Individual variation of human sphingosine 1-phosphate receptor 1 coding sequence leads to heterogeneity in receptor function and drug interactions

Hideru Obinata[1], Sarah Gutkind[1], Jeremiah Stitham[2], Toshiaki Okuno[3], Takehiko Yokomizo[3], John Hwa[2], Timothy Hla[1]*

Supplementary Materials:

Primers used for mutagenesis

A11D

Forward: 5'-cgctggtcaaggaccaccgcagctc-3'

Reverse: 5'-gagctgcggtggtccttgaccagcg-3'

R13G

Forward: 5'-gtcaaggcccacggcagctcggtct-3'

Reverse: 5'-agaccgagctgccgtgggccttgac-3'

A39T

Forward: 5'-cacgggaaagctgaatatcagcacggataaagaaaatagtataaa-3'

Reverse: 5'-tttatactattttctttatccgtgctgatattcagctttcccgtg-3'

I45T

Forward: 5'-ggacaaggagaacagcactaaactgacctcggtgg-3'

Reverse: 5'-ccaccgaggtcagtttagtgctgttctccttgtcc-3'

P79S

Forward: 5’-ggaaaaccaagaaattccaccgaagcatgtactattttattggcaatc-3’

Reverse: 5’-gattgccaataaaatagtacatgcttcggtggaatttcttggttttcc-3’

R120P

Forward: 5’-cccagtggtttctgccggaagggagtatgtt-3’

Reverse: 5’-aacatactcccttccggcagaaaccactggg-3’

Y198C

Forward: 5’-caccgtgctgccgctctgccacaagcac-3’

Reverse: 5’-gtgcttgtggcagagcggcagcacggtg-3’

R238C

Forward: 5’-cgccgcctgacgttctgcaagaacatttcca-3’

Reverse: 5’-tggaaatgttcttgcagaacgtcaggcggcg-3’

S245N

Forward: 5’-gcaagaacatttccaaggccaaccgcagctctg-3’

Reverse: 5’-cagagctgcggttggccttggaaatgttcttgc-3’

G305C

Forward: 5’-ctgtgctcaactcctgcaccaaccccatc-3’

Reverse: 5’-gatggggttggtgcaggagttgagcacag-3’

P332R

Forward: 5’-gctgcaagtgccggagcggagactc-3’

Reverse: 5’-gagtctccgctccggcacttgcagc-3’

R342P

Forward: 5’-ctggcaaattcaagccacccatcatcgccgg-3’

Reverse: 5’-ccggcgatgatgggtggcttgaatttgccag-3’

G347S

Forward: 5’-cgacccatcatcgccagcatggaattccacc-3’

Reverse: 5’-ggtggaattccatgctggcgatgatgggtcg-3’

S351G

Forward: 5’-cgccggcatggaattcggccgcagcaaa-3’

Reverse: 5’-tttgctgcggccgaattccatgccggcg-3’

Legends for supplemental figures

Fig. S1. Internalization of $S1P_1$ SNP mutants after S1P stimulation.

293T cells were transduced with lentivirus vectors for the expression of GFP-tagged $S1P_1$ mutants, starved overnight, then stimulated with 200 nM S1P for 1 h. The cells were fixed with paraformaldehyde, and the GFP signal was observed for the localization of the $S1P_1$ mutants under a confocal microscope system. Data are the representative of at least 3 independent experiments with the same tendency.

Fig. S2. Expression levels of wild type $S1P_1$, I45T, R120P, and G305C mutants in CHO-K1 cells

CHO-K1 cells were transduced with lentivirus vectors for the expression of GFP-tagged $S1P_1$ mutants, and selected by puromycin. Expression levels of each mutant were examined by western blot analysis.

Fig. S3. Internalization of $S1P_1$ SNP mutants after FTY720-P stimulation.

293T cells were transduced with lentivirus vectors for the expression of GFP-tagged $S1P_1$ mutants, starved overnight, and stimulated with 10 nM FTY720-P for 1 h. The cells were fixed with paraformaldehyde, and the GFP signal was observed for the localization of the $S1P_1$ mutants under a confocal microscope system. Data are the representative of at least 3 independent experiments with the same tendency.

Fig. S4. Comparison between wild type $S1P_1$, R13G, I45T and G305C mutants in TEER.

