Supplemental figure 1. Effect of myriocin on the cellular viability. LDH release to the culture medium was analyzed. BHK/ABCB4 and BHK/ABCA1 cells were treated without (empty bars) and with 10 nM mifepristone (filled bars) for 16 h and then incubated with 0-80 μ M myriocin for 24 h. Experiments were performed in triplicate, and the average values are shown with the S.D.

Supplemental figure 2. Effect of myriocin on ABC protein-independent release of PC and cholesterol from HEK293 cells. HEK293 cells were pre-treated with 0 or 20 μM myriocin for 24 h. Then, cells were incubated in the absence or presence of 1 mM NaTC (A) or 10 μg/ml apoA-I (B) for 24 h. Amounts of PC (A) and cholesterol (B) in the medium were measured.

Supplemental figure 3. Effect of myriocin on the amount of ABCB4 and ABCA1 expressed in HEK293 cells. HEK293 cells were treated with 0 or 20 μM myriocin for 24 h. Cell lysates (10 μg of protein) were separated by 7% polyacrylamide gel electrophoresis, and ABCB4 and ABCA1 were analyzed by Western blotting. Vinculin was analyzed as a loading control.

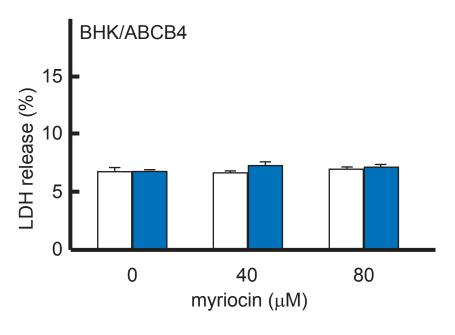
Supplemental figure 4. Mifepristone-induced ABCB4 and ABCA1 expression in BHK cells. BHK/ABCB4 (A) and BHK/ABCA1(B) cells were treated with 10 nM mifepristone for 24 h and ABCB4 and ABCA1 were analyzed by Western blotting. Vinculin was analyzed as a loading control.

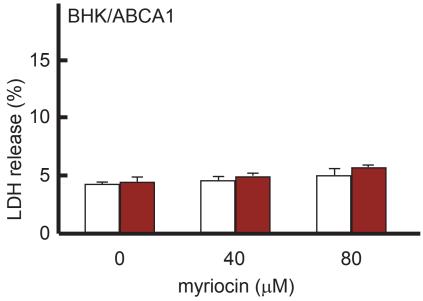
Supplemental figure 5. ABCA1-dependent PC (A) and cholesterol (B) efflux to apoA-I from BHK/ABCA1 cells. BHK/ABCA1 cells were pre-treated with 0 or 10 nM mifepristone for 24 h. Then, cells were incubated with (filled bars) or without (empty bars) 10 μg/ml apoA-I in the absence or presence of 40 μM myriocin for 16 h. Then PC (A) and cholesterol (B) contents in the medium were measured.

Supplemental figure 6. Effect of HPA-12 on ABC protein-independent release of PC and cholesterol from BHK/ABCA1 cells. BHK/ABCA1 cells were pre-treated with 0 or 10 μ M HPA-12 for 24 h. Then, cells were incubated with 0 or 10 μ M HPA-12 in the absence or presence of 1 mM NaTC or 10 μ g/ml apoA-I for 24 h. Cholesterol (A, C) and PC (B, D) in the medium were then measured.

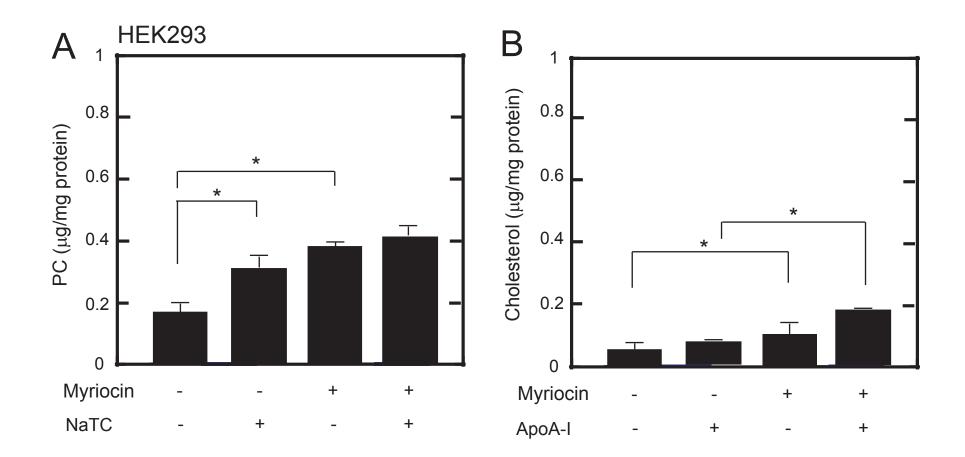
Supplemental figure 7. Effect of myriocin and HPA-12 on ABCB4 and ABCA1 expression. BHK/ABCB4 (A) and BHK/ABCA1 (B) cells were incubated with or without 40 μM myriocin or 10 μM HPA-12 in the presence of 10 nM mifepristone for 24 h. In the case of HPA-12, cells were pretreated with HPA-12 before the addition of mifepristone. Expression of ABCB4 and ABCA1 were then analyzed by Western blotting. Vinculin was analyzed as a loading control. Lower panels show the relative expression of ABCB4 and ABCA1.

