

Supplementary Information For

Functional characterization of *ABCA4* genetic variants related to Stargardt disease

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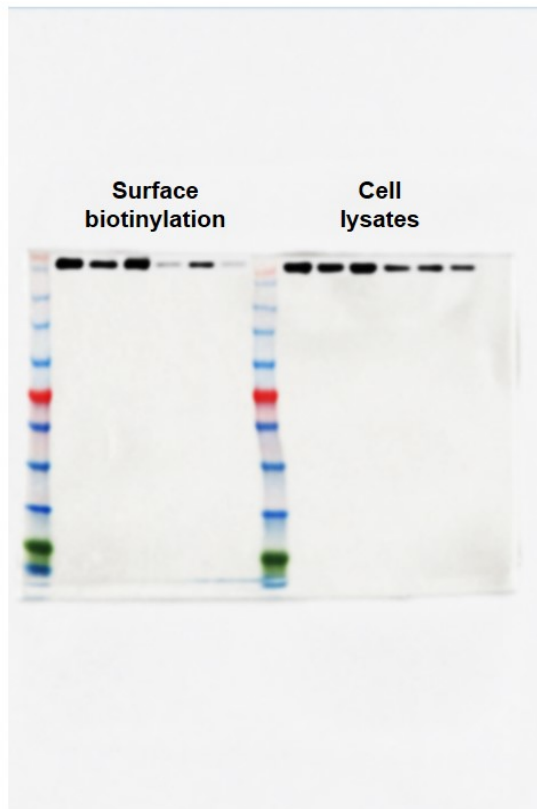
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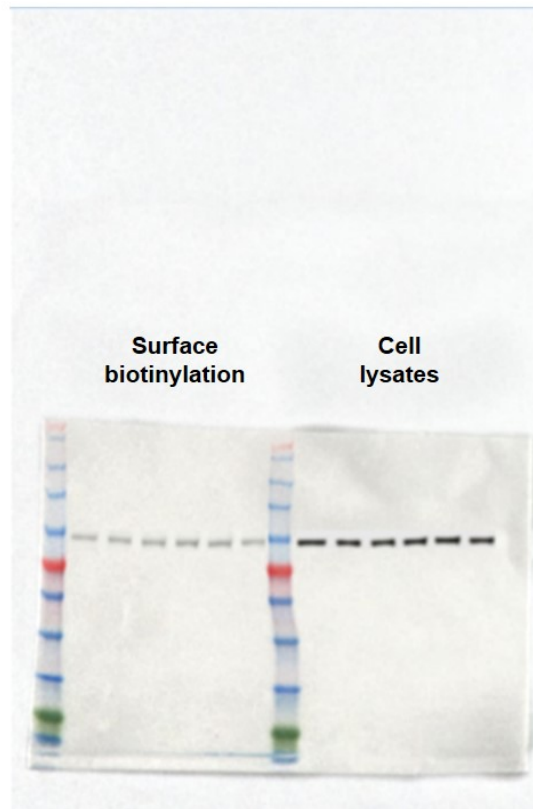
Supplementary Figure 1. Effect of *ABCA4* variants on *ABCA4* surface expression. HEK-293T cells were transfected with *ABCA4* wild-type or variant plasmids, and a surface biotinylation assay was conducted. Among figures, the figures named 'Repeat 1' were used in the main manuscript.

