Supplementary Fig. 1. ApoA-I-dependence of ABCA1- and ABCB4-mediated lipid efflux. The efflux of cellular free cholesterol (A) and choline phospholipids (B) was analyzed. HEK/ABCA1-GFP cells, HEK/ABCA1-MM-GFP cells, and HEK/ABCB4 cells were incubated for 24 hours in DMEM containing 0.02% BSA (open bars) or 0.02% BSA and 5 μg/ml apoA-I (hatched bars). Experiments were performed in triplicate, and the average values are represented with the S.E. **, $p<0.001$, significantly different from values in the presence of BSA.

Supplementary Fig. 2. Effect of mutations on apoA-I binding to ABCA1-expressing cells. HEK/ABCA1-GFP, HEK/ABCA1-MM-GFP, or HEK/ABCA1-W590S-GFP cells were incubated with 5 μg/ml Alexa-546-labeled apoA-I for 15 min at 37°C. Bar, 20 μm.

Supplementary Fig. 3. Time-dependence of ABCA1-mediated lipid efflux. The efflux of cellular free cholesterol (A) and choline phospholipids (B) was analyzed. HEK/ABCA1-GFP cells were incubated for 12 or 24 hours with DMEM containing 0.02% BSA, 0.02% BSA and 1 mM NaTC (filled bars), or 0.02% BSA and 5 μg/ml apoA-I (hatched bars). NaTC- or apoA-I-dependent lipid efflux was calculated by subtracting the amount of lipid secreted into BSA. Experiments were performed in triplicate, and the average values are represented with the S.E. *, $p<0.05$; **, $p<0.001$, significantly different from values at 12h.

Supplementary Fig. 4. Effects of NaTC on the cellular viability. The LDH release to culture medium was analyzed. HEK cells, HEK/ABCA1-GFP cells, HEK/ABCA1-MM-GFP cells and HEK/ABCA1-W590S-GFP cells were incubated for 24 hours in DMEM containing

0.02% BSA (open bars) or 0.02% BSA and 1mM NaTC (filled bars). Experiments were performed in triplicate, and the average values are represented with the S.E.

Supplementary Fig. 5. Lipid efflux to NaTC-PC complexes. The efflux of cellular free cholesterol was analyzed. HEK cells and HEK/ABCA1-GFP cells were incubated for 24 hours in DMEM containing 0.02% BSA, 0.02% BSA and 1mM NaTC + 2 μM PC, or 0.02% BSA and 1mM NaTC. The values for free cholesterol efflux from HEK/ABCA1-GFP cells were shown for comparison. Experiments were performed in triplicate, and the average values are represented with the S.E. **, $p<0.001$, significantly different from values in the presence of BSA.

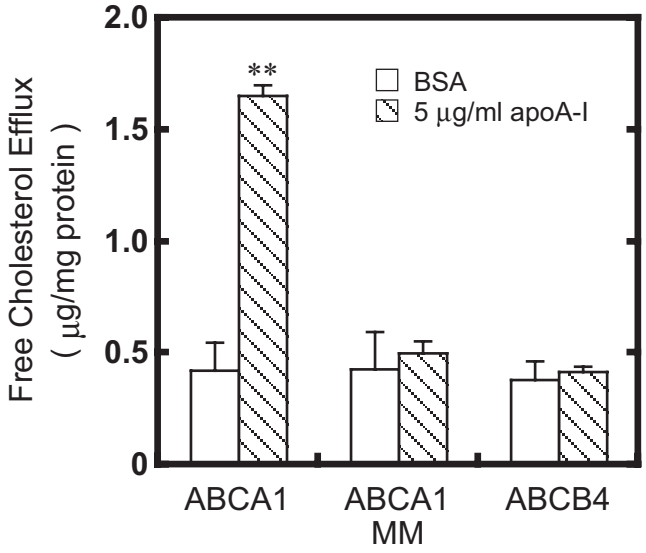
Supplementary Fig. 6. ABCA1-mediated lipid efflux at 27°C. The efflux of cellular free cholesterol was analyzed. HEK/ABCA1-GFP cells, HEK/ABCA1-W590S-GFP cells, and HEK/ABCA1-MM-GFP cells were incubated for 8 hours in DMEM containing 0.02% BSA and 10 μg/ml apoA-I at 27°C (open bars) or 37°C (filled bars). Experiments were performed in triplicate, and the average values are represented with the S.E.

