TMEM16A(a)/anoctamin-1 Shares a Homodimeric Architecture with CLC Chloride Channels

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**Figure S2.** The oligomeric structure of mTMEM16A(ac) in HEK 293 cells. Flp-In HEK 293 cells stably expressing the mTMEM16A(ac)-His channel were induced with tetracycline for 36 h, surface-labeled with Cy5-NHS ester, and homogenized with digitonin or dodecyl maltoside (DDM) as indicated. The orange ovals schematically illustrate the homodimeric and the protomeric structure of the mTMEM16A(ac) channel. (A) The mTMEM16A(ac)-His channel was purified with non-denaturing Ni-NTA chromatography and resolved with BN-PAGE in the non-denatured and partially SDS-denatured state to determine its higher-ordered structure. In contrast to the digitonin-solubilized mTMEM16A(ac), which is entirely homodimeric without SDS-pretreatment, a fraction of the dodecyl maltoside-solubilized mTMEM16A(ac) migrates as a protomer even without SDS pretreatment (B) HEK 293 cells were homogenized in the presence of the indicated concentration of glutaraldehyde (GA) using digitonin or dodecyl maltoside (DDM) as the detergent for protein extraction, as indicated. The proteins were purified with non-denaturing Ni-NTA chromatography and resolved in the fully SDS-denatured state with reducing SDS-urea-PAGE. (C) The same digitonin-solubilized mTMEM16A(ac) samples used in (B) were resolved in the non-denatured and partially SDS-denatured state with BN-PAGE. The oocyte-expressed concatenated TMEM16A(a) homodimer (2mer) was also analyzed to indicate the position of the wild-type homodimer.