

Supplemental material (Figures):

Fig. S1. Diagram of the structure of biotinylated lipids used in this study.

Fig. S2. FACS plots depicting binding of ceramide, ceramide-1-phosphate (C1P), lactosylceramide (LC), and diacylglycerol (DG) to HPCA displayed on yeast. All the lipids were tested at a concentration of 800 nM. Lipid binding is represented in the y-axis and surface expression level (Xpress epitope) is represented in the x-axis.

Fig. S3. Relative binding of ceramide, ceramide-1-phosphate (C1P), lactosylceramide (LC), and diacylglycerol (DG) to the 20 tested clones. Values are expressed as a ratio of the lipid-binding MFI to the Xpress epitope (surface expression) MFI normalized to an empty pYD1 vector display control.

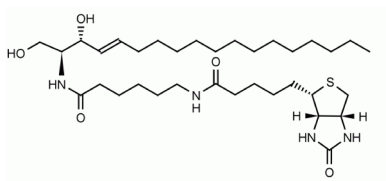
Fig. S4. Diagram showing a 3-dimensional representation of amino acid residues in bovine neurocalcin (NCALD) and human NCS1 that correspond to mutations in HPCA that reduce ceramide binding. Yellow residues are equivalent to residues that were confirmed to reduce binding in the context of HPCA 1+2 or HPCA 3+4. Red residues are equivalent to residues confirmed to reduce binding in the context of HPCA 1+2 or HPCA 3+4 as well as in combination in the context of full-length HPCA. The first and second EF-hands (roughly equivalent to HPCA 1+2) are shown in green and the third and fourth EF-hands (roughly equivalent to HPCA 3+4) are shown in cyan. Calcium ions are shown in yellow. A, Amino acids in bovine neurocalcin equivalent to positions whose mutation reduce ceramide binding in HPCA. Diagram is based on previously published crystal structure of bovine neurocalcin (38), (RCSB PDB 1BJF). B, Amino

acids in human NCS1 equivalent to positions whose mutation reduce ceramide binding in HPCA.

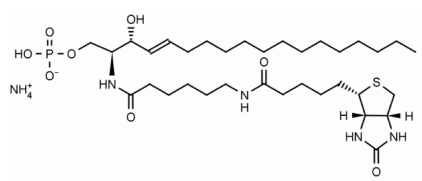
Diagram is based on previously published crystal structure of human NCS1 (39), (RCSB PDB 1G8I).

Fig. S1.

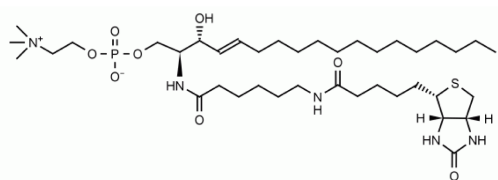
Ceramide-biotin



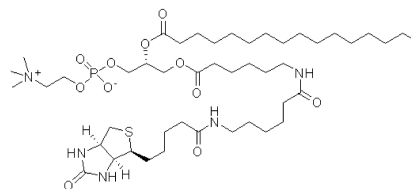
C1P-biotin



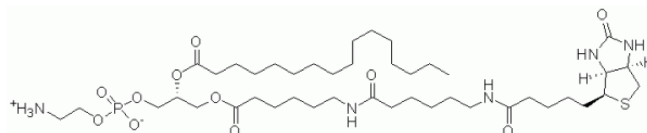
Sphingomyelin-biotin



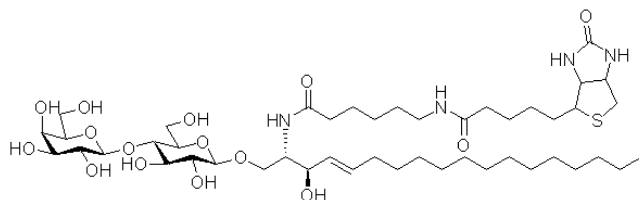
Phosphatidylcholine-biotin



Phosphatidylethanolamine-biotin



Lactosylceramide-biotin



Diacylglycerol-biotin

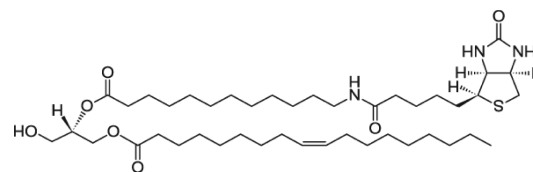


Fig. S2.

