		Primer (5'→3')
L2187A	Forward	TCTTCATTATCTTCTGCCGCCAGGATATTCAGC
	Reverse	GCTGAATATCCTGGCGGCAGAAGATAATGAAGA
L2194A	Forward	GATATTCAGCATCGCCTCCCAGAGCAAAA
	Reverse	TTTTGCTCTGGGAGGCGATGCTGAATATC
L2201A	Forward	GAGCAAAAAGCGAGCCCACATAGAAGACT
	Reverse	AGTCTTCTATGTGGGCTCGCTTTTTGCTC
ABCA1 ∆C-ter	Forward	TCTAGAGGATCCCCGGGATC
	Reverse	CAGGTCCGGGTTGGACC
ABCA2 C-ter	Forward	TCCAACCCGGACCTGGTGAAGGACGTGGTGCG
	Reverse	CGGGGATCCTCTAGAGCAGAGCGTGTCCGTGT
ABCA3 C-ter	Forward	TCCAACCCGGACCTGGCGCTGGAGGAGTTCAAG
	Reverse	CGGGGATCCTCTAGATCGCCCCTCCTCTGC
ABCA4 C-ter	Forward	TCCAACCCGGACCTGCTTCCTGACCTGAACCCTGTG
	Reverse	CGGGGATCCTCTAGAGTCCTGGGCTTGTCGACTG
ABCA5 C-ter	Forward	TCCAACCCGGACCTGGAAGTAGACCGCCTTCAAAG
	Reverse	
ABCA6 C-ter	Forward	TCCAACCCGGACCTGGTGACTTTGGTCCACACTGAGA
	Reverse	CGGGGATCCTCTAGAAGGTTCATCTGAATGAGGGAGG
ABCA7 C-ter	Forward	TCCAACCCGGACCTGCAGCCGGCAGCGG
	Reverse	CGGGGATCCTCTAGAGAGCACAGTCTCGGCAGTG
ABCA8 C-ter	Forward	TCCAACCCGGACCTGGTGGAGCCCCTCCATGC
	Reverse	CGGGGATCCTCTAGAAGGCTCTTCCTGGGGGA
ABCA9 C-ter	Forward	TCCAACCCGGACCTGATGGAGCCCCTCCATGC
	Reverse	CGGGGATCCTCTAGAAGGCTCTTCCTGCAGGAGG
ABCA10 C-ter	Forward	TCCAACCCGGACCTGGTGGAAGCTCTCCACACAGAG
	Reverse	
ABCA12 C-ter	Forward	TCCAACCCGGACCTGGAGACCCTCACAAAGTTCATGC
	Reverse	CGGGGATCCTCTAGAAGACTCCATCTGGTCATCTTGTG
ABCA13 C-ter	Forward	TCCAACCCGGACCTGTGCACTGTTTCTGACCACTTG
	Reverse	CGGGGATCCTCTAGAGATGGGCAAGTGATGTGTGT
ABCA1(6A)	Forward	GCAGCAGCTGCTGCAGCCAAGGACCAAAGTGATGA
	Reverse	TGCAGCAGCTGCTGGTCAAGTGTTGTCTGAG
ΑΒCA1(Δ40),(Δ46)	Forward	TCTAGAGGATCCCCGGCGGA
ABCA1(Δ40)	Reverse	CGGGGATCCTCTAGAGGCAAAGTTCACAAATACTTGG
ABCA1(Δ46)	Reverse	CGGGGATCCTCTAGATTGGTCAAGTGTTGTCTGAG

Supplementary Table 1. List of primers used for plasmid construction.



Supplementary Figure 1. Western blot analysis of chimeras and mutants of ABCA1. HEK293 cells transiently expressing chimeras or mutants of ABCA1 were lysed with 1% TritonX-100/PBS supplemented with protease inhibitor. The lysates were analyzed by Western blotting using MT25 antibody.



Supplementary Figure 2. Cholesterol efflux activity of ABCA1 in the presence or absence of apoA-I. The percentage of cholesterol efflux in the presence or absence of apoA-I was measured as described in the legend of Figure 3 and indicated in filled bars and empty bars, respectively. Experiments were performed in triplicate, and mean values are shown  $\pm$  SE.



Supplementary Figure 3. **Plasma membrane localization of ABCA1 mutants.** Plasma membrane localization of ABCA1 mutants were analyzed by flow cytometry using MT25 antibody as described in the legend of Figure 2.



Supplementary Figure 4. Alexa 647-labeled PFO-D4 binding to FreeStyle 293-F cells. Alexa 647-labeled PFO-D4 binding to FreeStyle 293-F cells was analyzed by flow cytometry. Both axes are displayed in logarithmic scale. Warmer colors indicate higher density of counts. FreeStyle 293-F cells with fluorescence intensities greater than 50,000 a.u. were defined as GFP-positive, and the others as GFP-negative. The median value of the Alexa647 fluorescence intensity of each population is shown.



Supplementary Figure 5. ApoA-I-dependent cholesterol efflux activity of ABCA1(V2218L) and ABCA1(V2218I). The percentage of cholesterol efflux from HEK293 cells expressing ABCA1(V2218L)-GFP and ABCA1(V2218I)-GFP was measured as described in the legend of Figure 3. Experiments were performed in triplicate, and mean values are shown  $\pm$  SE. \*P < 0.01 compared with control.