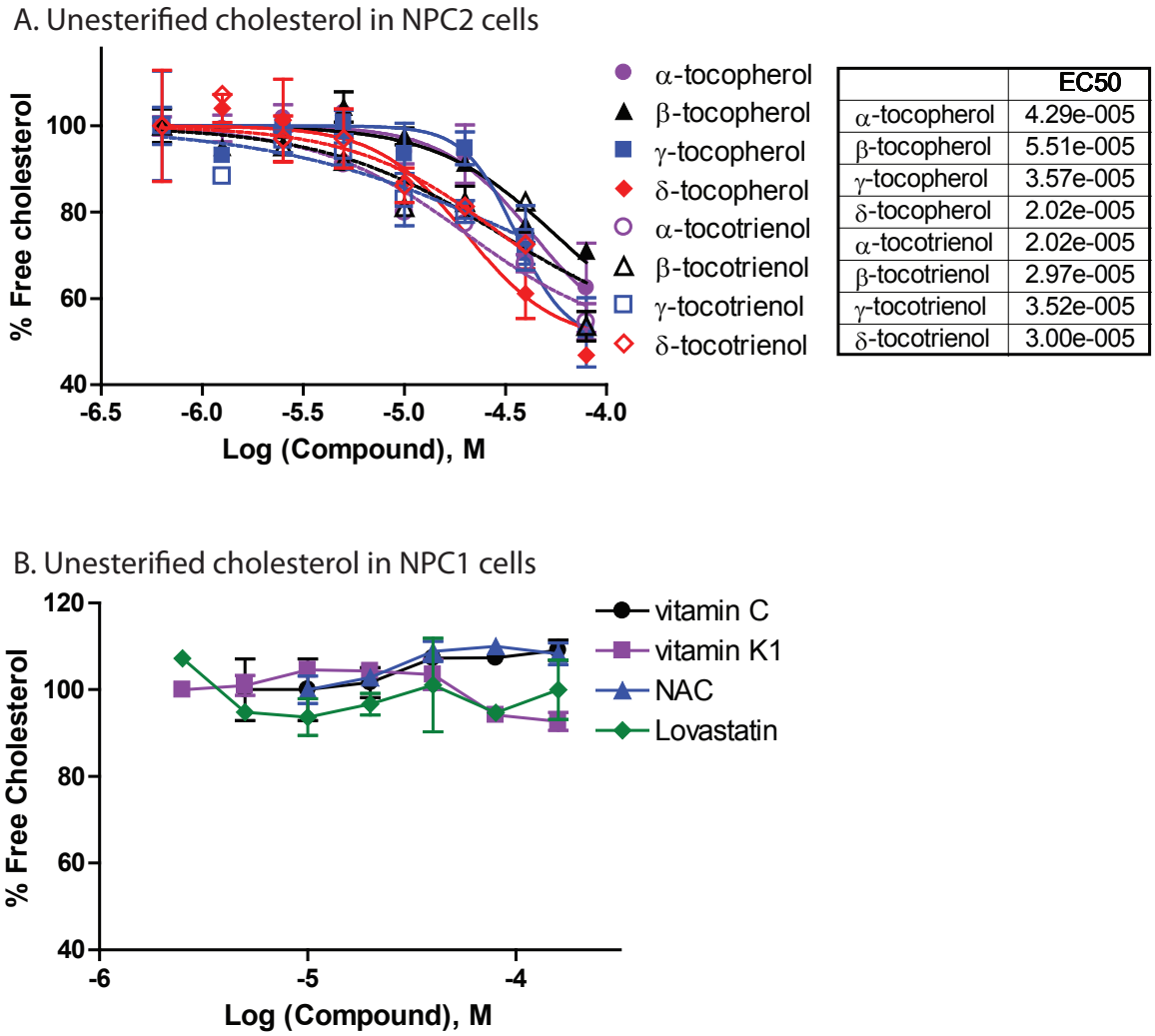
SUPPLEMNTAL MATERIALS

Supplemental Table:

Table S1. Fibroblast cell lines from Coriell Cell Repository.

