Supporting Information

The extracellular domain of angulin-1 and palmitoylation of its cytoplasmic region are required for angulin-1 assembly at tricellular contacts

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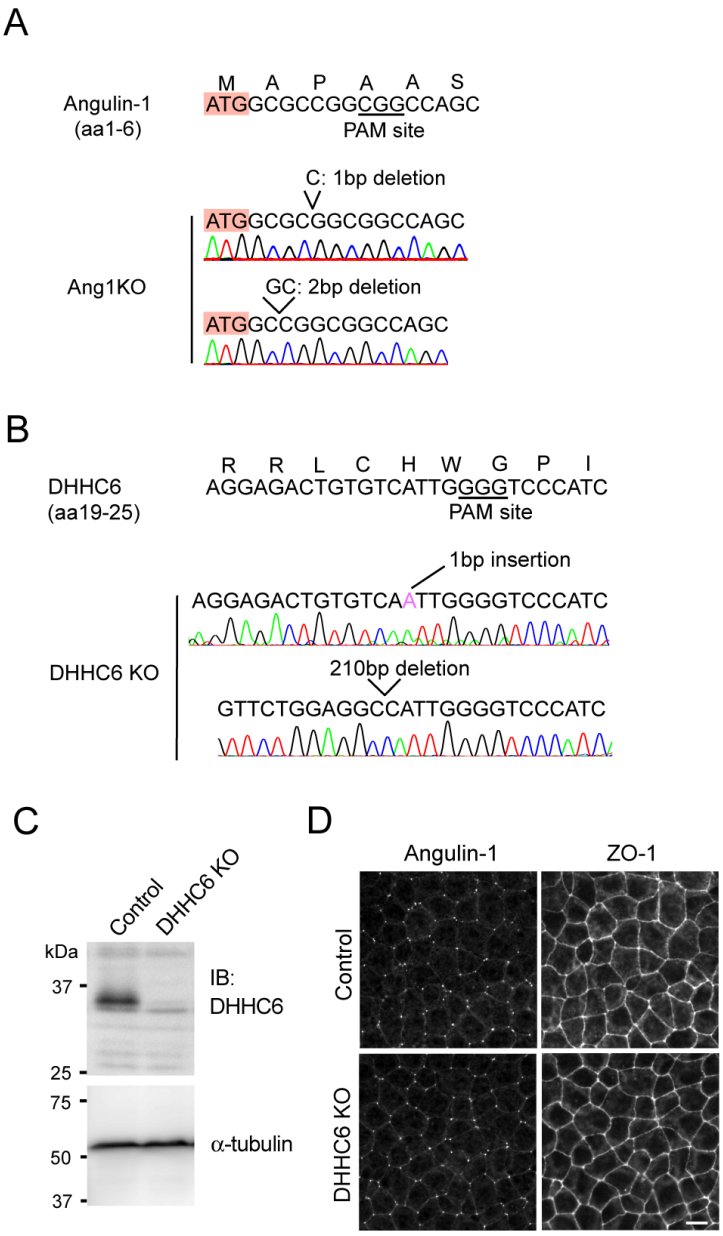

Figure S1. (A) Angulin-1 gene mutations in Ang1KO cells generated by CRISPR/Cas9-mediated genome editing. The nucleotide sequence corresponding to the N-terminal six amino acids of mouse angulin-1 is indicated. The PAM sequence was selected at 8 bases downstream of the 1st ATG. Two frameshift mutations near the 1st ATG were introduced. (B) DHHC6 gene mutations in DHHC6 KO cells generated by CRSPR/Cas9-mediated genome editing. The nucleotide sequence corresponding to amino acid 19-25 of mouse DHHC6 is indicated. The PAM sequence was selected around the codons for tryptophan 24. In one of the obtained DHHC6 KO clones, mutations with 1 nucleotide insertion and 210 nucleotides deletion mutations were introduced near the 1st ATG. (C) Western blotting of control EpH4 cells and DHHC6 KO cells with an anti-DHHC6 and anti-α-tubulin antibodies. (D) Immunofluorescence staining of control EpH4 cells and DHHC6 KO cells with anti-angulin-1 and anti-ZO-1 antibodies. Scale bar: 10 μm.