Supplementary Fig. 7. Time-dependent apoA-I binding to ABCA1-expressing cells. Cells expressing ABCA1 or ABCA1-W590S, fused with GFP, were incubated with 5 μg/ml Alexa-546-labeled apoA-I for the indicated times at 27°C. Bar, 20 μm.

Supplementary Fig. 8. Time-dependent apoA-I dissociation from ABCA1-expressing cells. Cells expressing ABCA1 or ABCA1-W590S, fused with GFP, were incubated with 5

μg/ml Alexa-546-labeled apoA-I for 15 min at 37°C and further incubated with 25 μg/ml unlabeled apoA-I for the indicated times at 27°C. Bar, 20 μm.

A



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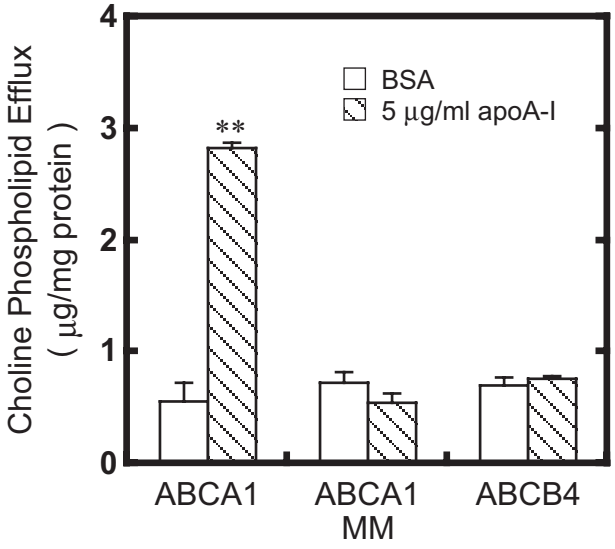
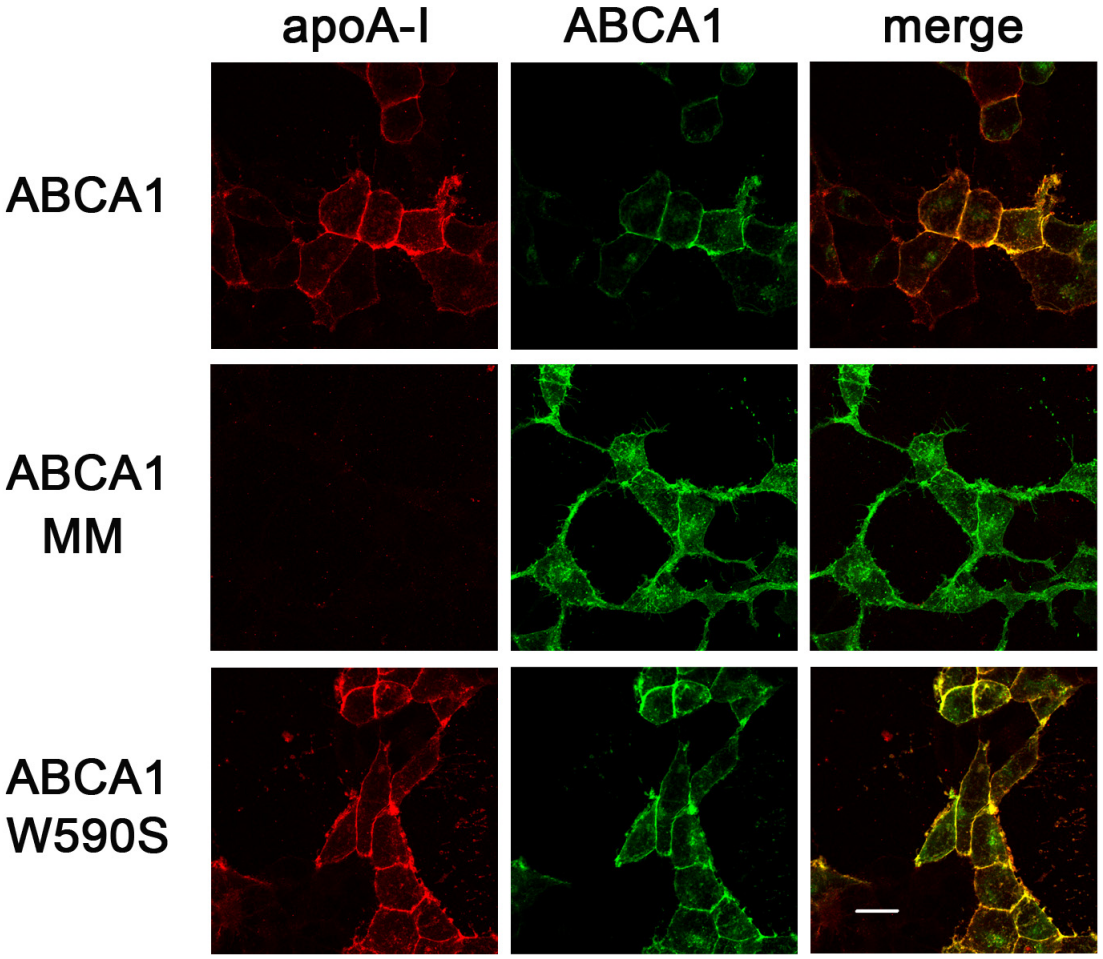
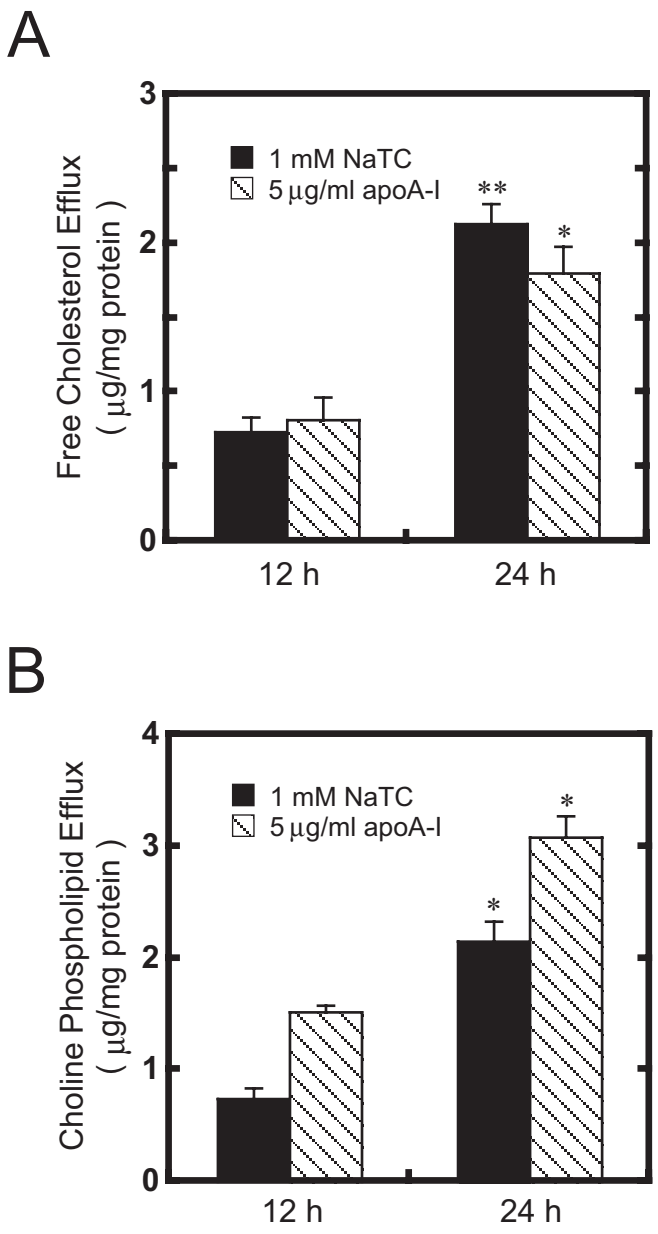


