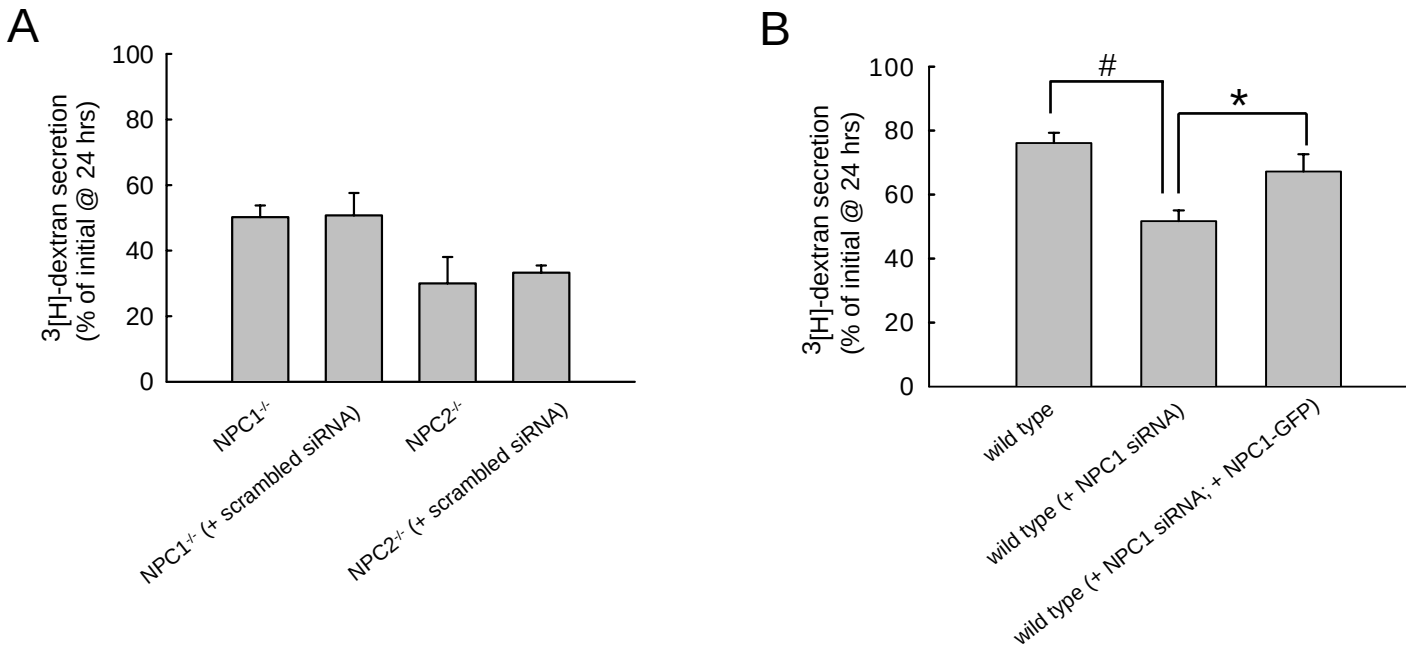
SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Specificity of siRNA for NPC1 and NPC2. *A*, transfection with scrambled siRNA does not affect ^{3}[H]-dextran secretion. NPC1$^{-/-}$ fibroblasts with and without treatment with scrambled siRNA secrete similar amounts of ^{3}[H]-dextran. Similarly, NPC2$^{-/-}$ fibroblasts with and without treatment with scrambled siRNA secrete comparable amounts of ^{3}[H]-dextran. *B*, wild type cells release approximately 80% of their total dextran at 24 hrs. Wild type cells treated with NPC1 siRNA exhibit significantly impaired cumulative dextran release at 24 hrs relative to wild type cells (#, $p < 0.01$). Cells transfected with functional mouse NPC1-GFP for 48 hrs and subsequently treated with NPC1 siRNA, however, have cumulative dextran secretion at the 24 hr time point that is significantly higher than siRNA treated wild type cells (*, $p < 0.05$). Data shown are means ± S.E. from experiments run in triplicate.

