Supplemental table 1. Oligonucleotide primers designed for PCR amplification of cDNAs

| Gene | Sequence | Restriction site |
|---|---|---|
| mouse | 5'-gccaagcttaaccatggaaccagaatgccc-3' | *Hin*d III |
| *Sphk1* | 5'-ggcgcggccgcttaaagagcgtaatctggaacatcgtatgggtatggttcttctggaggtggcccccgc-3' | *Not* I |
| mouse | 5'-gccaagcttgggtcatggccccaccaccac-3' | *Hin*d III |
| *Sphk2* | 5'-ggcgcggccgctcaaagagcgtaatctggaacatcgtatgggtaggcttgtggcttttgacc-3' | *Not* I |
| human | 5'-gccgctagcgggccggcatgatgtgc-3' | *Nhe* I |
| *SPNS2* | 5'-ctagcggccgctcagactttcacagatgcgggcg-3' | *Not* I |
| mouse | 5'-cggactagtaccatggcttgttggcctcagttaaggct-3' | *Spe* I |
| *Abca1* | 5'-cgctcgagctgtgtgaacgggattcttcatacataac-3' | *Xho* I |
| human | 5'-gccgctagccgttccaaggagcgcgaggtcgg-3' | *Nhe* I |
| *ABCB1* | 5'-ggcctcgagttcactggcgctttgttccagc-3' | *Xho* I |
| mouse | 5'-cggaagcttccatcatggcgctgcgcagcttc-3' | *Hin*d III |
| *Abcc1* | 5'-ggcgcggccgctcacaccaagccagcatcc-3' | *Not* I |
| human | 5'-ggcaagcttctctccagatgtcttccag-3' | *Hin*d III |
| *ABCG2* | 5'-ggcggatccttaagaatatttttttaagaaataac-3' | *Bam*H I |

Supplemental Figure 1. Intracellular contents of sphingolipids. The CHO (mock), CHO/SPHK1 (SPHK1) and CHO/SPHK1/hSPNS2 (SPHK1/SPNS2) cells were collected, and the intracellular contents of S1P, DH-S1P, Sph and DH-Sph were measured by HPLC. $C_{17}$-Sph and $C_{17}$-S1P were used as the internal standard. Experiments were performed more than three times and *error bars* indicate the standard deviation.

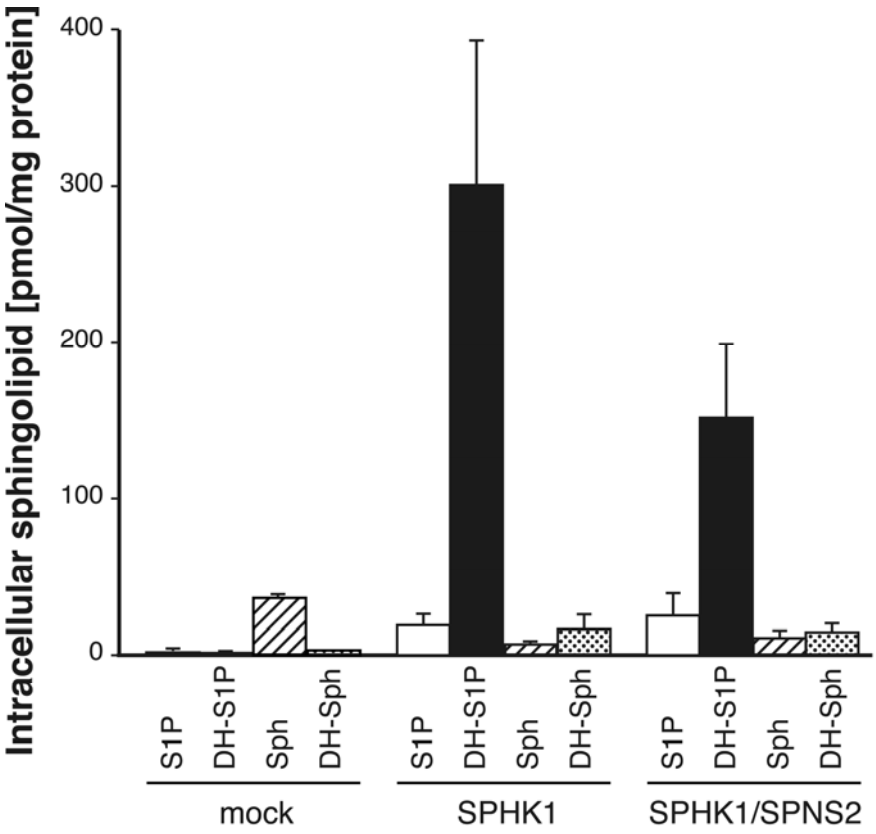
Supplemental Figure 2. hSPNS2 does not transport dephosphorylated forms of sphingolipids. CHO/SPHK1 (mock) or CHO/SPHK1/hSPNS2 (SPNS2) cells were grown for 2 days and incubated with releasing medium containing Sph, FTY720, DH-Sph, phyto-Sph or $C_{17}$-Sph at 5 μM for 1 h. Subsequently, cells were washed twice and incubated with releasing medium for 2 h. Releasing media were collected and sphingolipid content was measured by HPLC. $C_{17}$-Sph and $C_{17}$-S1P were used as the internal standard for measuring S1P, FTY720-P, DH-S1P and phyto-S1P, whereas phyto-Sph and phyto-S1P were used as the internal standard for measuring $C_{17}$-Sph and $C_{17}$-S1P.

