Expanded View Figures

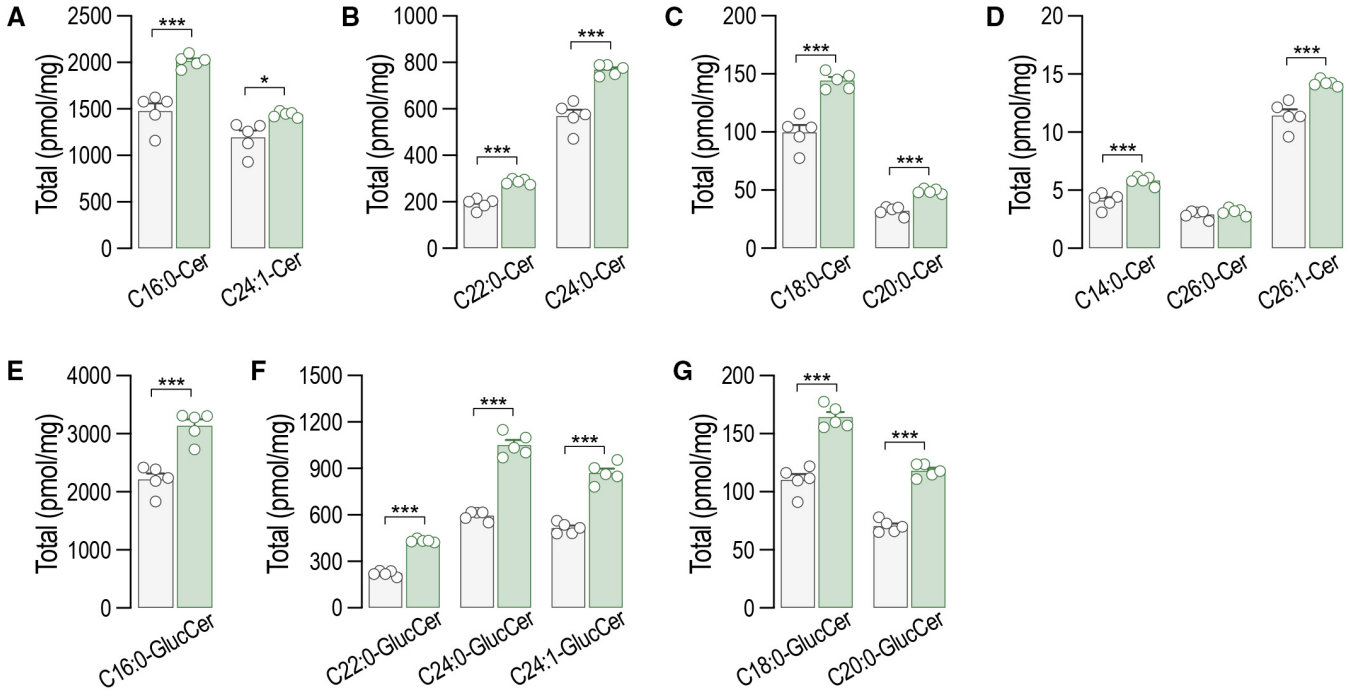


Figure EV1. Ceramide and glucosylceramide levels are increased in S1pr1^{ECKO} mEC compared to S1pr1$^{f/f}$.

A–G LC–MS/MS quantification of total and specific (A–D) ceramides and (E–G) glucosylceramides in S1pr1$^{f/f}$ and S1pr1^{ECKO} mEC after 4-OHT (1 μM, 72 h) treatment (n = 5/group from two independent EC isolations/group; four mice/EC isolation).

Data information: Data are expressed as mean ± SEM. ***$P \leq 0.001$. Statistical significance was determined by unpaired t-test.
Source data are available online for this figure.