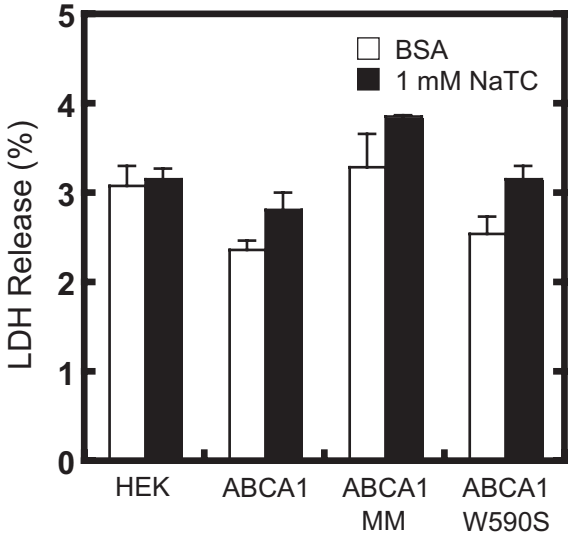
Supplementary Figure 1. Nagao,K et al.



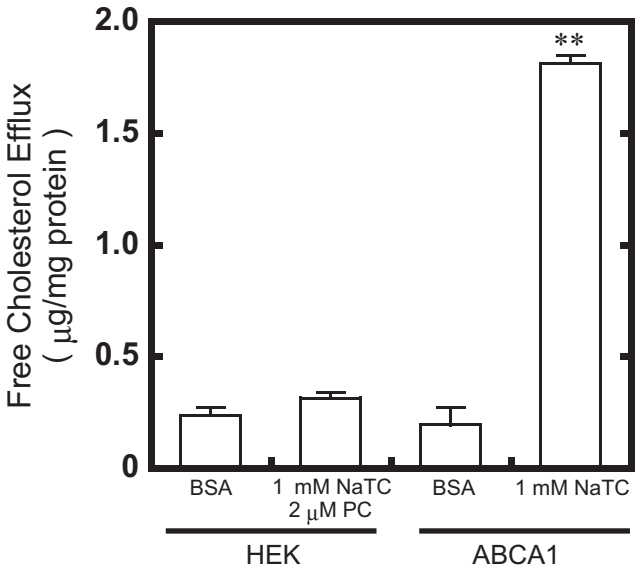
Supplementary Figure 2. Nagao,K et al.



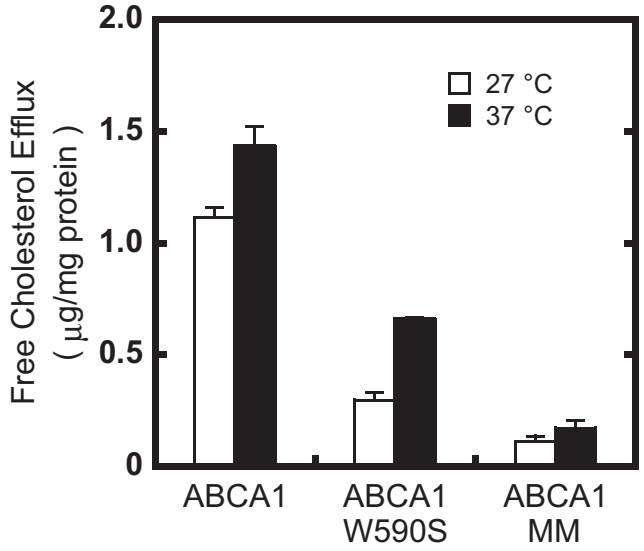
Supplementary Figure 3. Nagao,K et al.