Supplemental Figure 3. Activity of several different ABC transporters. *A*. ApoA-I dependent cholesterol efflux assay was performed with ABCA1-expressing CHO cells as reported previously (1). Briefly, CHO/SPHK1(mock) and CHO/SPHK1/ABCA1 (ABCA1) cells were incubated with F-12 medium containing 10% FBS and 0.5 μCi/ml [$^3$H] cholesterol (PerkinElmer Life Sciences) at 37 °C for 24 h. Cells were washed with F-12 medium containing 0.2 % BSA three times and incubated with medium containing 0.2 % BSA and 10 μg/ml apoA-I (Calbiochem) at 37 °C for 6 h in the presence or absence of glyburide (Sigma). Cholesterol efflux was indicated as the percentage of total radiolabeled cholesterol in cells and medium. *B*. Calcein-AM export assay was performed to measure ABCB1 and ABCC1 activity as reported previously (2). Briefly, CHO/SPHK1 (mock), CHO/SPHK1/ABCB1 (ABCB1) and CHO/SPHK1/ABCC1 (ABCC1) cells were washed with warmed HBSS (10 mM HEPES) three times and preincubated in HBSS (10 mM HEPES) at 37 °C for 30 min. After preincubation, calcein-AM (MoBiTec) was added at 1 μM and incubated at 37 °C for 60 min. After incubation, the cells were washed with ice-cold HBSS (10 mM HEPES) twice and lysed with 1 % triton X-100. The fluorescence of the calcein generated inside the cells was measured with a fluorescence spectrophotometer (excitation 485 nm; emission 535 nm). *C*. Hoechst 33342 export assay was used to measure ABCG2 activity as reported previously (3). The CHO/SPHK1 (mock) and CHO/SPHK1/ABCG2 (ABCG2) cells were washed with HBSS three times and preincubated in HBSS at 37 °C for 30 min. After preincubation, Hoechst 33342 (Invitrogen) was added at 1 μM and incubated at 37 °C for 90 min. Subsequently, cells were washed with ice-cold HBSS three times and incubated at 37 °C for 30 min in