Eponym of disease	*Disease name*	*Abbreviation*	*Affected gene*	*Protein*	*Accumulated Lipid*	*Genotype*	*Coriell Catalog #*
Batten	Ceroid lipofuscinosis, neuronal 2	CLN2	*TPP1*	Tripeptidyl peptidase I	lipopigments (lipofuscin)	p.R127X, p.R208X	GM16485
Fabry	Alpha-galactosidase A deficiency		*GLA*	Alpha galactosidase A	globotriaosylceramide	p.W162X, rs2071397 rs2071228	GM00107
Farber	Lipogranulomatosis		*AC*	Acid ceramidase (N-acylsphingosine amidohydrolase)	ceramide	p.Y36C, p.Y36C	GM20015
Niemann-Pick, type C1		NPC1	*NPC1*	NPC1	Unesterified cholesterol	p.P237S, p.I1061T	GM03123
Niemann-Pick, type C2		NPC2	*NPC2*	NPC2	Unesterified cholesterol	p.C93F, p.C93F	GM17910
Niemann-Pick, type A		NPA	*ASM*	Acid sphingomyelinase	Sphingomyelin	p.L302P, p.L302P	GM16195
Sanfilippo type B	Mucopolysaccharidosis III type B	MPS IIIB	*NAGLU*	N-acetyl-alpha-D-glucosaminidase	Partially degraded heparan sulfate	p.R297X, p.R643H	GM02552
Tay-Sachs	GM2 gangliosidosis	TSD		Beta hexosaminidase A	GM2 ganglioside	c.1278insTATC c.1278insTATC	GM00221
Wolman	Lysosomal acid lipase deficiency		*LAL*	Lysosomal acid lipase	Cholesteryl ester & triglycerides	unknown	GM11851

Supplemental Figures:



	EC50
α-tocopherol	4.29e-005
β-tocopherol	5.51e-005
γ-tocopherol	3.57e-005
δ-tocopherol	2.02e-005
α-tocotrienol	2.02e-005
β-tocotrienol	2.97e-005
γ-tocotrienol	3.52e-005
δ-tocotrienol	3.00e-005

Figure S1. Levels of cellular unesterified (free) cholesterol in NPC2 fibroblasts after the treatments with eight vitamin E isoforms and in NPC1 fibroblasts after the treatments with other vitamins. **(A)** Concentration-response curves of eight vitamin E isoforms on cellular unesterified cholesterol level in the NPC2 fibroblasts were determined by the Amplex cholesterol assay. The cells were treated with compounds with concentration ranging from 0.63 to 80 μM except γ-tocotrienol and δ-tocotrienol that ranged from 0.63 to 40 μM. δ-T was the most potent compounds with an EC_{50} value of 20.2 μM. **(B)** Concentration-response curves of vitamin C, vitamin K, NAC and lovastatin on cellular unesterified cholesterol level in the NPC1 fibroblasts determined by the Amplex-red cholesterol assay. These four compounds did not reduce the unesterified cholesterol level in the NPC1 fibroblasts.

