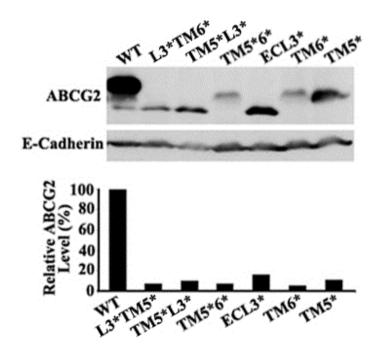
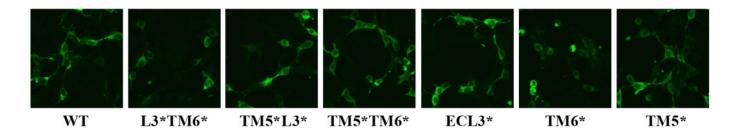
## **Supplemental Figures**

Human WLQYFSIPRYGFTALQHNEFLGQNFCPGLNATGNNPCNY--ATCTGEEYLVKQGIDLSPWGLWK Mouse WLQYFSIPRYGFTALQYNEFLGQEFCPGFNVTDNSTCVNSYAICTGNEYLINQGIELSPWGLWK Rat WLQYFSIPRYGFTALQHNEFLGQEFCPGLNVTMNSTCVNSYTICTGNDYLINQGIDLSPWGLWR

**Figure S1. Alignment of the ECL3 sequences of human, rat, and mouse ABCG2.** The amino acid sequences are denoted by single letter code. The red letters indicates the conserved QXXS motif and cysteine residues.



**Figure S2. Western blot analysis of ABCG2 expression in stable cell lines.** Plasma membranes were isolated from HEK293 cells with stable expression of ABCG2<sup>Myc-WT</sup>, ABCG2<sup>Myc-L3\*TM6\*</sup>, ABCG2<sup>Myc-TM5\*L3\*</sup>, ABCG2<sup>Myc-TM5\*6\*</sup>, ABCG2<sup>Myc-ECL3\*</sup>, ABCG2<sup>Myc-TM6\*</sup>, and ABCG2<sup>Myc-TM5\*</sup> followed by Western blot analysis of ABCG2 expression probed with Myc antibody. Membrane level of E-Cadeherin was used as a loading control.



**Figure S3.** Analysis of ABCG2 subcellular localization using immunofluorescence staining. HEK293 cells transfected with ABCG2<sup>Myc-WT</sup>, ABCG2<sup>Myc-L3\*TM6\*</sup>, ABCG2<sup>Myc-TM5\*L3\*</sup>, ABCG2<sup>Myc-TM5\*6\*</sup>, ABCG2<sup>Myc-TM5\*6\*</sup>, ABCG2<sup>Myc-TM5\*</sup> were stained with Myc antibody and FITC-conjugated secondary antibody followed by imaging using a confocal microscope.