Repeat 1

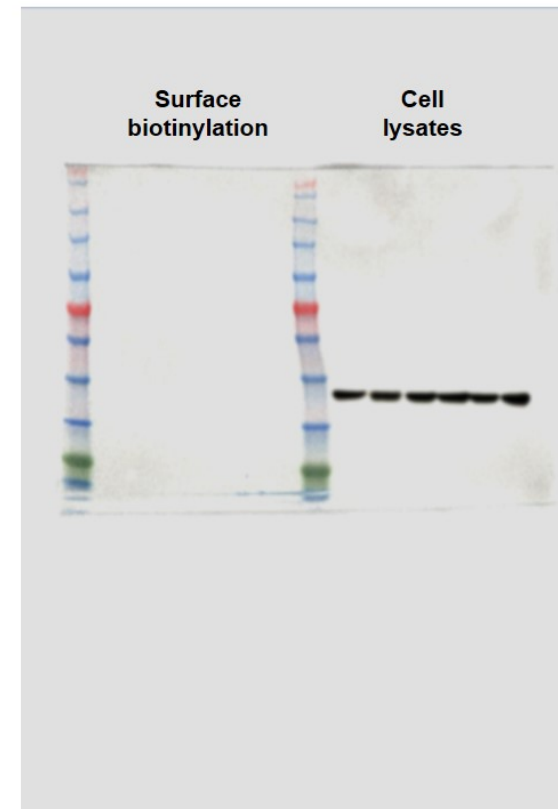
Blot: ABCA4



Blot: Na⁺/K⁺ ATPase

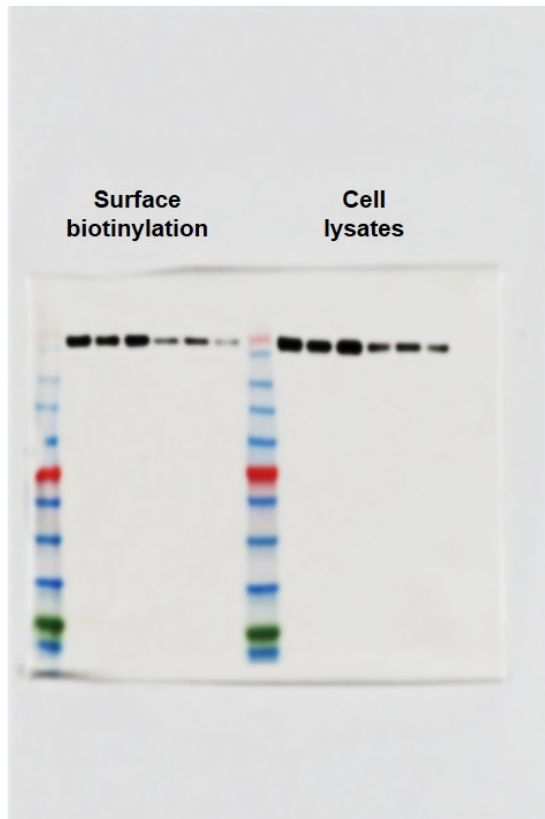


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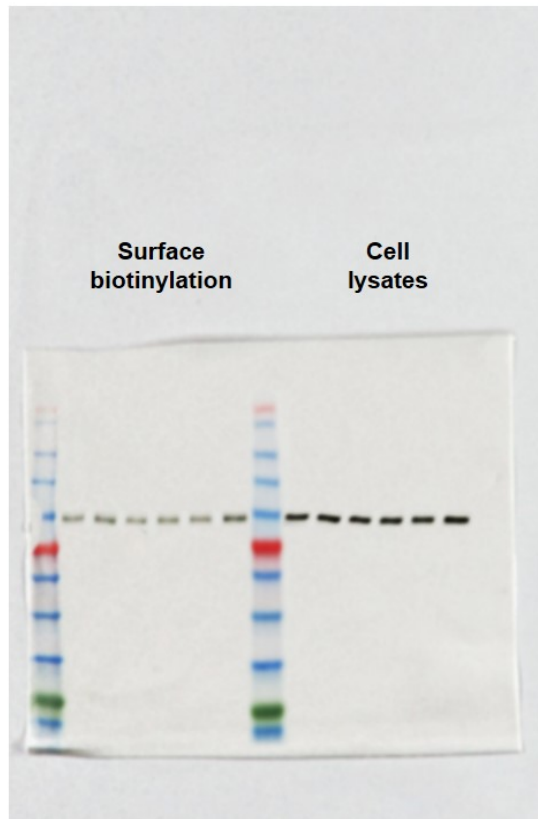


Repeat 2

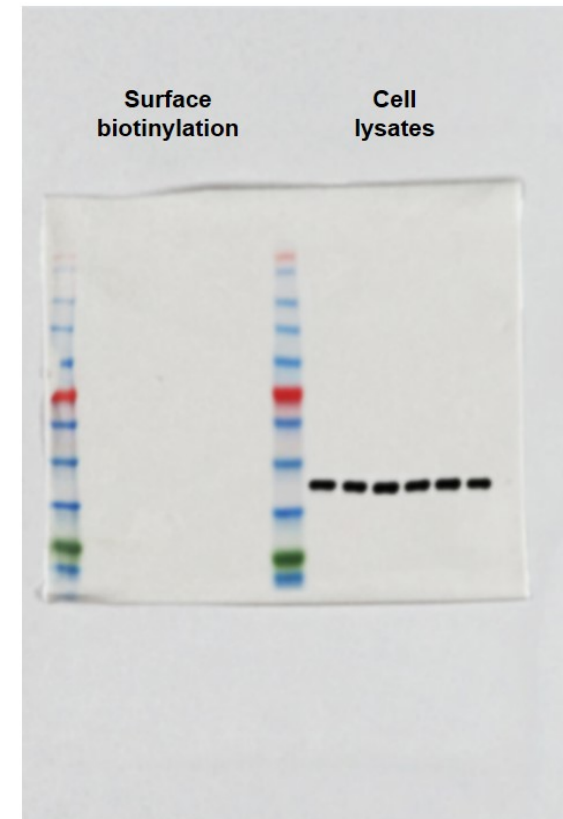
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Blot: Na⁺/K⁺ ATPase