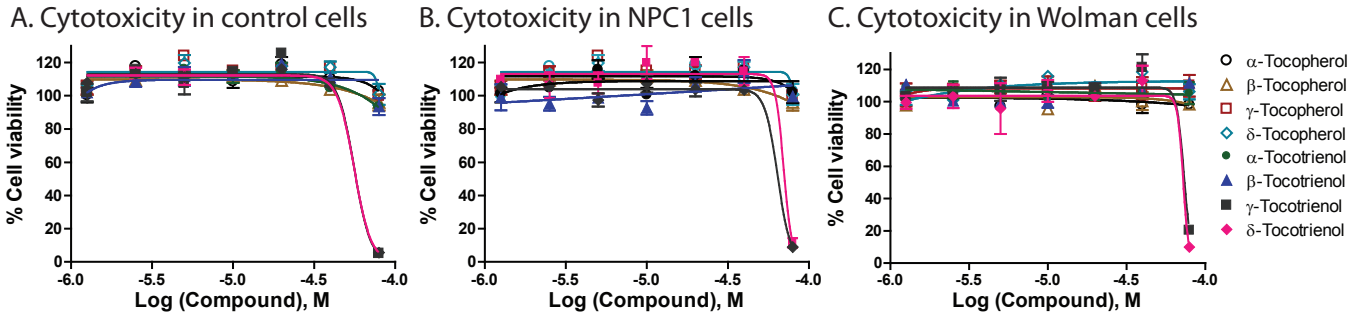


Figure S2. Cytotoxicity of eight vitamin E isoforms in control, NPC1 and Wolman firbroblasts. Cytotoxicity of eight vitamin E isoforms was measured by an ATP content assay in the control **(A)**, NPC1 **(B)**, and Wolman **(C)** fibroblasts. Except γ-tocotrienol and δ-tocotrienol which were cytotoxic at 80 μM (but not cytotoxic at the concentrations less then 40 μM), the other six isoforms of vitamin E including δ-T and α-T were not cytotoxic at the concentration up to 80 μM after a 4-day treatment in these cells.