Supplemental figure 8. The extracted ion chromatograms for standards of SM and PC. Mixture of SM d18:1/16:0 and PC 16:0/18:1 (1 ng each) was subjected to LC-ESI-MS/MS analysis. SM and PC standards were detected by scanning for neutral loss of 74 Da in the negative ion mode.

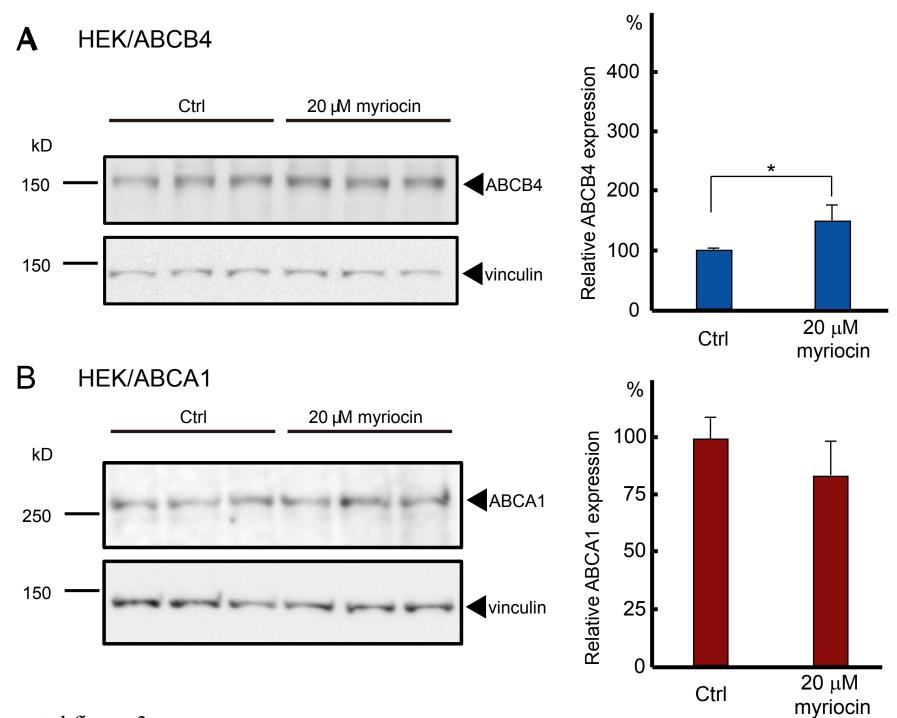