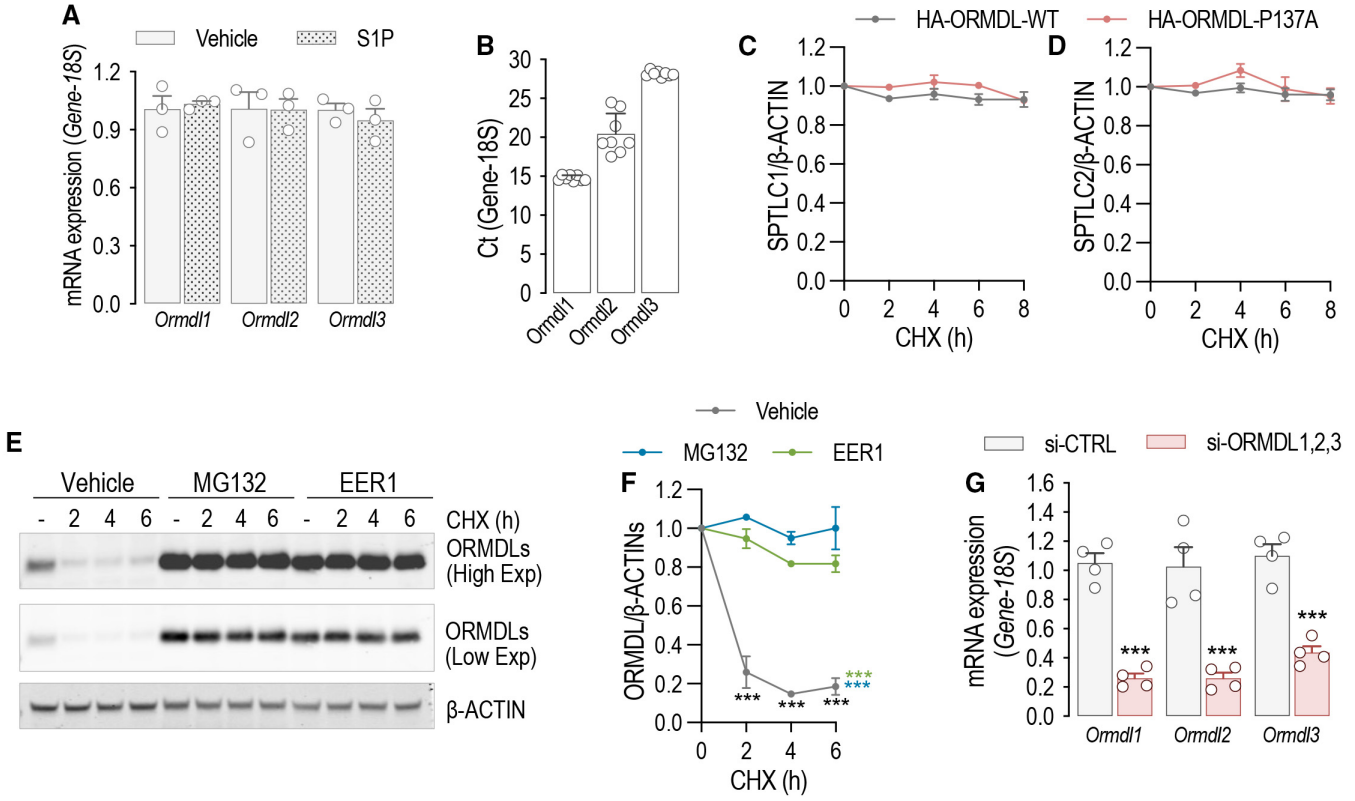


Figure EV2. S1P inhibits SPT activity via ORMDLs stabilization.

A RT-PCR for Ormdl1, Ormdl2, and Ormdl3 in the presence or absence of S1P (300 nM, 30′) ($n = 3$ biological replicates).

B Ormdl isoforms mRNA abundance determined subtracting the Ct of housekeeping 18S from the Ct of Ormdls ($n = 8$ biological replicates).

C, D Quantification of © SPTLC1 and (D) SPTLC2 levels from Fig 1G.

E, F (E) Western blot analysis for ORMDLs in HUVEC lysates treated with CHX (10 μM) for the indicated period of time, in the presence or absence of MG132 (10 μM) or EER1 (10 μM) and (F) relative quantification.

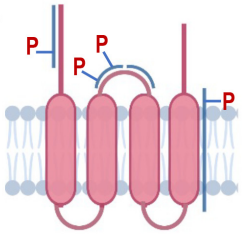
G RT-PCR for Ormdl1, Ormdl2, and Ormdl3 before and after independent silencing with the corresponding siRNA (40 nM, 72 h) ($n = 4$ biological replicates).

Data information: Western blot and RT-PCR are representative of three or more independent biological replicates. β-ACTIN, loading control. Data are expressed as mean ± SEM. ***$P \leq 0.001$. Statistical significance was determined by two-way ANOVA with Tukey's post-test (C, D, F) and unpaired t-test (A, G).
Source data are available online for this figure.