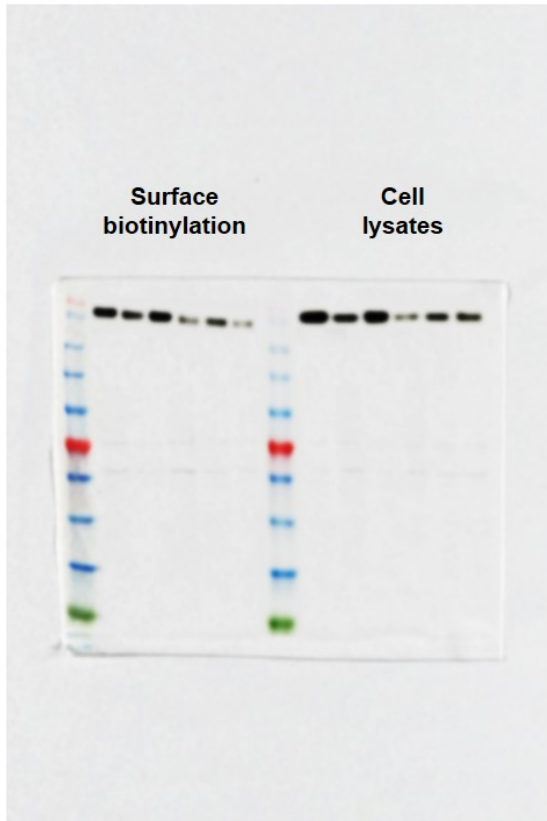


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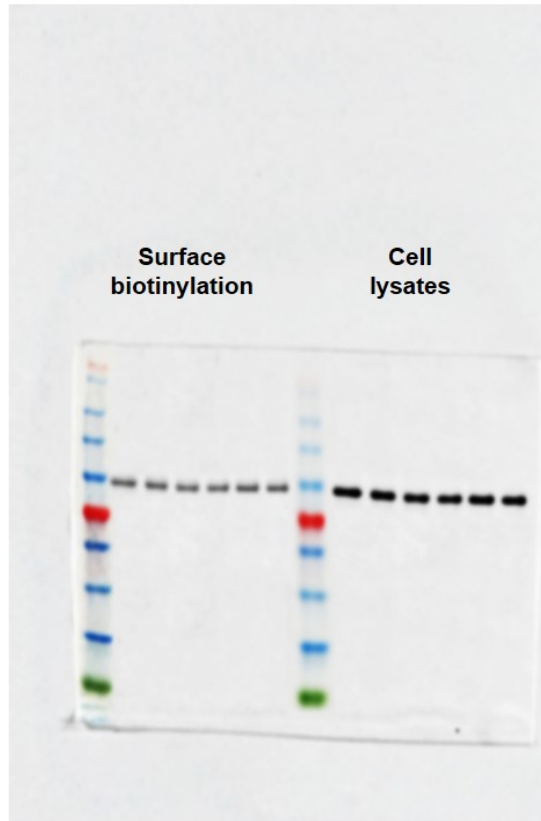


Repeat 3

Blot: ABCA4



Blot: Na⁺/K⁺ ATPase



Blot: Aldolase A

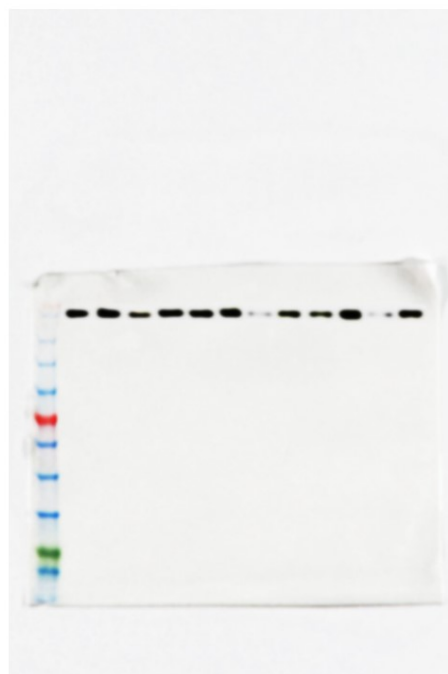


Supplementary Figure 2. Effect of MG132 or bafilomycin A₁ on ABCA4 expression. *ABCA4* expression was examined after transfection with *ABCA4* wild-type or variant plasmids. Immunoblotting was performed after treatment with MG132 **(a)** or bafilomycin A₁ **(b)**. Among figures, the figures named 'Repeat 1' were used in the main manuscript.