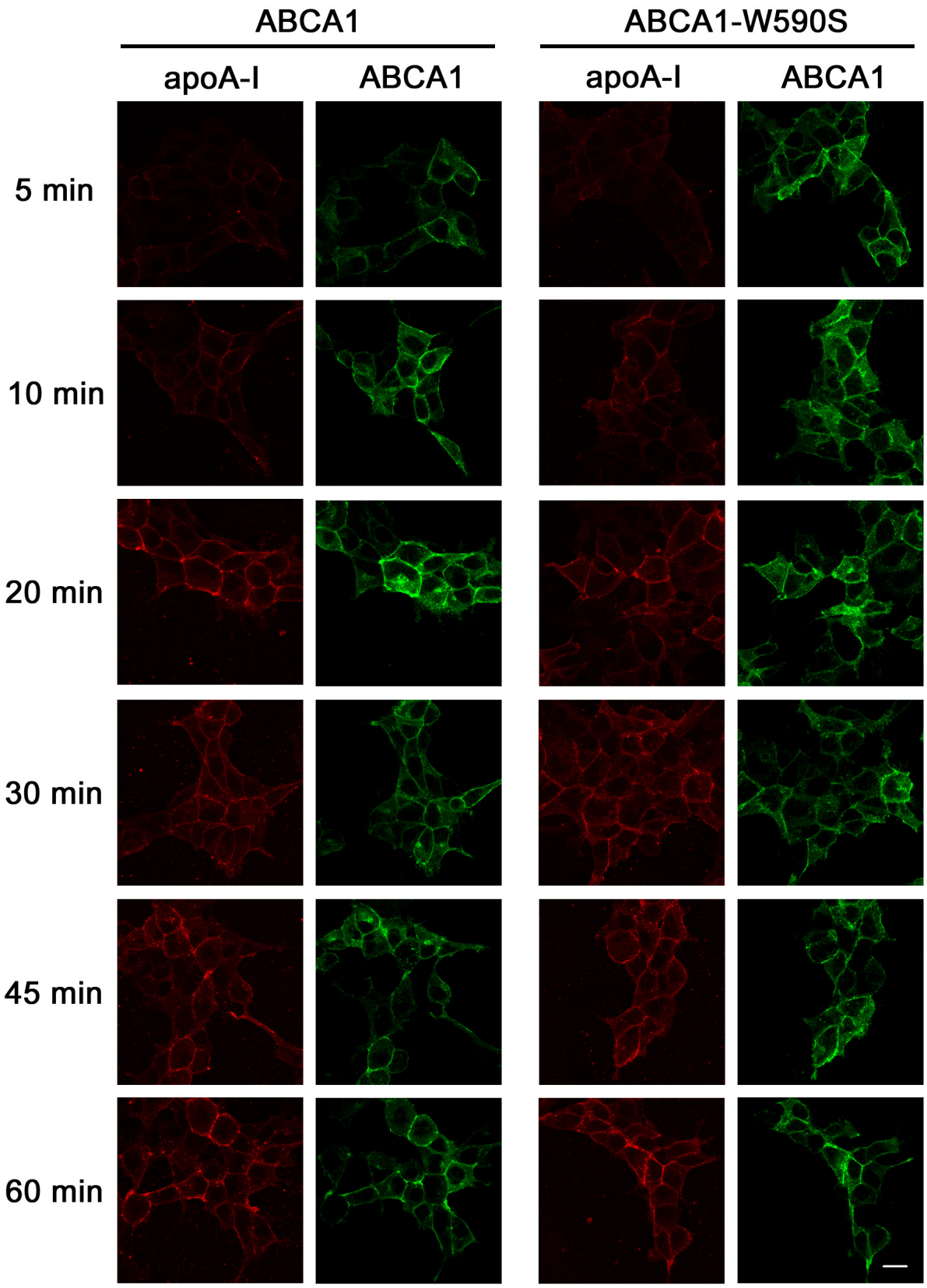
Supplementary Figure 4. Nagao,K et al.