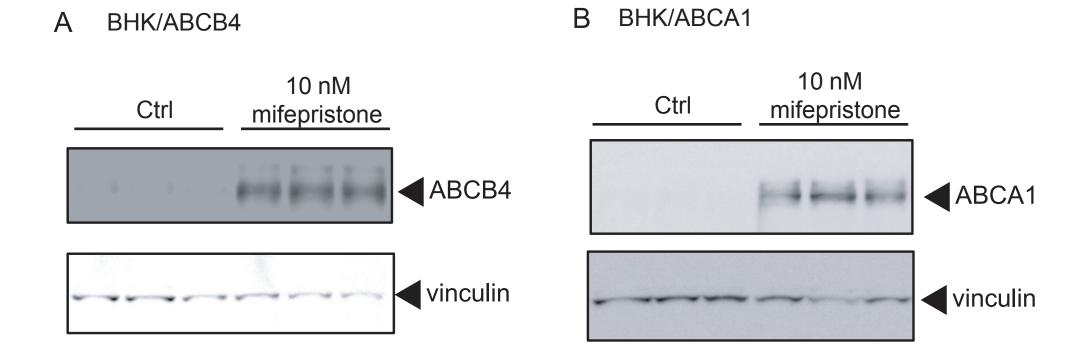


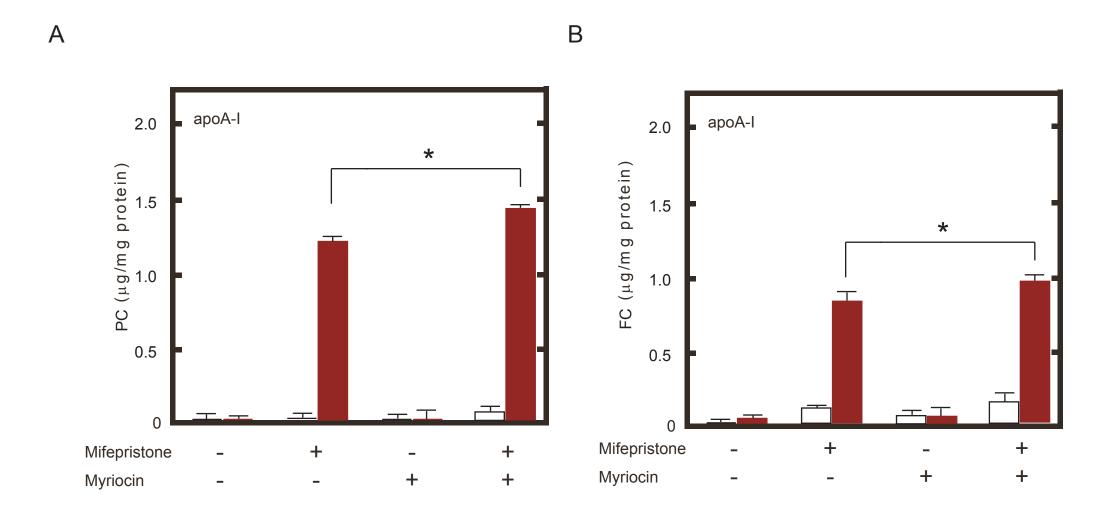
Supplemental figure 1

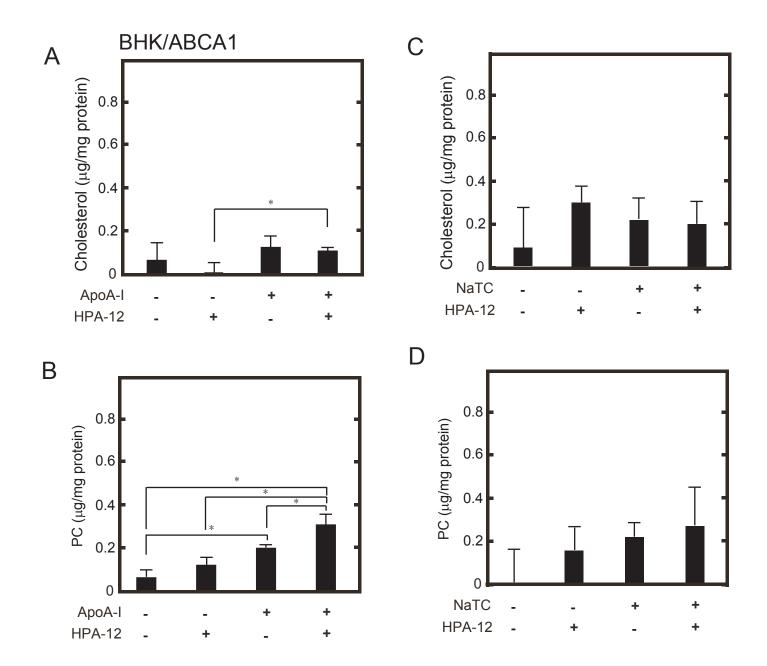


Supplemental figure 2

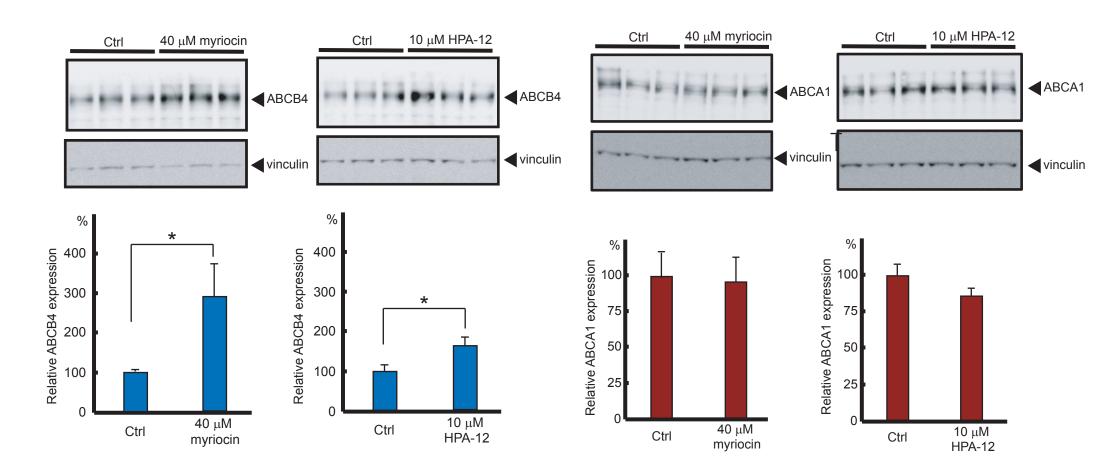


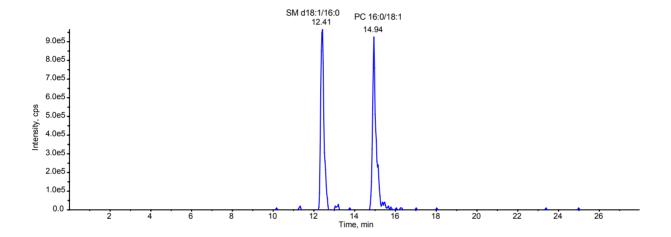






Supplemental figure 6





Supplemental figure 8