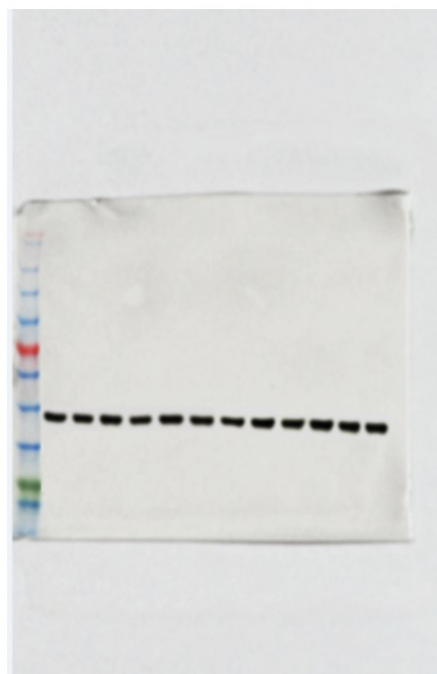
(a)

Repeat 1

Blot: ABCA4

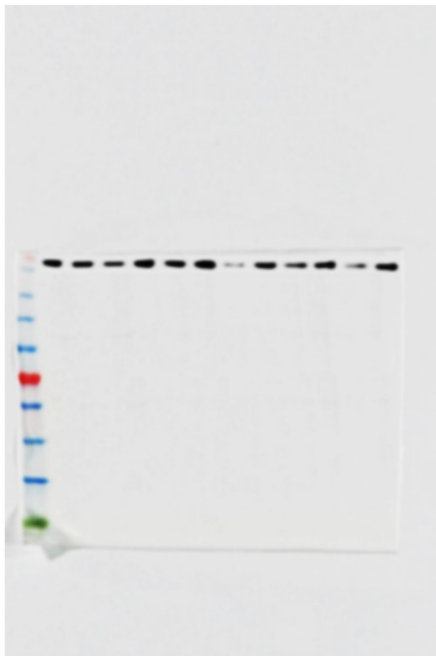


Blot: Actin

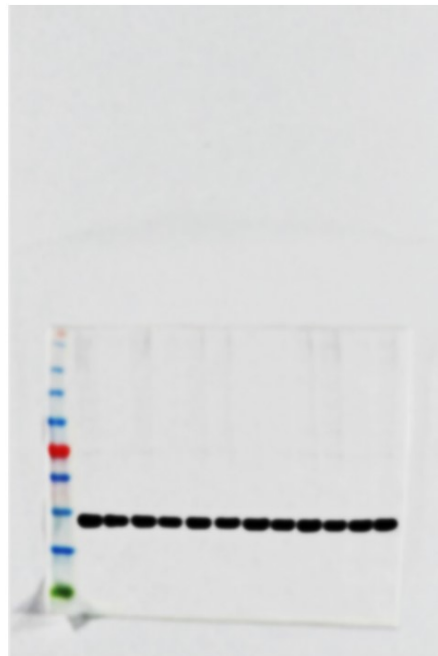


Repeat 2

Blot: ABCA4

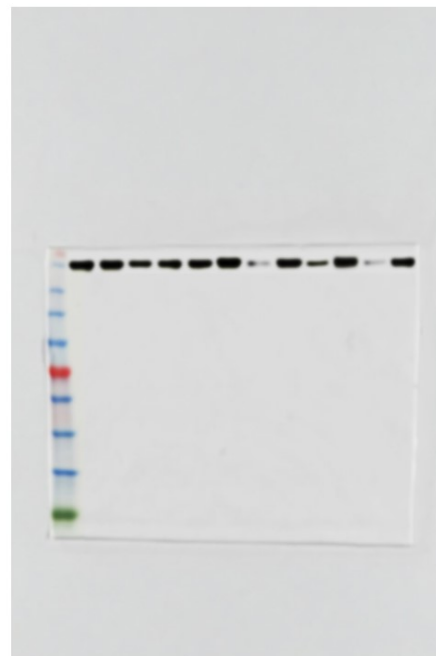


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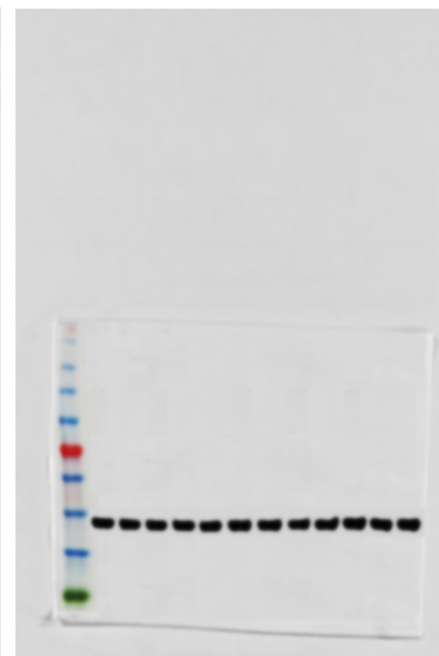