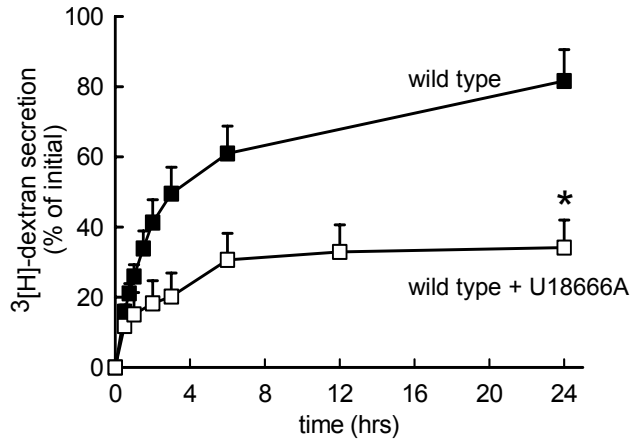
Fig. S2. Release of lysosomal ^{3}[H]-dextran polymers from treated wild type fibroblasts. *A*, wild type cells (■) release about 80% of their initial dextran over a 24 hr time period. Cells treated with 10 μM U18666A for six hrs (□) exhibit significantly reduced dextran release. *B*, wild type cells treated with (□) or without (■) 10 μg/mL progesterone exhibit significantly reduced dextran release. All data points represent average ± S.D. from three independent experiments (*, $p < 0.05$ by unpaired *t*-test).

Fig. S3. Delivery of LDL-derived cholesterol is impaired in cells with deficient NPC1 and NPC2. Cholesterol efflux was measured as described in Experimental Procedures. Wild type fibroblasts are able to mobilize LDL-derived cholesterol to the plasma membrane whereas cells with mutations in NPC1 and NPC2 exhibit significantly impaired efflux rates. NPC2 mutated cells treated with NPC1 siRNA also show decreased rates of cholesterol efflux as compared to normal cells (*, $p < 0.05$ by unpaired *t*-test).

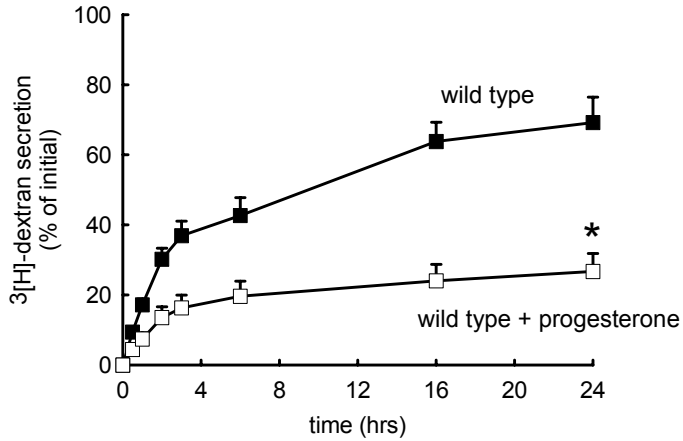
Fig. S4. Delivery of LDL-derived cholesterol to the ER is impaired in cells with deficient NPC1 and NPC2. Cholesterol esterification rates were measured as described in Experimental Procedures. Wild type fibroblasts are able to mobilize LDL-derived cholesterol to the ER whereas cells with mutations in NPC1 and NPC2 exhibit significantly impaired rates. NPC2 mutated cells treated with NPC1 siRNA also show decreased rates of cholesterol efflux as compared to normal cells (*, $p < 0.05$ by unpaired *t*-test).