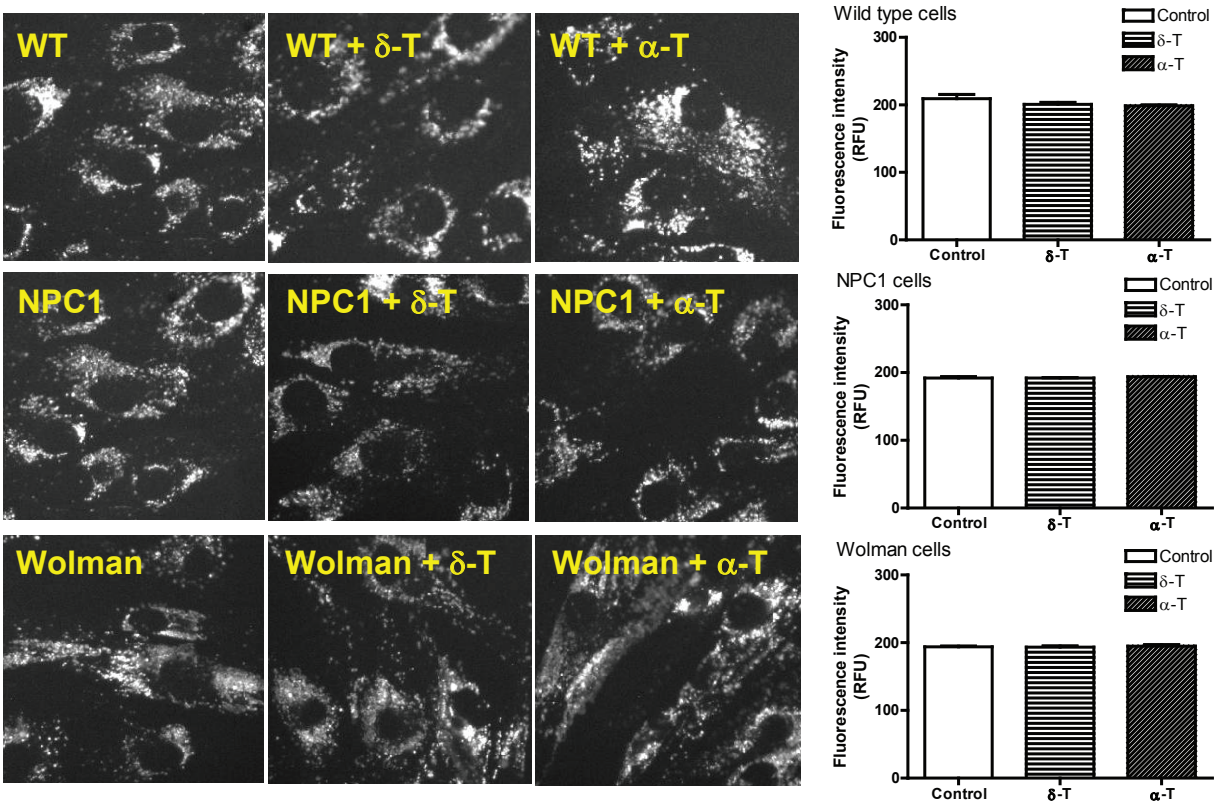


Figure S3. Lysosomal pH indicated by a LysoSensor dextran dye in NPC1 and Wolman fibroblasts. The method was described previously by Chen and colleagues (Chen Q, Zhang CL, Luther RW and Mixson AJ. Nucleic Acids Res 30:1338-45, 2002). Briefly, cells were incubated with 40 μM δ-T for 24 hrs and the indicator dye (catalog # L-22460, Invitrogen) for 16 hrs. The images were taken using Incell2000 (GE healthcare) with an excitation of 360 nm, emission-1 of 530 and emission-2 of 450 nm. The imaging analysis was performed using the software provided with the Incell2000 imaging analyzer. The fluorescence intensity at emission 530 nm represents dye staining in late endosomes/lysosomes and endocytic vesicles that becomes strong at acidic pH. The data indicated that the treatment with δ-T in these cells does not alter pH in late endosomes/lysosomes compared with untreated cells. The fluorescence intensity at emission 450 nm (dye staining in non-acidic compartments) was much weak because the dye was concentrated in the acidic compartments including late endosomes/lysosomes (images not shown).

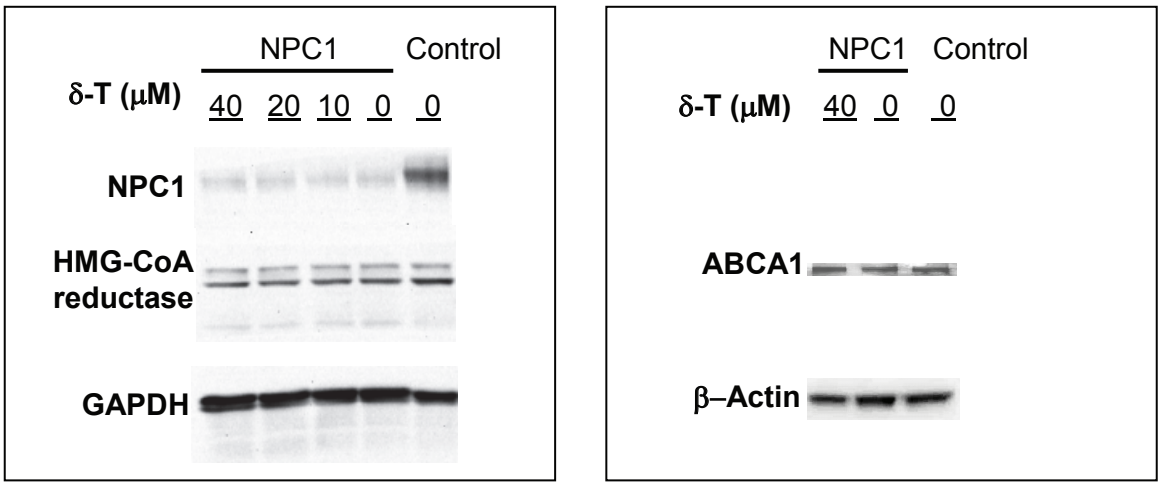


Figure S4. Protein levels of NPC1, HMG-CoA reductase and ABCA1 in response to δ-T treatment. Compared with control cells, 40 μM δ-T treatment for four days did not alter the protein levels of NPC1, HMG-CoA reductase and ABCA1 steroid transporter in NPC1 fibroblasts.

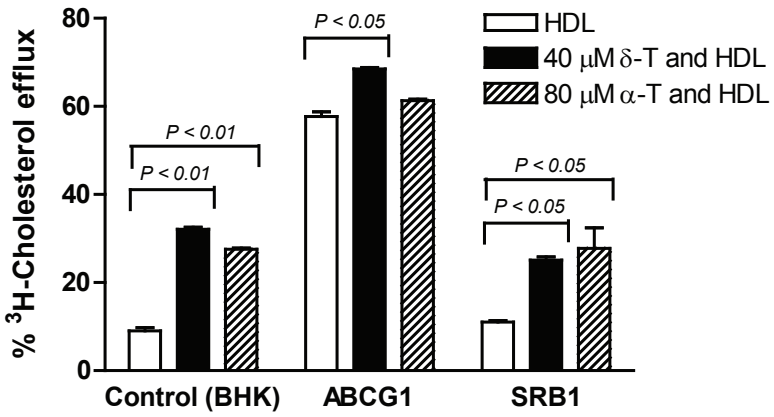
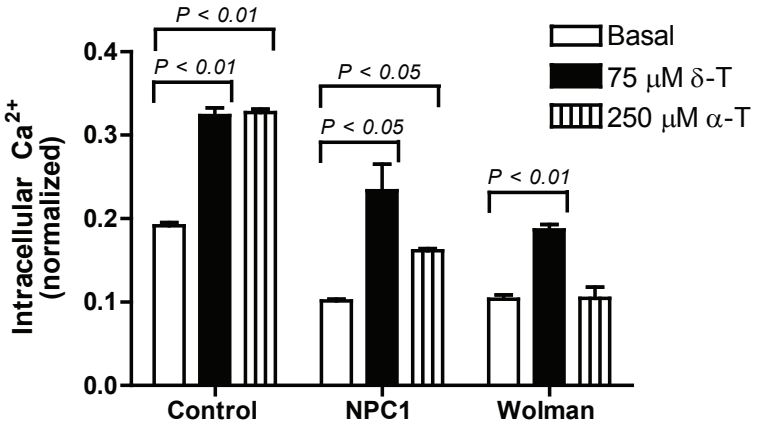