Repeat 3

Blot: ABCA4



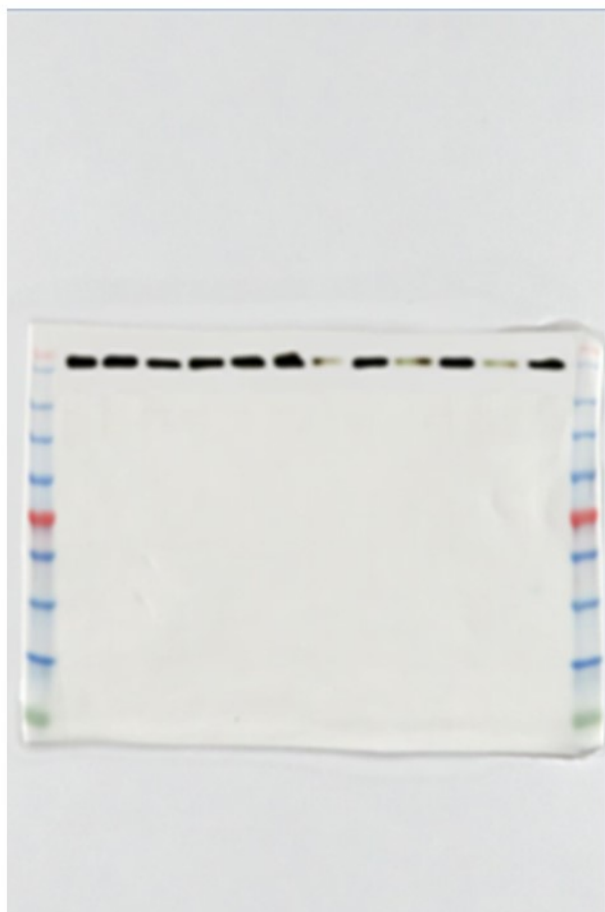
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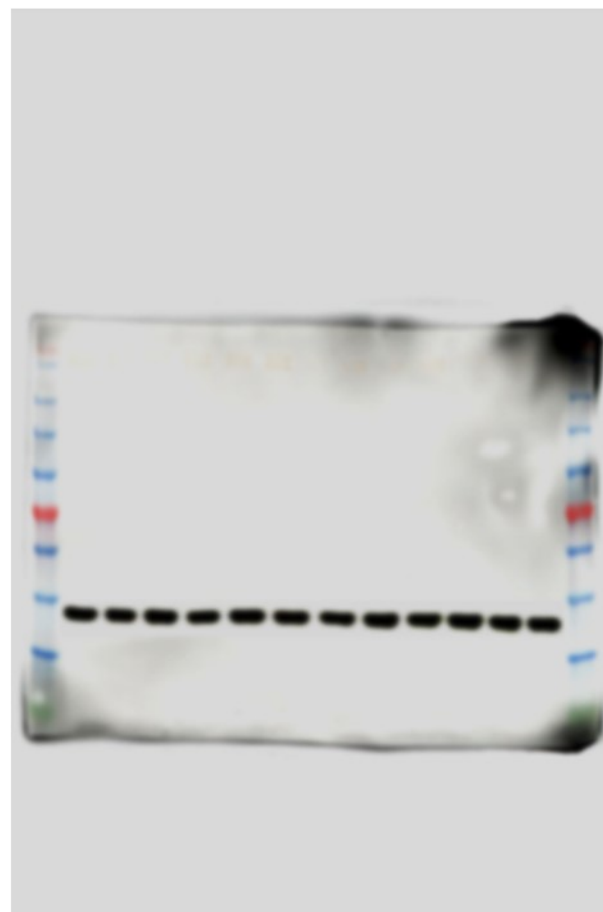
(b)