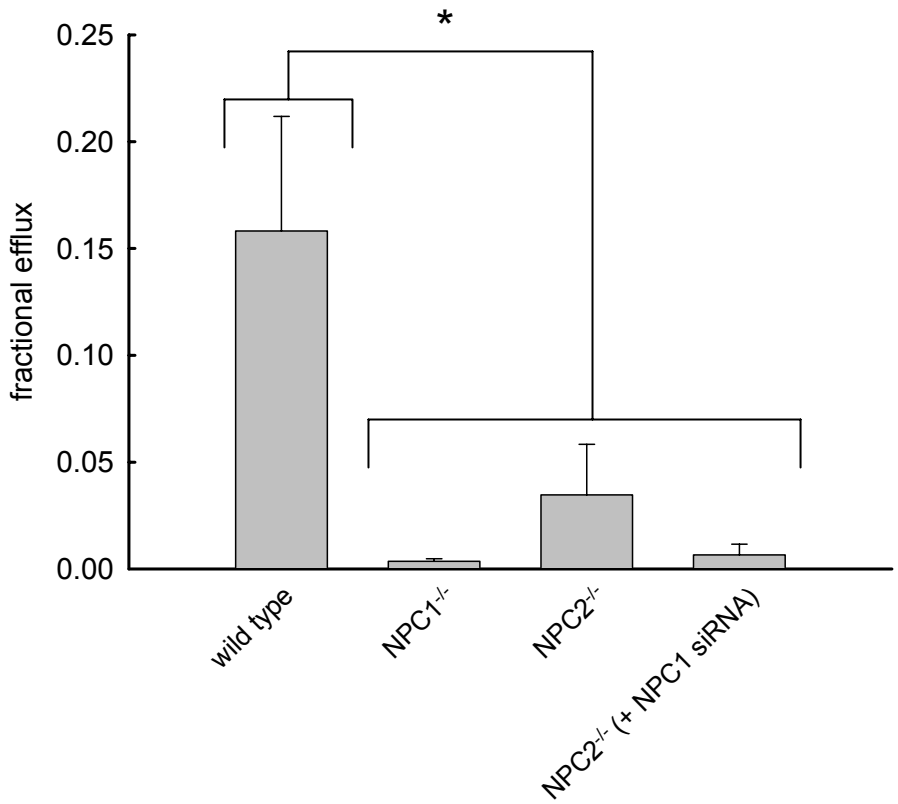

A
3[H]-dextran secretion
(% of initial @ 24 hrs)
100
80
60
40
20
0
NPC1-/-
NPC1-/- (+ scrambled siRNA)
NPC2-/-
NPC2-/- (+ scrambled siRNA)
B
3[H]-dextran secretion
(% of initial @ 24 hrs)
100
80
60
40
20
0
#
*
wild type
wild type (+ NPC1 siRNA)
wild type (+ NPC1 siRNA; + NPC1-GFP)

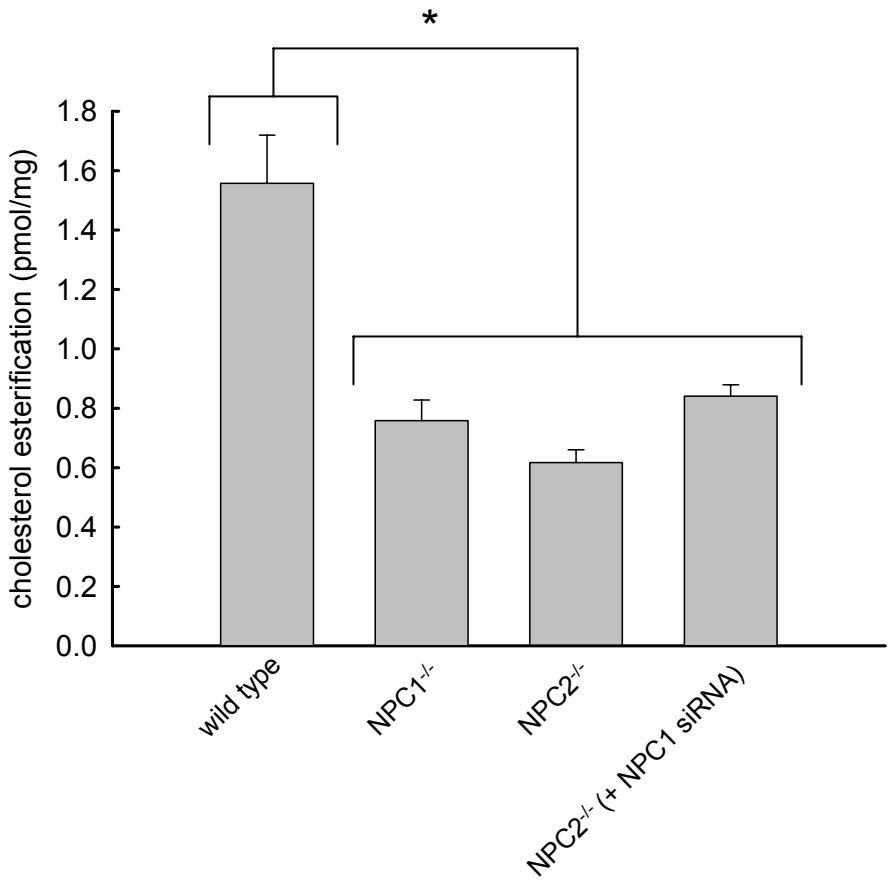
A


100
80
60
40
20
0
0
4
8
12
16
20
24
time (hrs)
3[H]-dextran secretion
(% of initial)
wild type
wild type + U18666A
*

B


100
80
60
40
20
0
0
4
8
12
16
20
24
time (hrs)
3[H]-dextran secretion
(% of initial)
wild type
wild type + progesterone
*


*
0.25
0.20
0.15
0.10
0.05
0.00
fractional efflux
wild type
NPC1-/-
NPC2-/-
NPC2-/- (+ NPC1 siRNA)


*
cholesterol esterification (pmol/mg)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
wild type
NPC1-/-
NPC2-/-
NPC2-/- (+ NPC1 siRNA)