Figure S5. [^{3}H] -cholesterol efflux in the ABCG1 and SRB1 overexpressing cells in the presence of or absence of δ-T and α-T. ^{3}H-cholesterol efflux greatly increased in the ABCG1 transformed cells and slightly increased in the SR-BI transformed cells. δ-T was able to further increase the cholesterol efflux in both transformed cell lines.

A. Cytosolic Ca^{2+} response in the absence of extracellular Ca^{2+}



B. Lysosomal Ca^{2+} release in the absence of extracellular Ca^{2+}

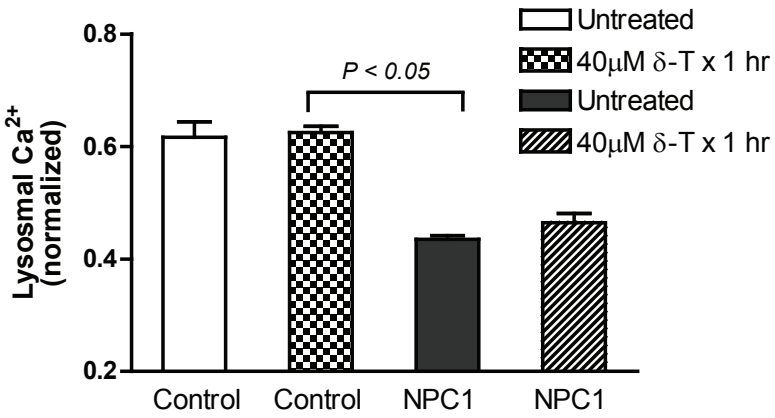


Figure S6. Cytosolic Ca^{2+} response to δ-T and α-T, lysosomal Ca^{2+} release induced by GPN in the absence extracellular Ca^{2+}. **(A)** Similarly as in the presence of extracellular Ca^{2+} (Fig. 4B), 75 μM δ-T significantly stimulated cytosolic Ca^{2+} release in the absence of extracellular Ca^{2+} in both NPC1 and Wolman fibroblasts. **(B)** Lysosomal Ca^{2+} release was induced by 200 nM Gly-Phe β-naphthylamide (GPN) in the absence of extracellular Ca^{2+} in NPC1 fibroblasts. In NPC1 cells, lysosomal Ca^{2+} release in response to GPN was 30 % less than that of control cells. This reduction of lysosomal Ca^{2+} release in NPC1 cells was not significantly altered after the cells were treated with 40 μM δ-T for 1 hour.