Repeat 1

Blot: ABCA4



Blot: Actin

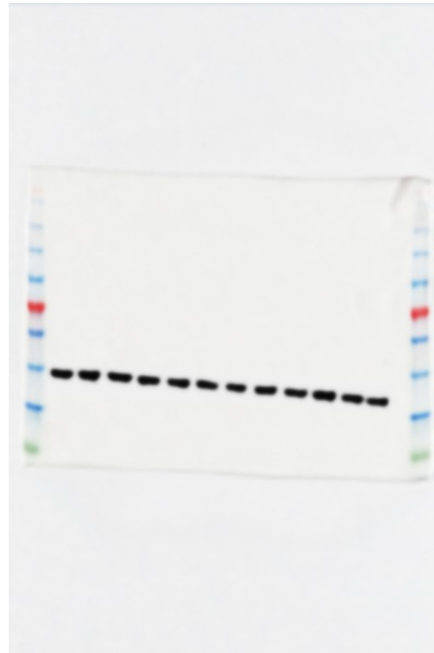


Repeat 2

Blot: ABCA4

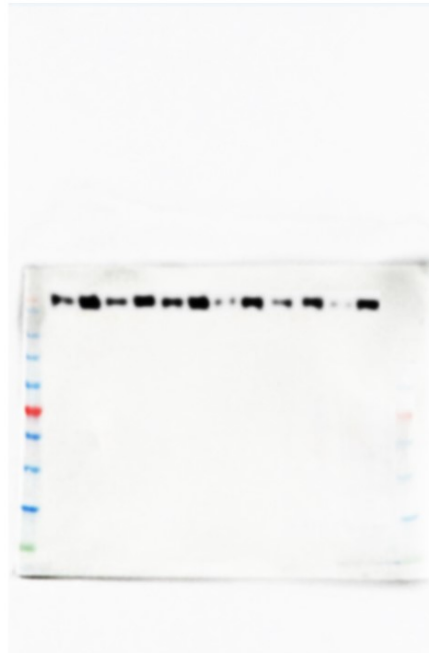


Blot: Actin

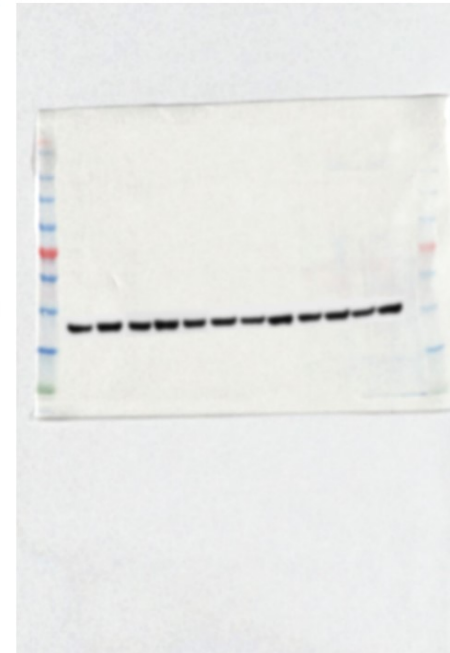


Repeat 3

Blot: ABCA4



Blot: Actin



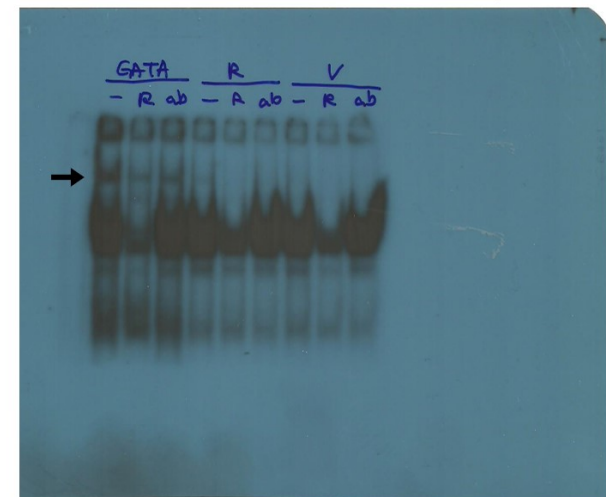
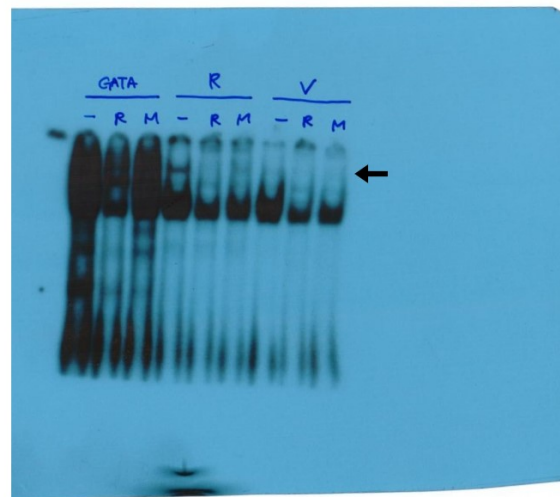
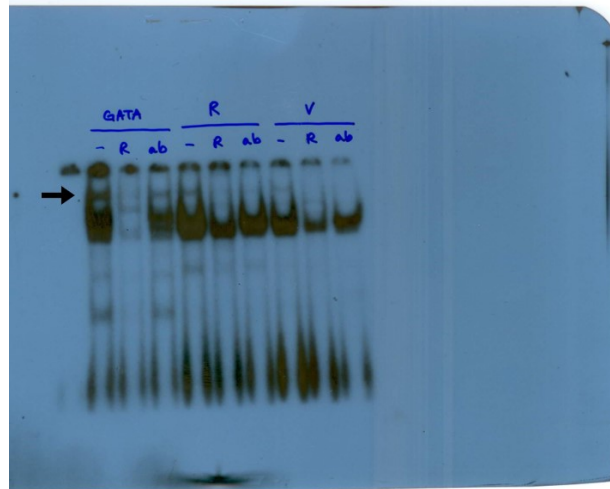
Supplementary Figure 3. Gel shift assays. 32 P-labeled oligonucleotides [lanes 1-3, GATA-2 consensus; lanes 4-6, wild-type c.-1086A; lanes 7-9, c.-1086C variant, **(a)**, lanes 1-3, HLF consensus; lanes 4-6, wild-type c.-900A; lanes 7-9, c.-900T variant, **(b)**, lanes 1-3, GATA-2 consensus; lanes 4-6, wild-type c.-761C; lanes 7-9, c.-761A variant, **(c)**] were incubated with nuclear protein extracts. Competition and supershift assays were performed. Each arrow indicates the band of the DNA-protein complex. Among figures, the figures named 'Repeat 1' were used in the main manuscript.