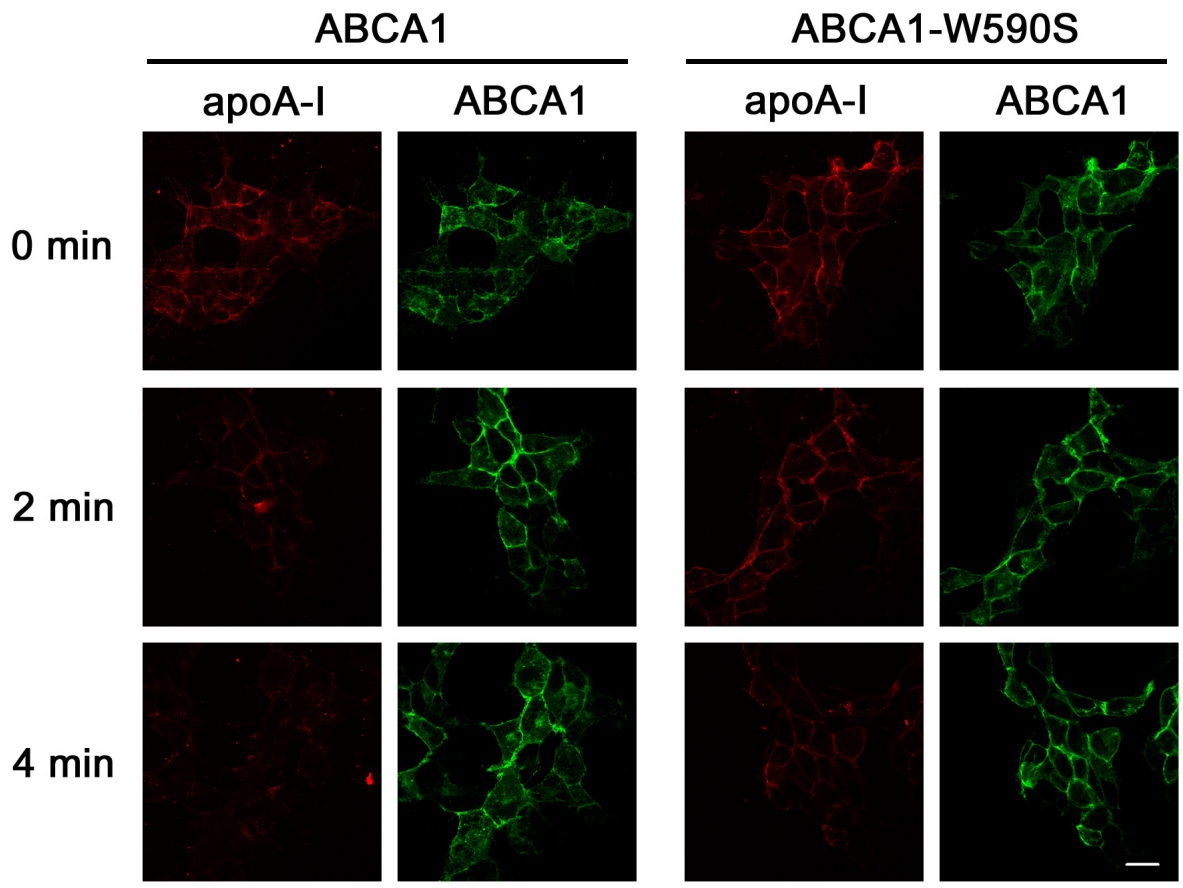
Supplementary Figure 5. Nagao,K et al.



Supplementary Figure 6. Nagao,K et al.



Supplementary Figure 7. Nagao,K et al.



Supplementary Figure 8. Nagao,K et al.