HUVECs were transduced with lentivirus vectors for the expression of GFP-tagged SNP mutants together with a vector for endogenous $S1P_1$-specifc shRNA. (A) Western blot analysis was performed to check the overexpression of wild type $S1P_1$, I45T and G305C, and the suppression of endogenous $S1P_1$ by shRNA. The cells were starved, and stimulated with 100 nM BSA-bound S1P (B) or HDL-bound S1P (C). The changes in TEER were monitored in an ECIS θ system (Applied Biophysics). Data are the representative of 3 independent experiments with the same tendency.

Supplemental figures
Fig. S1

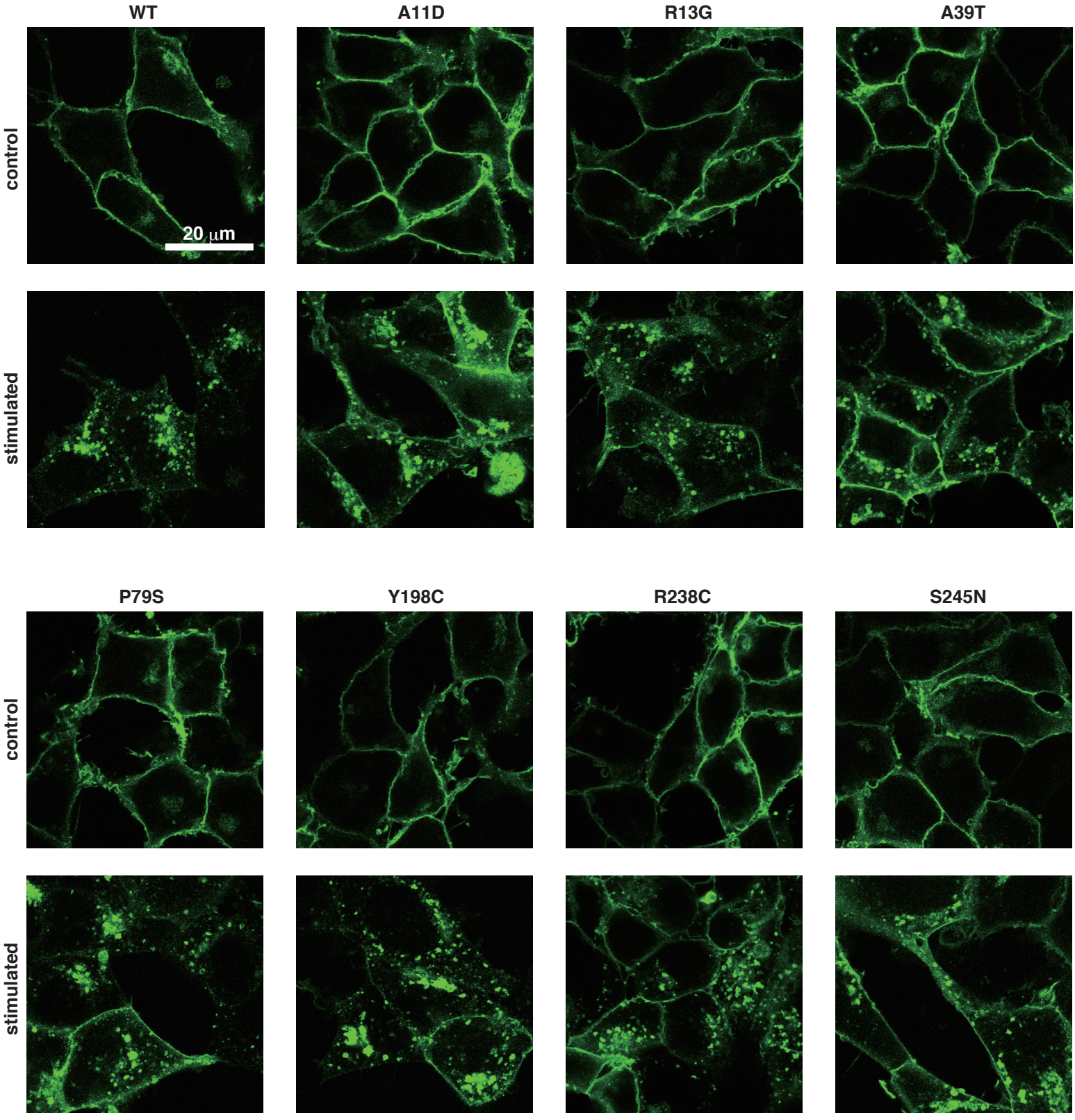


Fig. S1 (continued)