(a)

Repeat 1

Repeat 2

Repeat 3

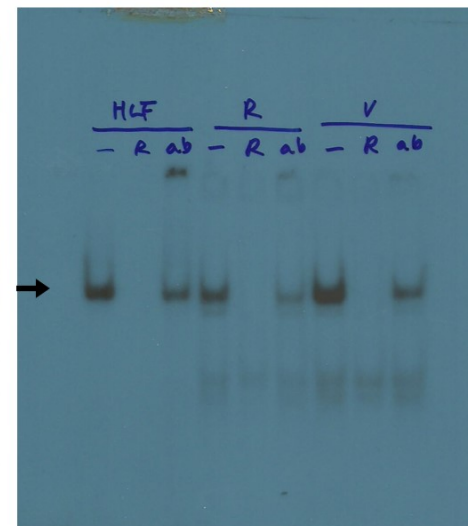
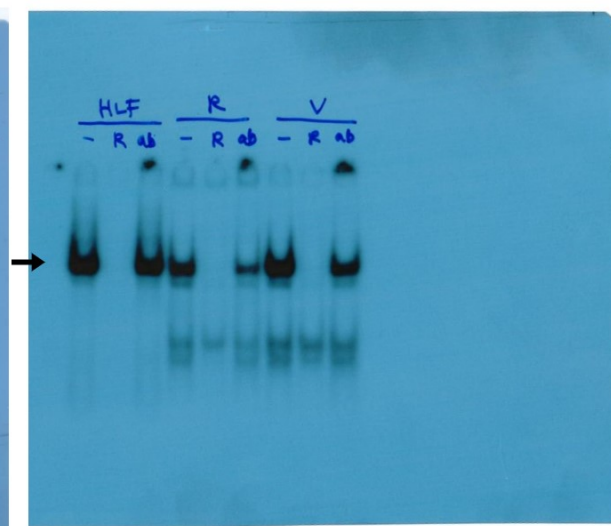
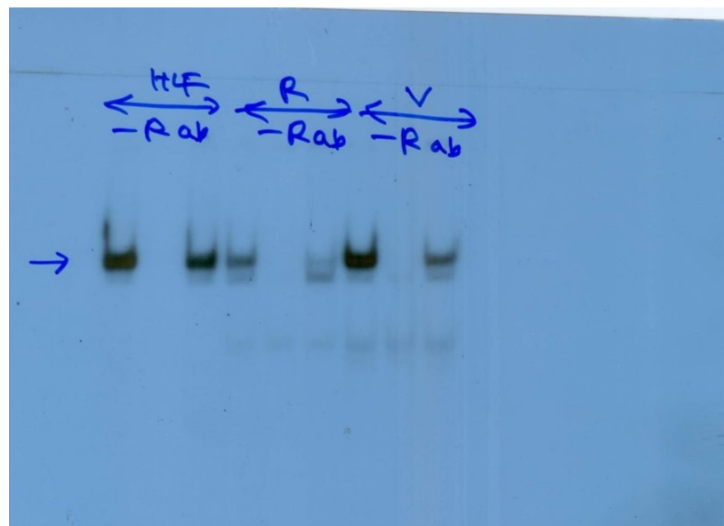


(b)