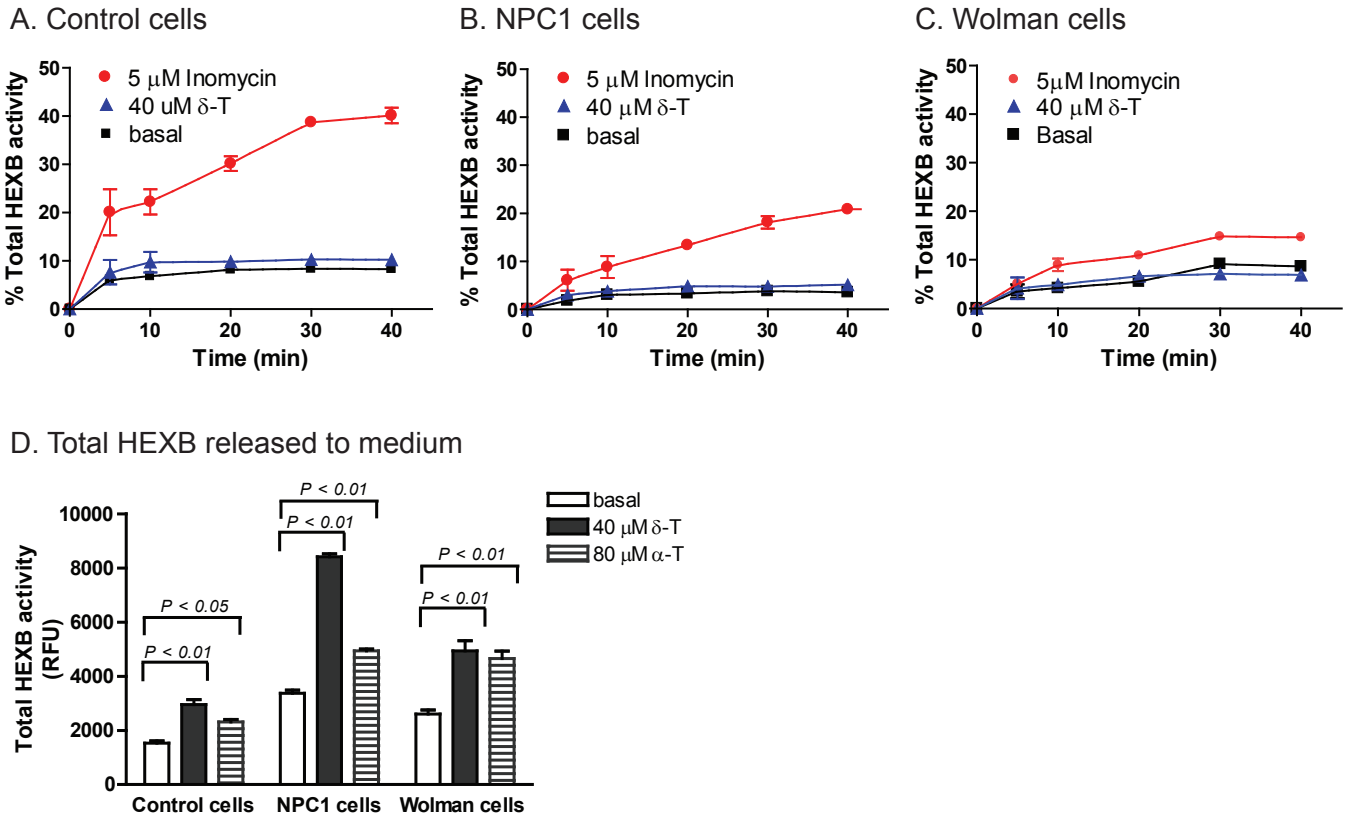


Figure S7. Lysosomal exocytosis in NPC1 and Wolman fibroblasts after one hour treatment with δ-T or α-T. When both compounds were added to NPC1 **(B)** and Wolman **(C)** fibroblasts as well as control fibroblasts **(A)** for 1 hour, 40 μM δ-T or 80 μM α-T did not alter the activity of HEXB (a lysosomal enzyme) in extracellular medium. This data indicated that 40 μM δ-T or 80 μM α-T did not have the immediate effect on exocytosis in these cells, which was differently from the significant enhancement of exocytosis observed after a 24-hour treatment of 40 μM δ-T or 80 μM α-T in these cells (Fig. 5). (D) Increase in the total HEXB enzyme activity secreted into the medium in 30 min from the fibroblasts after a 24 hr pretreatment with 40 μM δ-T or 80 μM α-T. The experiment was performed in 24-well plates with cells at 30,000 cells/well. In all three cell types, δ-T and α-T significantly stimulated the secretion of HEXB into the medium. The basal HEXB secreted into the medium in both NPC1 and Wolman was also significantly higher than that in control cells ($P<0.01$ in both). The data shown are mean of three experiments and the error bars represent SD.