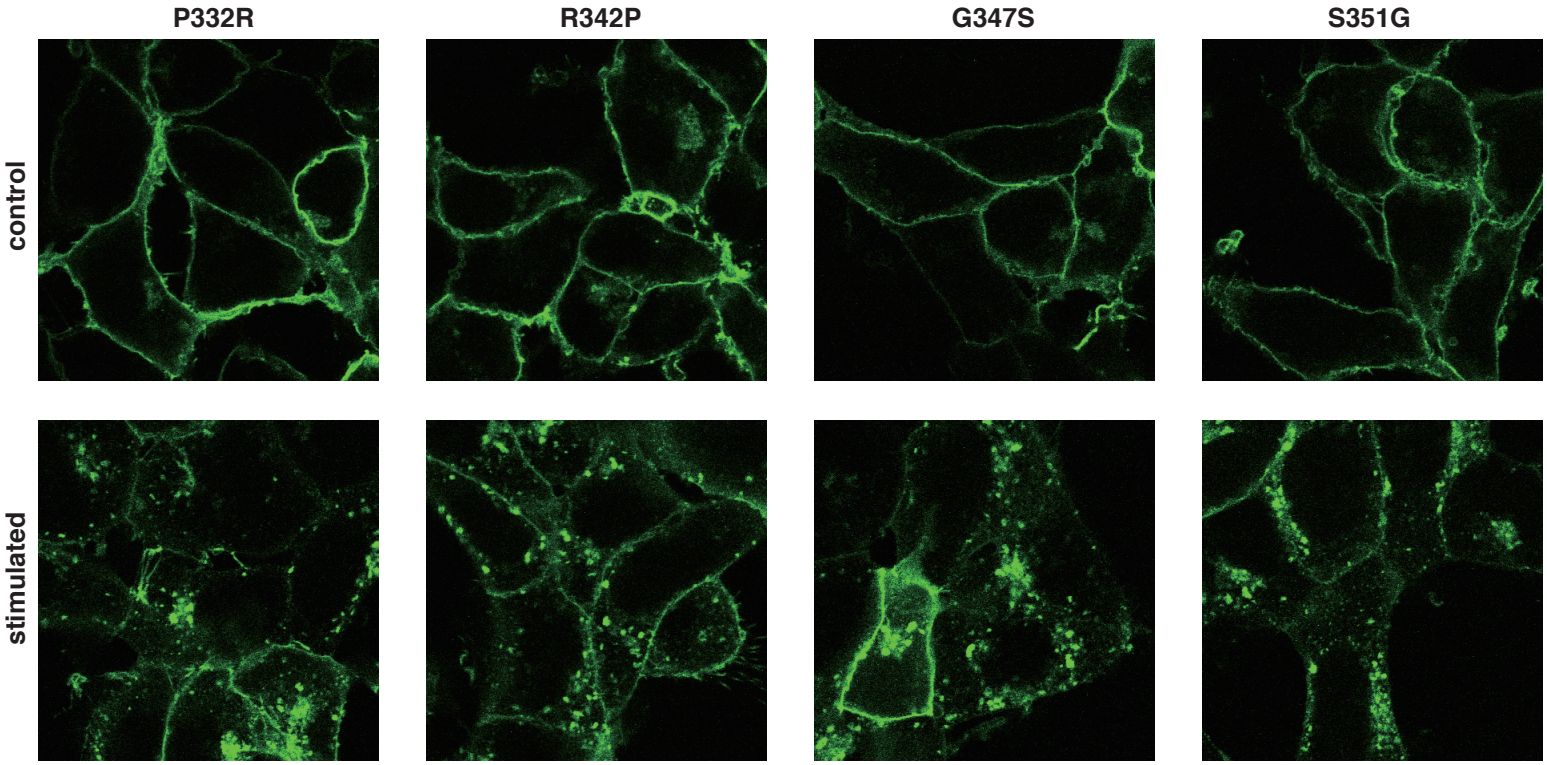


Fig. S2

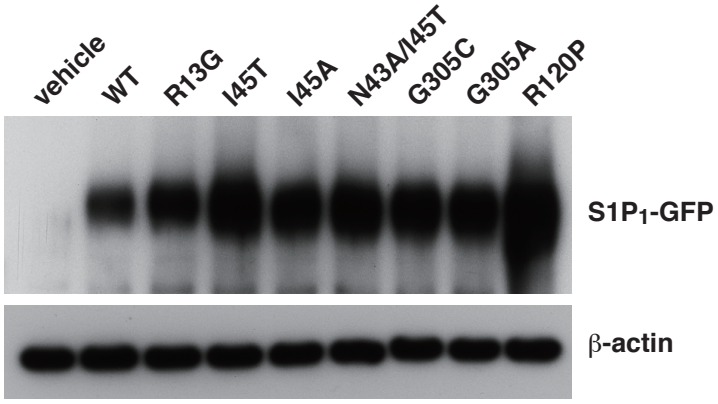

vehicle
WT
R13G
I45T
I45A
N43A/I45T
G305C
G305A
R120P
S1P1-GFP
β-actin

Fig. S3