A

MYPYDVPDYANVGTAHSEVN**P**NTRVMNSRGIWLSYVLAIGLLHIVLLSIPFVSVPVVWTLTNLIHNMGMYIFLHTVKGT**P**FET**P**DQ GKARLLTHWEQMDYGVQFTASRKFLTITPIVLYFLTSFYTKYDQIHFVLNTVSLMSVLI**P**KLPQLHGVRIFGINKY

B



C Peptide summary

Sequence: YDQIHFVLNTVSLMSVLIPK, P137-oxidation (15.99492 Da)

Charge: +2, Monoisotopic m/z: 1167.1286 Da (-3.72 mmu/-3.19 ppm), MH+: 2333.24993 Da, RT: 72.7453 min

Identified with: Sequest HT (v1.17); Xcorr: 3.44, Percolator q-Value: 7.1e-4, Percolator PEP: 4.0e-3

Fragment match tolerance used for search: 0.6 Da

Fragments used for search: -H_2O; v; -NH_3; v; b; b; -H_2O; b; -NH_3; v

D

Position	b+	b2+	aa	y+	y2+
119	164.0706	82.5389	Y		
120	279.0976	140.0524	D	2170.1940	1085.6007
121	407.1561	204.0817	Q	2055.1671	1028.0872
122	520.2402	260.6237	I	1927.1085	964.0579
123	657.2991	329.1532	H	1814.0245	907.5159
124	804.3675	402.6874	F	1676.9655	838.9864
125	903.4359	452.2216	V	1529.8971	765.4522
126	1016.5200	508.7636	L	1430.8287	715.9180
127	1130.5629	565.7851	N	1317.7447	659.3760
128	1231.6106	616.3089	T	1203.7017	602.3545
129	1330.6790	665.8431	V	1102.6540	551.8307
130	1417.7110	709.3592	S	1003.5856	502.2965
131	1530.7951	765.9012	L	916.5536	458.7804
132	1661.8356	831.4214	M	803.4695	402.2384
133	1748.8676	874.9375	S	672.4291	336.7182
134	1847.9360	924.4717	V	585.3970	293.2022
135	1961.0201	981.0137	L	486.3286	243.6679
136	2074.1042	1037.5557	I	373.2446	187.1259
137	2187.1518	**1094.0796**	**P**	260.1605	130.5839
138			K	147.1128	74.0600

E

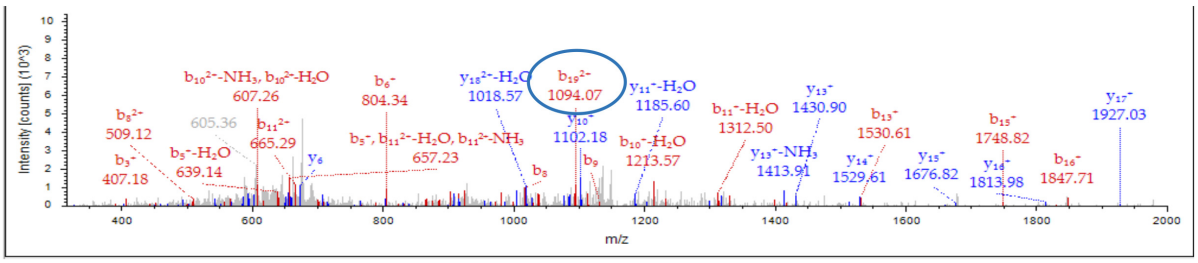


Figure EV3. ORMDL3 is hydroxylated at P137.

A HA-ORMDL3 sequence. In green HA-tag; in blue the peptides identified by MS; in red the prolines identified my MS.
B Graphical representation of the peptides identified by MS and prolines localization.
C Summary for the peptide containing P137.
D Ion masses for the amino acids in the peptide.
E Representative mass spectrometry showing the P137 hydroxylated.

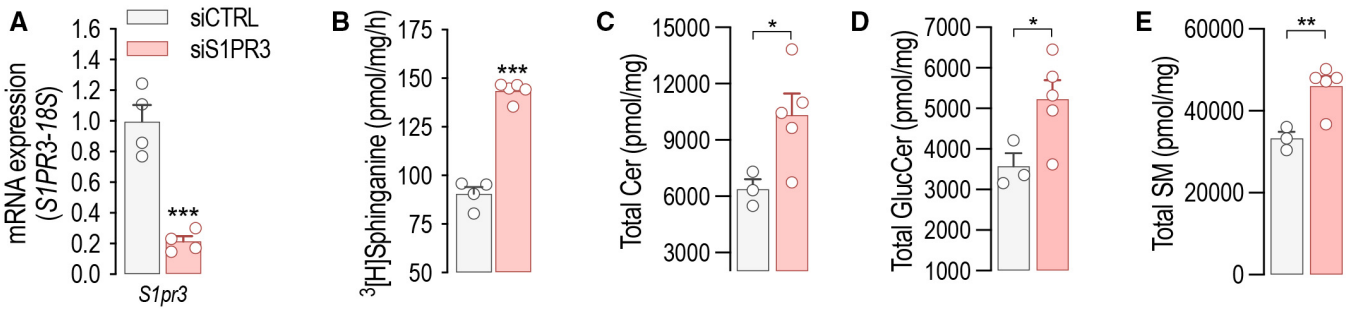


Figure EV4. S1pr3 deletion leads to increased SPT activity and SL levels.

