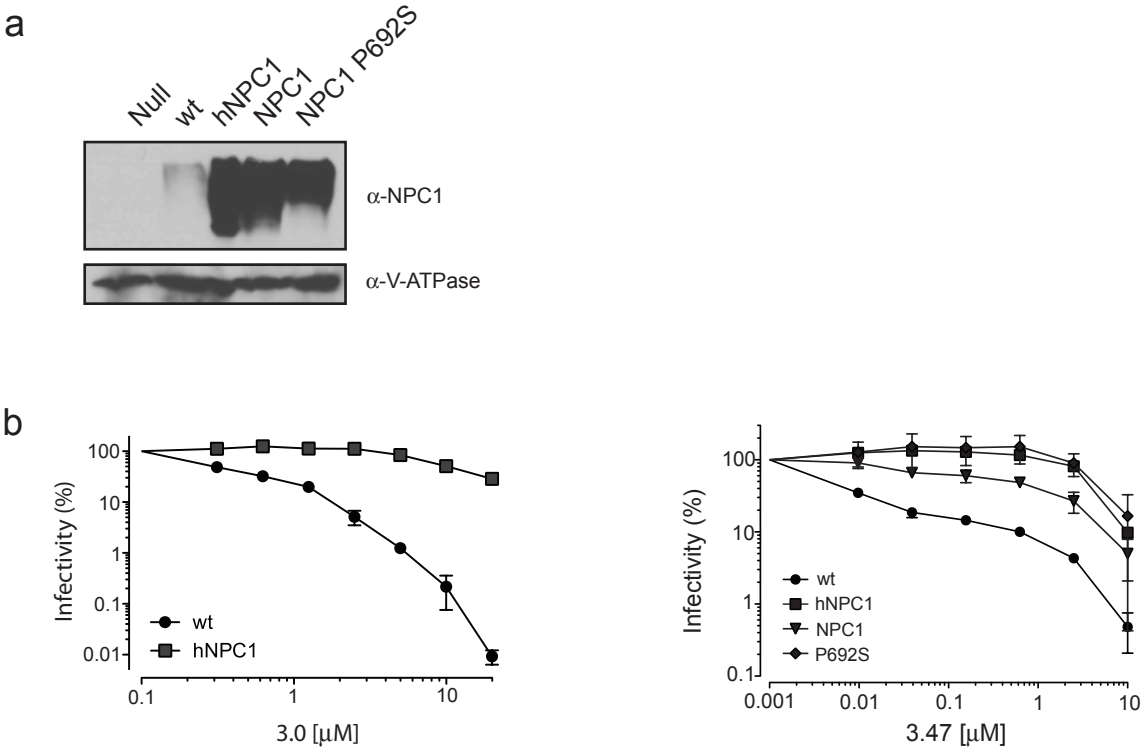


Figure S8. Effect of NPC1 expression on antiviral activity.

a, CHO_{null}, CHO_{wt}, CHO_{NPC1}, CHO_{hNPC1} and CHO_{P692S} cells were homogenized, and membranes in the post-nuclear supernatant were pelleted at 15000 x g. NPC1 and V-ATPase B1/2 in the pelleted membranes were detected by immunoblot as in Figure 3b.

b, CHO_{wt}, CHO_{hNPC1}, CHO_{NPC1}, CHO_{P692S} cells were incubated in the presence of increasing concentrations of 3.0 (left), 3.47 (right) or vehicle prior to the addition of VSV particles pseudotyped with EboV GP. Infection was calculated as in Fig 1b. Data is mean ± s.d. (n=4) and is representative of 3 experiments.