Supplementary Materials: Clinical and Molecular Evidence of ABCC11 Protein Expression in Axillary Apocrine Glands of Patients with Axillary Osmidrosis

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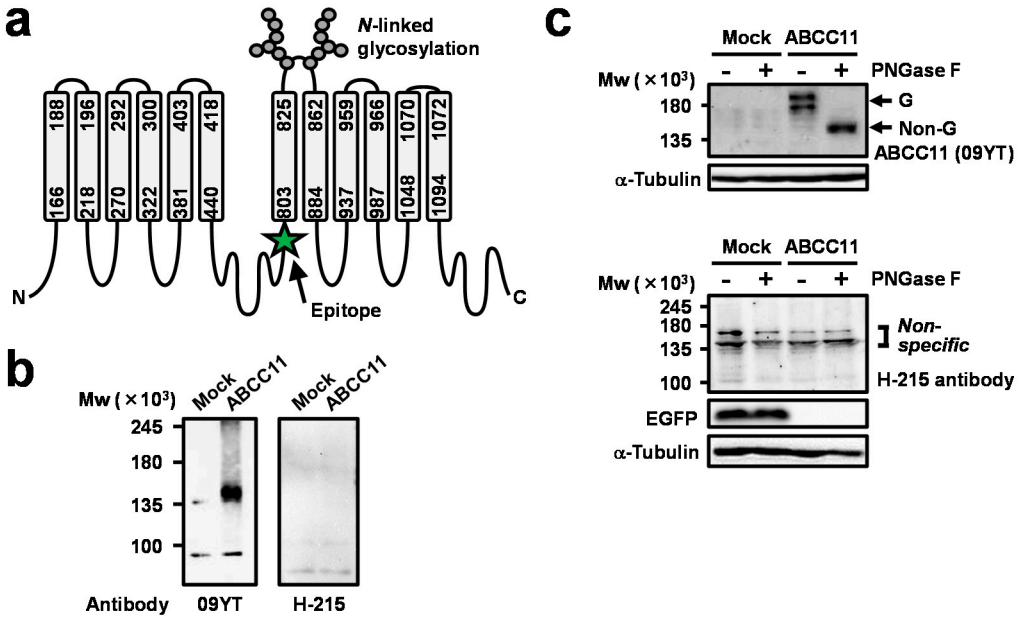

Figure S1. Generation and validation of the anti-ATP-binding cassette C11 (ABCC11) polyclonal antibody. (**a**) Schematic illustration of the ABCC11 protein with the position of the epitope indicated. The putative amino acid sequence in the transmembrane helices was estimated by using the SOSUI program; (**b**,**c**) Validation of the anti-ABCC11 polyclonal antibody (09YT) with immunoblotting using ABCC11-expressing Sf9 cells (**b**) or 293A cells (**c**). In the result from the whole cell lysate of ABCC11-expressing Sf9 insect cells that were derived from our previous study (Toyoda et al., *FASEB J* **2009**, *23*, 2001–2013.), the anti-ABCC11 antibody (09YT) showed a single strong signal with a molecular weight of about 150,000 that corresponds to the non-glycosylated ABCC11 protein. This antibody could recognize both the *N*-glycosylated and non-glycosylated ABCC11 protein exogenously expressed in mammalian 293A cells. The *N*-linked glycosylated forms of ABCC11 (G) were shortened to the non-glycosylated form (Non-G) with peptide *N*-glycosidase F (PNGase F) treatment. On the other hand, H-215—a commercially available antibody—did not detect the ABCC11 protein in our experiments. The enhanced green fluorescent protein (EGFP)-expressing adenovirus was used as a mock virus in (**c**), and adenovirus-mediated protein expression (17 MOI) was confirmed with immunoblotting using an anti-EGFP antibody. α-Tubulin: a loading control.

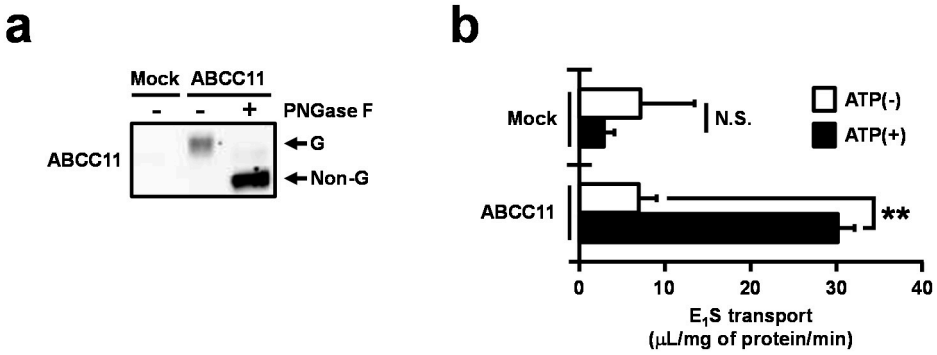

Figure S2. Validation of the ABCC11-expressing adenoviruses. (**a**) Expression of matured-ABCC11 wild-type (WT) protein on the membrane vesicles. ABCC11 WT-expressing or mock membrane vesicles were subjected to immunoblotting analysis with the anti-ABCC11 antibody (09YT). G: glycosylated, Non-G: non-glycosylated form of the ABCC11 protein; (**b**) Estrone sulfate (E_1S) transport activities of ABCC11 in the presence or absence of ATP. E_1S transport into membrane vesicles was measured for 10 min. ATP-dependent incorporation of E_1S was detected in the ABCC11 WT-expressing vesicles, but not in control vesicles. The values are expressed as the mean ± S.D., n = 3. Statistical analyses for significant differences were performed according to Student's t-test (**, $p < 0.01$; N.S., not significantly different between groups).

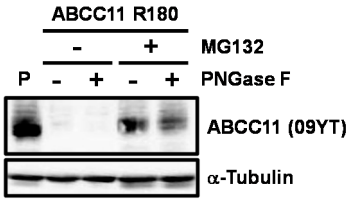

Figure S3. Generation of ABCC11 R180 variant from the adenovirus vector. Thirty-six hours after the infection, ABCC11 R180 variant-expressing Hepa1.6 cells were treated with or without 2 μM MG132 for further 12 hours, then subjected to immunoblotting analysis with the anti-ABCC11 antibody (09YT). P: whole cell lysate of ABCC11 WT-expressing Hepa1.6 cells with both MG132 and PNGase F treatment as a positive control for band position of non-glycosylated ABCC11. α-Tubulin: a loading control. The result showed that ABCC11 R180 protein was not matured as glycoprotein but degraded by MG132-sensitive pathway, which is consistent with the previous report showing that ABCC11 R180 variant is recognized as endoplasmic reticulum-associated protein degradation (ERAD) substrate (Toyoda et al., *FASEB J.* **2009**, *23*, 2001–2013.).