Repeat 1

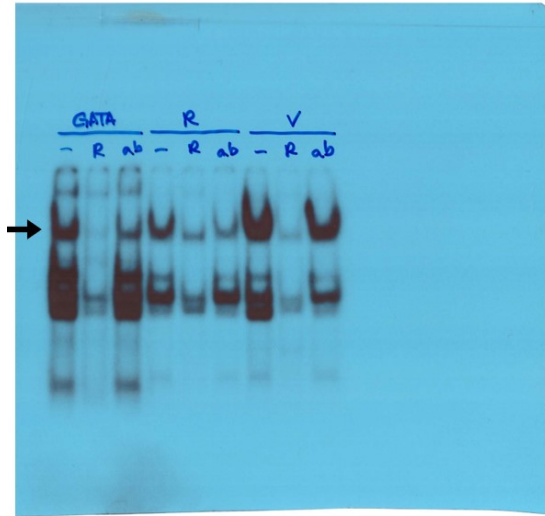
Repeat 2

Repeat 3

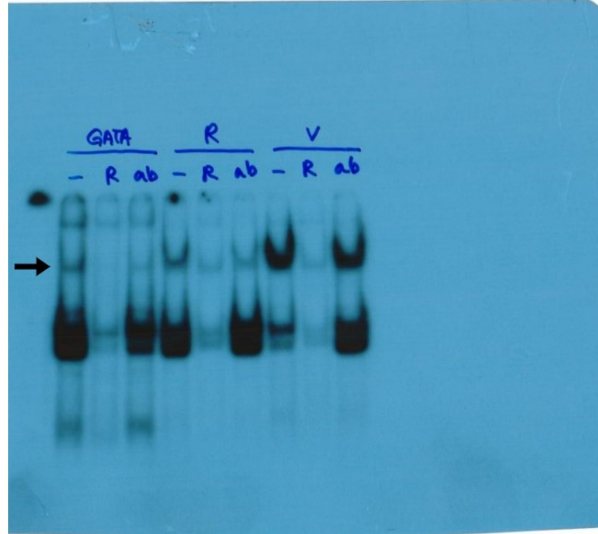


(c)

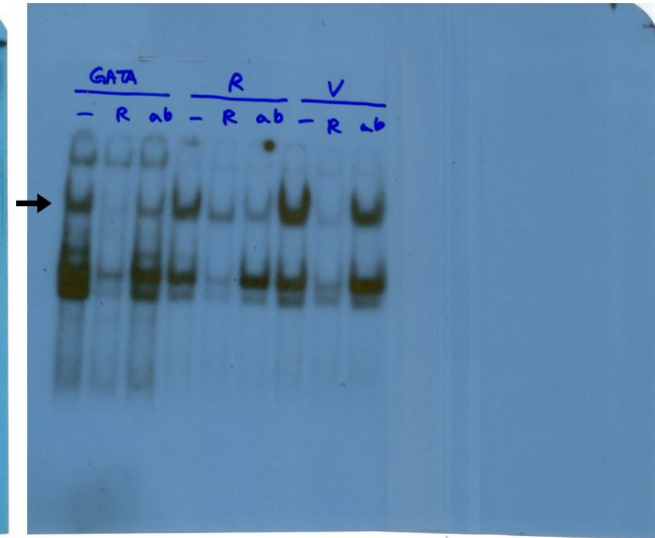
Repeat 1



Repeat 2



Repeat 3



Supplementary Table 1. Oligonucleotides used in the construction of *ABCA4* plasmids and or in gel shift assays

Primes for <i>ABCA4</i> promoter cloning ¹	
Sense (KpnI site)	5'-CGG GGT ACC GGA GCC TTT TTG CCA TCA TAA GC-3'
Antisense (NheI site)	5'-CTA GCT AGC AGG GTC CAG TTC TTC CAG AGC AA-3'
Primers for <i>ABCA4</i> mutagenesis PCR ²	
p.Arg290Gln (c.869G>A)	5'-TCA AGA GTT TAT CCA TCA GCC GAG TAT GCA GGA CT-3'
p.Asp645Asn (c.1933G>A)	5'-CC CTG CTT CGT GGA CAA TTC TTT CAT GAT CAT C-3'
p.Thr1117Ala (c.3349A>G)	5'-GAA CCA TCA TCA TGT CCG CTC ACC ACA TGG ACG AG-3'
p.Cys1140Trp (c.3420C>G)	5'-GAA GGC TCT ACT GGT CAG GCA CCC CAC-3'
p.Asn1588Tyr (c.4762A>T)	5'-TGG CCG GAT CAT GTA TGT GAG CGG GGG-3'
c.-1086A>C	5'-GCC TTT TTG CCA TCA TAA GCA GAA ACT CTC TCT CTC TTC TTG-3'
c.-900A>T	5'-GTC TAG AGT CTT TCA AAG AGT ACA CAT TCT GAG ATT TGA GG-3'
c.-761C>A	5'-CCA CCC CAT TGC AGG GAT GGA ATG ACA GTA ATG-3'
Oligonucleotides in gel shift assay	
c.-1086A (wild-type) ²	5'-TAA GCA GAA ACT ATC TCT CTC TTC T-3'
c.-1086C (variant) ²	5'-TAA GCA GAA ACT CTC TCT CTC TTC T-3'
c.-900A (wild-type) ²	5'-TCT TTC AAA GAG AAC ACA TTC TGA G-3'
c.-900T (variant) ²	5'-TCT TTC AAA GAG TAC ACA TTC TGA G-3'
c.-761C (wild-type) ²	5'-CCC ATT GCA GGG CTG GAA TGA CAG T-3'
c.-761A (variant) ²	5'-CCC ATT GCA GGG ATG GAA TGA CAG T-3'
GATA-2 consensus ³	5'-CAC TTG ATA ACA GAA AGT GAT AAC TCT-3'
HLF consensus ³	5'-CAG GGT TAC GTA ATC TGC T-3'

¹Restriction endonuclease sites, ²SNP sites, and ³consensus sequences of transcription factors are marked with bold-faced letters.