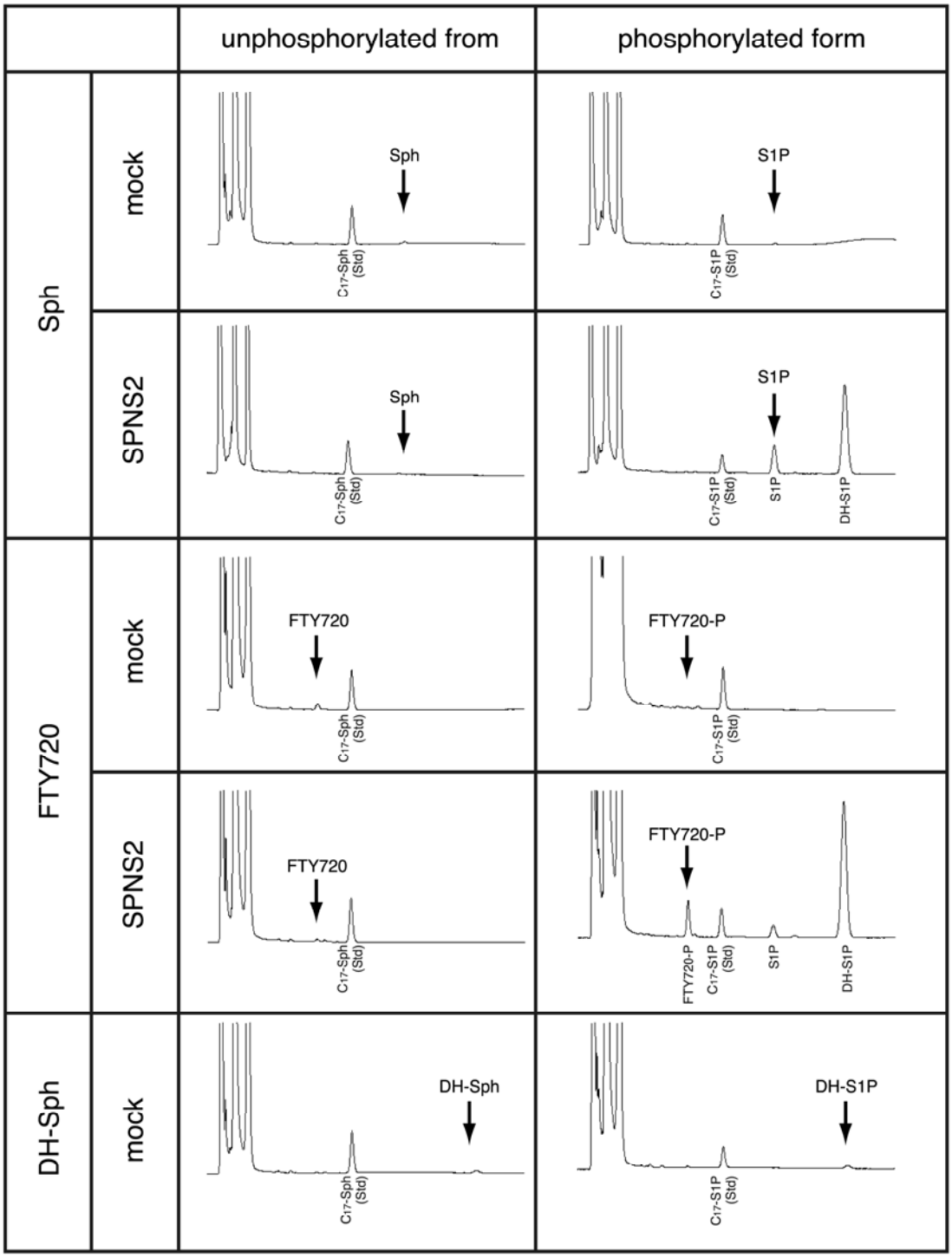
HBSS. After incubation, cells were washed with ice-cold HBSS three times and lysed with lysis buffer (0.1 % triton X-100 and 0.1 N NaOH). The fluorescence of the intracellular Hoechst 33342 was measured with a fluorescence spectrophotometer (excitation 370 nm; emission 450 nm).