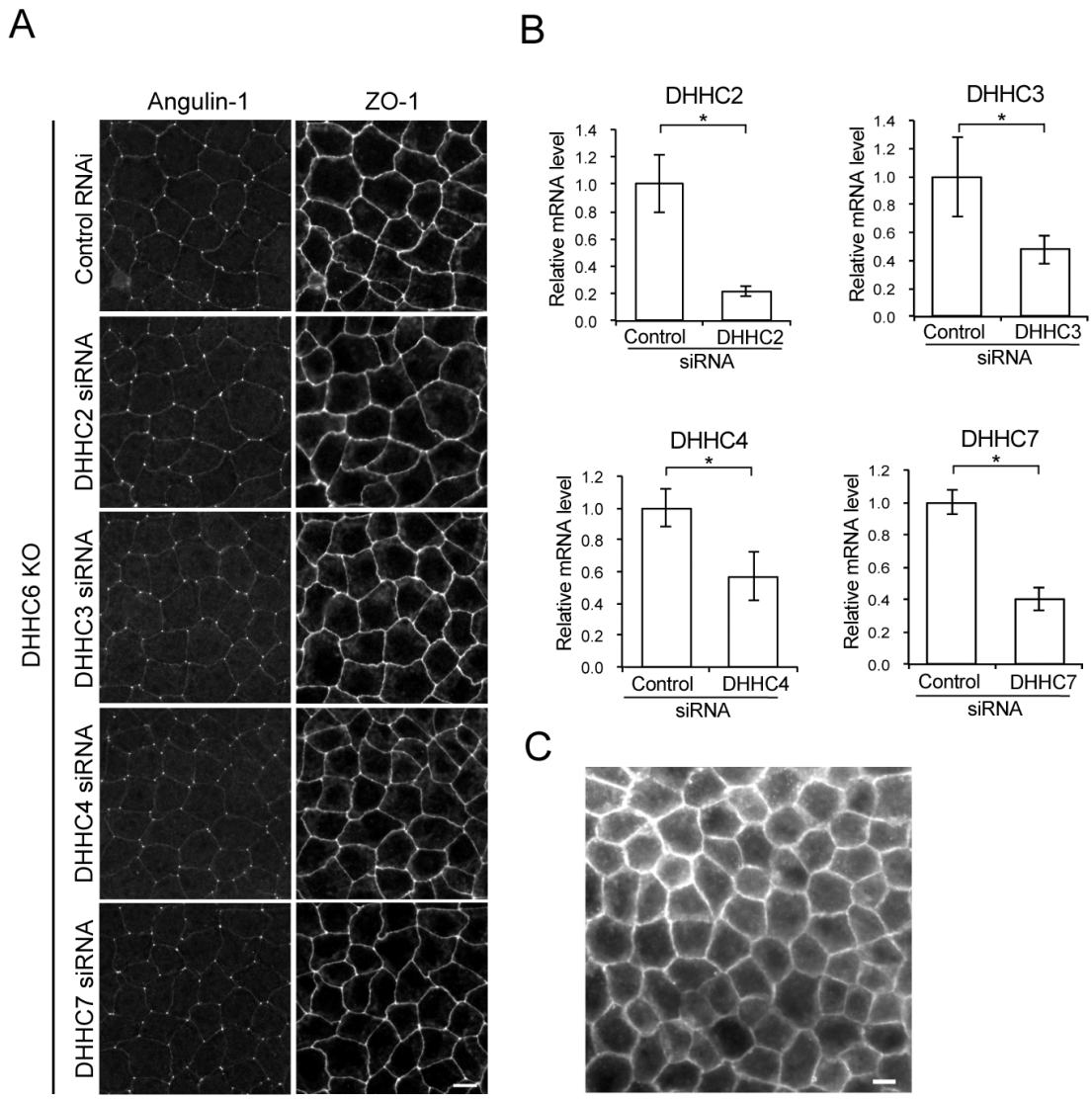

Figure S2. (A) Immunofluorescence staining of DHHC6 KO cells treated with control siRNA, DHHC2 siRNA, DHHC3 siRNA, DHHC4 siRNA, or DHHC7 siRNA with anti-angulin-1 and anti-ZO-1 antibodies. Scale bar: 10 μm. (B) Relative mRNA expression level of DHHC2, 3, 4 or 7 in DHHC6 KO cells treated with control siRNA or each DHHC siRNA was measured by qPCR. Ribosomal protein L5 expression was used for normalization. Graph represent mean ± SD (n=3 each). *p<0.05, compared by *t*-test. (C) EpH4 cells stained with filipin. Confluent EpH4 cells on coverslips were stained with 25 μg/ml filipin III (Sigma-Aldrich) for 10 min after fixation with 4% paraformaldehyde at room temperature for 10 min. Scale bar: 10 μm.