A, B (A) RT-PCR for S1pr3 and (B) SPT activity in mEC after siCTRL or siS1PR3 treatment (40 nM, 72 h) (n = 4 biological replicates).

C–E LC–MS/MS quantification of total (C) Ceramide, (D) Glucosylceramide, and (E) Sphingomyelin in murine endothelial cell (mEC) after siCTRL or siS1PR3 treatment (40 nM, 72 h) (n = 5/group from two independent EC isolations/group; 4 mice/EC isolation).

Data information: Data are expressed as mean ± SEM. *$P \leq 0.05$; ***$P \leq 0.001$. Statistical significance was determined by unpaired t-test.
Source data are available online for this figure.

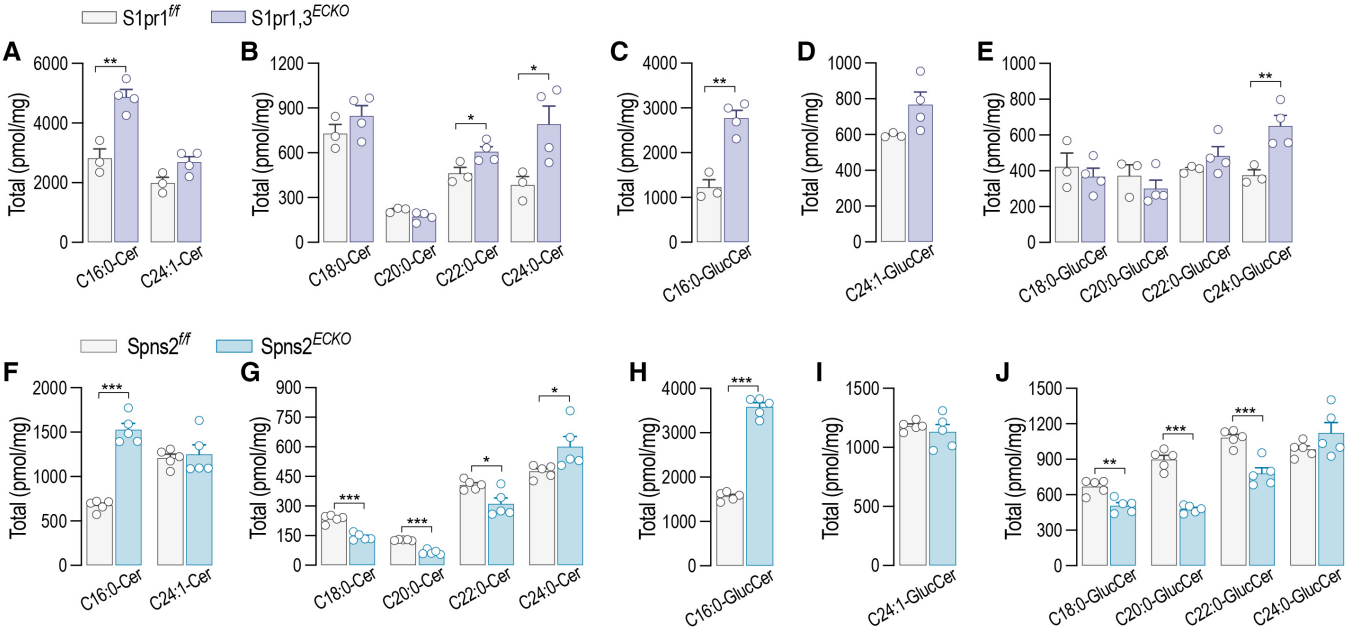


Figure EV5. Endothelial-derived S1P inhibits SL biosynthesis via S1PR1,3.

A–E LC–MS/MS quantification of specific (A and B) ceramides and (C–E) glucosylceramides in S1pr1,3$^{f/f}$ and S1Pr1,3^{ECKO} murine endothelial cell (mEC) after 4-OHT (1 μM, 72 h) and siS1PR3 (40 nM, 72 h) treatments (n = 5/group from two independent EC isolations/group; four mice/EC isolation).

F–J LC–MS/MS quantification of specific (F and G) ceramides and (H–J) glucosylceramides in Spns2$^{f/f}$ and Spns2^{ECKO} mEC after 4-OHT (1 μM, 72 h) treatment (n = 5/group from two independent EC isolations/group; four mice/EC isolation).

Data information: Data are expressed as mean ± SEM. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. Statistical significance was determined by unpaired t-test.
Source data are available online for this figure.