Supplemental Figure 4. ABCA7 knock-out mice were generated by replacing exons 15-17 with a neomycin cassette as described previously (4). Deletion of the corresponding gene was confirmed by Southern blotting and PCR. *A*. Platelets were prepared from wild type (WT), ABCA7 +/- (HET) and ABCA7 -/- (KO) mice with a modified protocol as described previously (5). Briefly, each mouse (8-10 weeks old) was anesthetized and whole blood (about 800 μl) was collected from the heart with acid citrate-dextran solution (ACD) used as an anticoagulant. Then collected blood was centrifuged at 150 × *g* for 15 min to obtain the platelet-rich plasma (PRP). Platelets were prepared by centrifugation of PRP at 1,000 × *g* for 10 min and washed with buffer A (20 mM HEPES-NaOH, pH 7.4, 3.3 mM $NaH_2PO_4$, 2.9 mM KCl, 1 mM $MgCl_2$, 138 mM NaCl and 1 mg/ml glucose) with ACD. Resulting platelets were suspended in buffer A with 1 % BSA. [$^3$H] Sph was added to the platelet suspension and the S1P export with (+) or without (-) thrombin was measured. Lipids were extracted from the platelets (P) and supernatant (S) and analyzed with thin-layer chromatography (TLC). S1P was added to the TLC plate as a standard; the positions of ceramide, Sph and S1P are indicated as Cer, Sph and S1P, respectively. *B*. Wild type (WT) and ABCA7 -/- mouse (KO) erythrocytes were prepared and used for a S1P transport assay as described previously (6). [$^3$H] Sph was added to the erythrocyte suspension and the cells and medium were separated at the indicated time point by centrifugation. Intracellular and extracellular amounts of Sph and S1P were determined by lipid extraction from the supernatant and cells followed by TLC analysis. Erythrocytes isolated from the KO mice synthesized S1P inside the cells and exported S1P to a similar degree as erythrocytes from wild type mice.

Supplemental Figure 1.

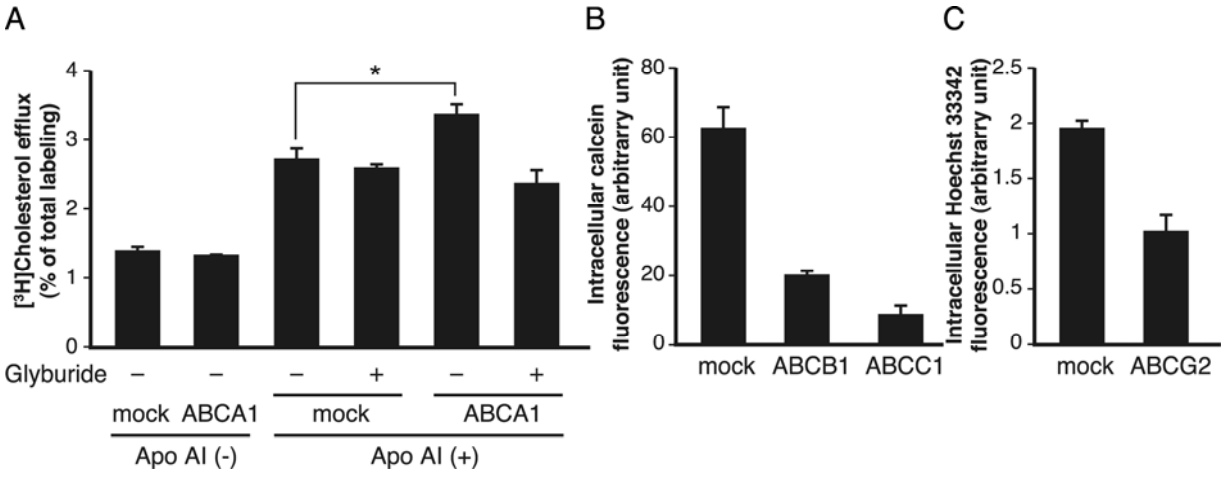


Supplemental Figure 2.



unphosphorylated from
phosphorylated form
DH-Sph
SPNS2
DH-Sph
C17-Sph (Std)
DH-S1P
C17-S1P (Std)
S1P
DH-S1P
phyto-Sph
mock
phyto-Sph
C17-Sph (Std)
phyto-S1P
C17-S1P (Std)
SPNS2
phyto-Sph
C17-Sph (Std)
phyto-S1P
phyto-S1P
C17-S1P (Std)
S1P
DH-S1P
C17-Sph
mock
C17-Sph
phyto-Sph (Std)
C17-S1P
phyto-S1P (Std)
SPNS2
C17-Sph
phyto-Sph (Std)
C17-S1P
phyto-S1P (Std)
C17-S1P
S1P
DH-S1P

Supplemental Figure 3.



Supplemental Figure 4.