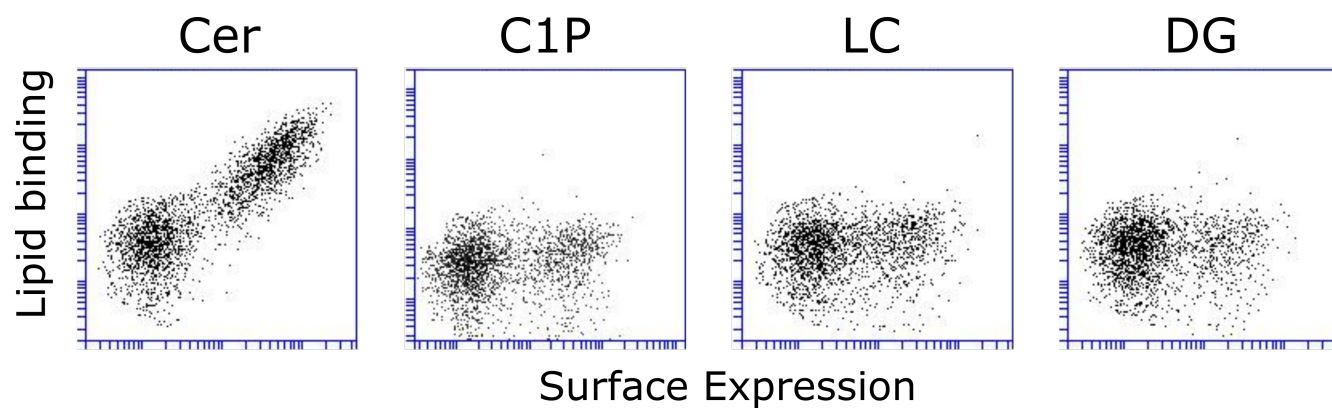
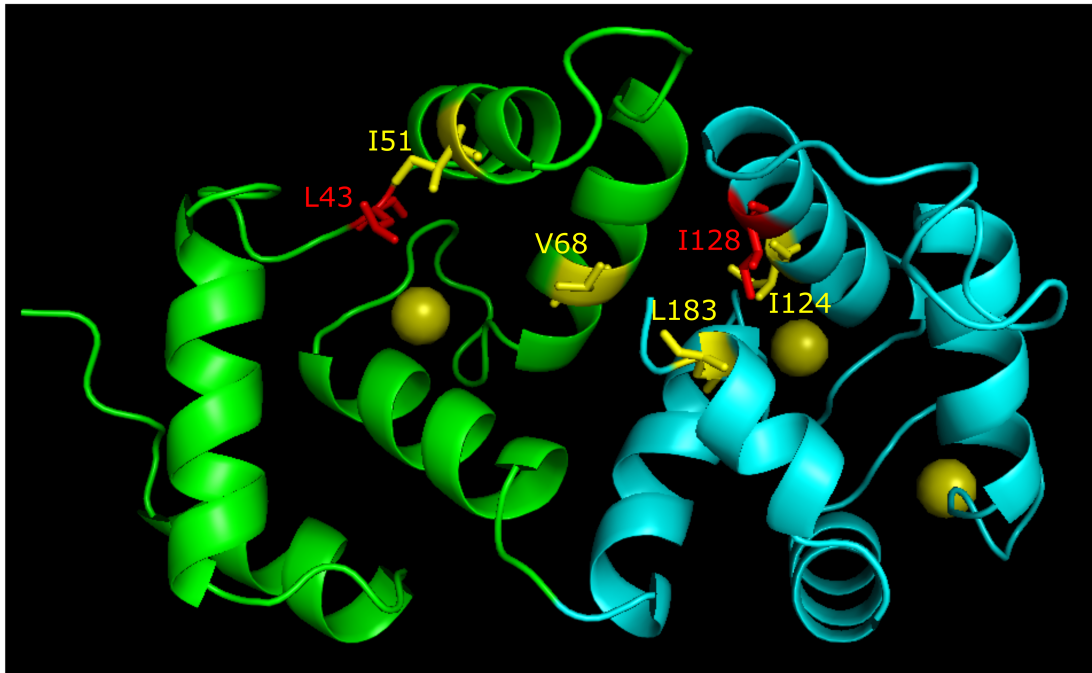


Fig. S3.

Protein	Cer	C1P	LC	DG
HSD17B4	6.33	1.50	1.61	0.79
HPCA	8.68	0.15	0.15	0.00
ST13	5.18	2.15	1.18	0.22
UBQLN2	16.10	2.18	1.96	0.80
PSAP	11.35	2.88	2.32	0.73
RAD23A	15.06	2.58	1.34	0.21
SGTA	4.67	1.45	0.92	0.27
HPCAL1	6.95	0.51	0.92	0.00
TTC22	6.24	1.23	1.41	0.62
ADRM1	1.49	1.06	0.86	0.32
UBQLN4	5.49	2.20	1.57	0.47
VSNL1	7.30	1.56	0.93	0.27
PTGDS	2.31	4.80	1.95	1.11
NCS	7.62	0.71	0.90	0.28
SIN3A	9.12	1.60	1.16	0.74
MYL6B	2.54	0.32	0.32	0.16
PPEF1	4.29	1.11	0.97	0.30
EFCAB6	0.89	0.45	0.35	0.17
CABP1	1.07	0.61	0.36	0.22
CALM3	0.00	0.00	0.00	0.02

Fig. S4.

A



B