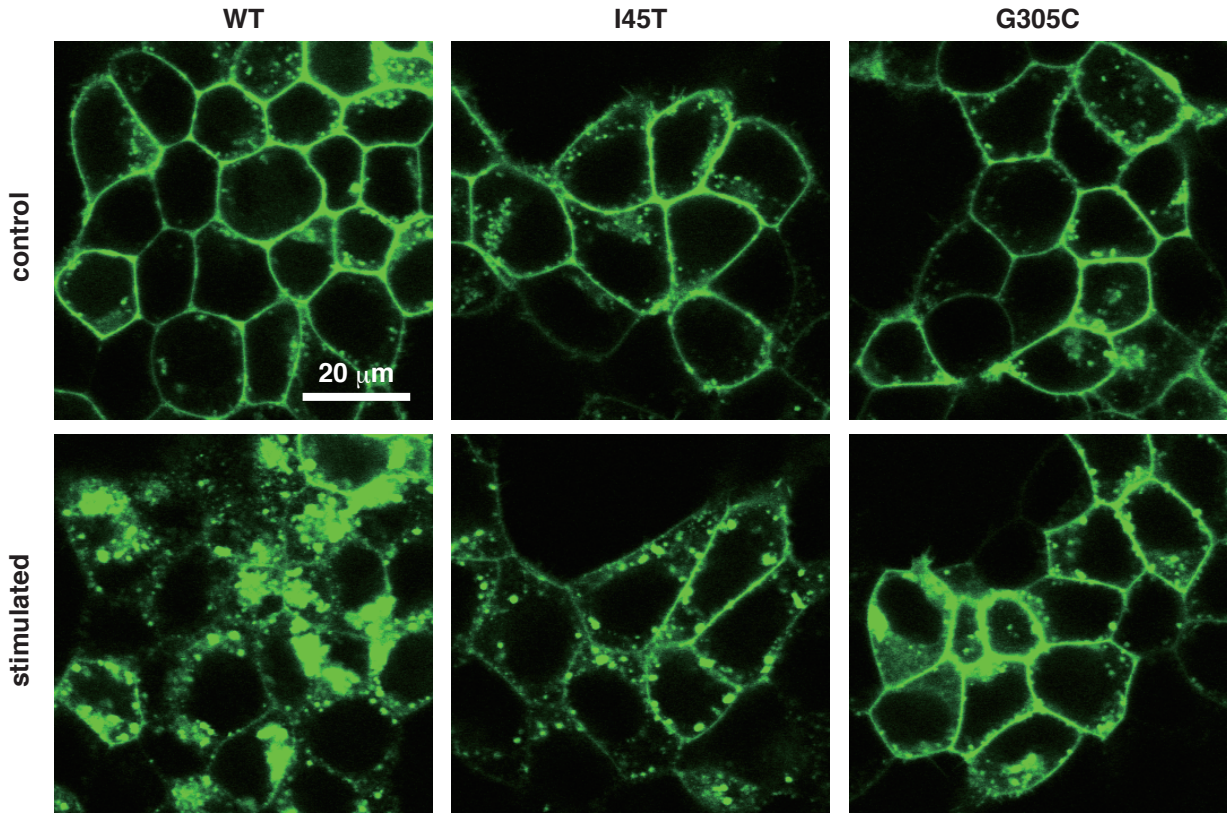
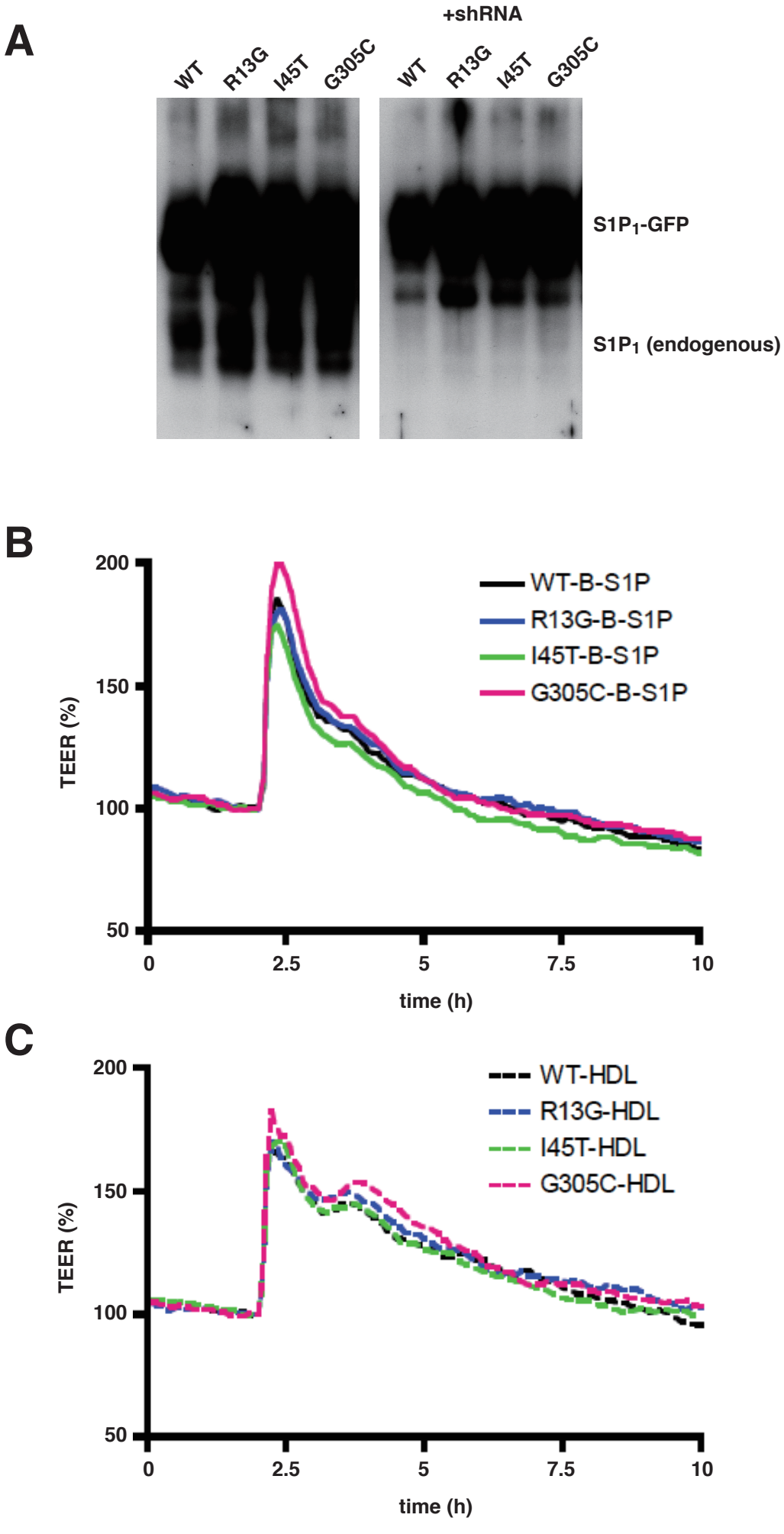


Fig. S4


A
+shRNA
WT
R13G
I45T
G305C
WT
R13G
I45T
G305C
S1P1-GFP
S1P1 (endogenous)
B
200
150
100
50
TEER (%)
0
2.5
5
7.5
10
time (h)
WT-B-S1P
R13G-B-S1P
I45T-B-S1P
G305C-B-S1P
C
200
150
100
50
TEER (%)
0
2.5
5
7.5
10
time (h)
WT-HDL
R13G-HDL
I45T-HDL
G305C-HDL