SUPPLEMENTARY DATA

Fig. S1. Immunoblot analysis of whole-cell lysates of oocytes expressing αβγ-ENaC (WT) or the triply-tagged channel αHA/FLAG-, βHA/His_6-, and γHA/V5-ENaC (3xTag). Oocytes were injected with 0.2 ng cRNA per subunit and incubated in low-sodium solution for two days. Whole-cell lysates of the oocytes were prepared and analyzed by SDS-PAGE followed by immunoblotting, using rabbit polyclonal anti-α- (*A*), anti-β- (*B*), or anti-γ-ENaC antibodies (*C*). *A*, the anti-α-ENaC antibody (directed against an epitope at the N-terminus) showed a specific signal for full-length α-ENaC at 95 kDa and an expected N-terminal furin cleavage fragment at 24 kDa in WT-ENaC and 25 kDa in the triply-tagged ENaC. (The slightly larger size is accounted for by the presence of the N-terminal HA-tag on the α-subunit). *B*, the anti-β-ENaC antibody (directed against an epitope at the C-terminus) detected a specific band at the full size (98 kDa). This band was stronger in oocytes expressing WT-ENaC compared with the triply-tagged channel, which is in good agreement with the ENaC whole-cell current data (see Fig. 1*A*, *B*). *C*, the anti-γ-ENaC antibody (directed against an epitope at the C-terminus) detected a full-length γ-ENaC band at 87 kDa and a C-terminal furin cleavage fragment at 76 kDa. The additional bands detected by the antibodies are also present in lysates of non-injected (NI) oocytes, and are therefore non-specific bands.

Fig. S2. Determination of the assembly state of ENaC homomers. *A*, low-magnification image of βHA/V5-ENaC. *B*, low-magnification image of γHA/V5-ENaC. *C*, low-magnification image of βHA/V5-ENaC that had been incubated with an anti-V5 antibody. Arrowheads indicate singly-decorated particles; the arrow indicates a doubly-decorated particle. *D*, low-magnification image of γHA/V5-ENaC that had been incubated with an anti-V5 antibody. Arrowheads indicate singly-decorated particles; the arrow indicates a doubly-decorated particle. Scale bars, 200 nm; shade-height scales, 0-4 nm.

Fig. S3. Discrimination between antibodies and Fab fragments. Low-magnification AFM images of anti-His_6 antibodies (*A*), anti-V5 Fab fragments (*B*), and a mixture of antibodies and Fab fragments (*C*). Arrows in (*C*) indicate antibodies; arrowheads indicate Fab fragments. Scale bars, 200 nm; shade-height scales, 0-4 nm.

Figure S1

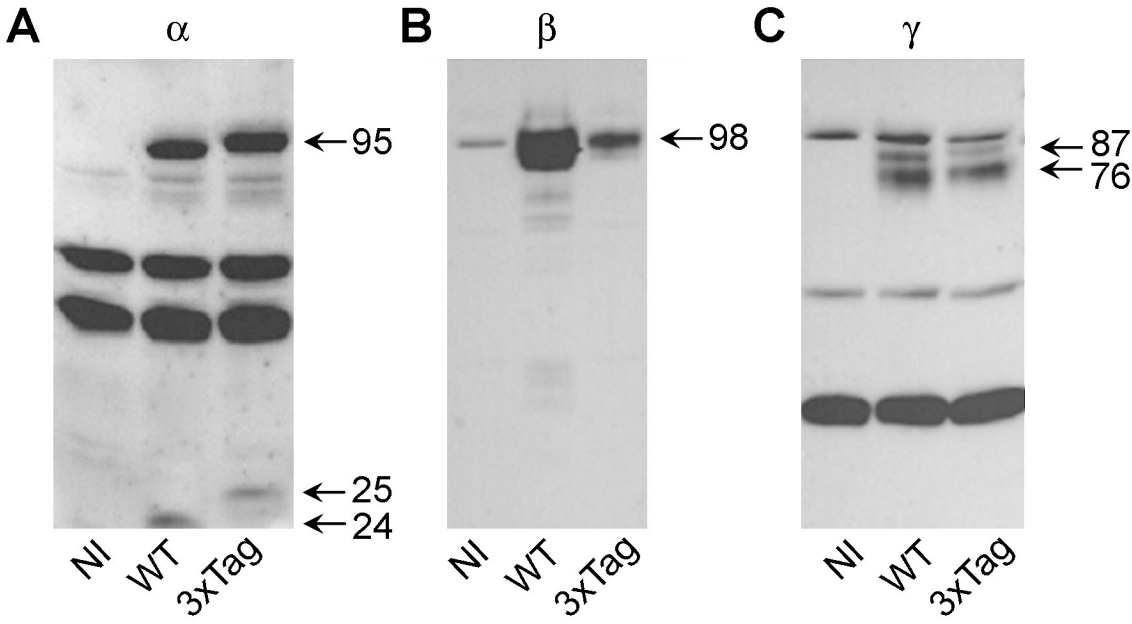

Figure S2

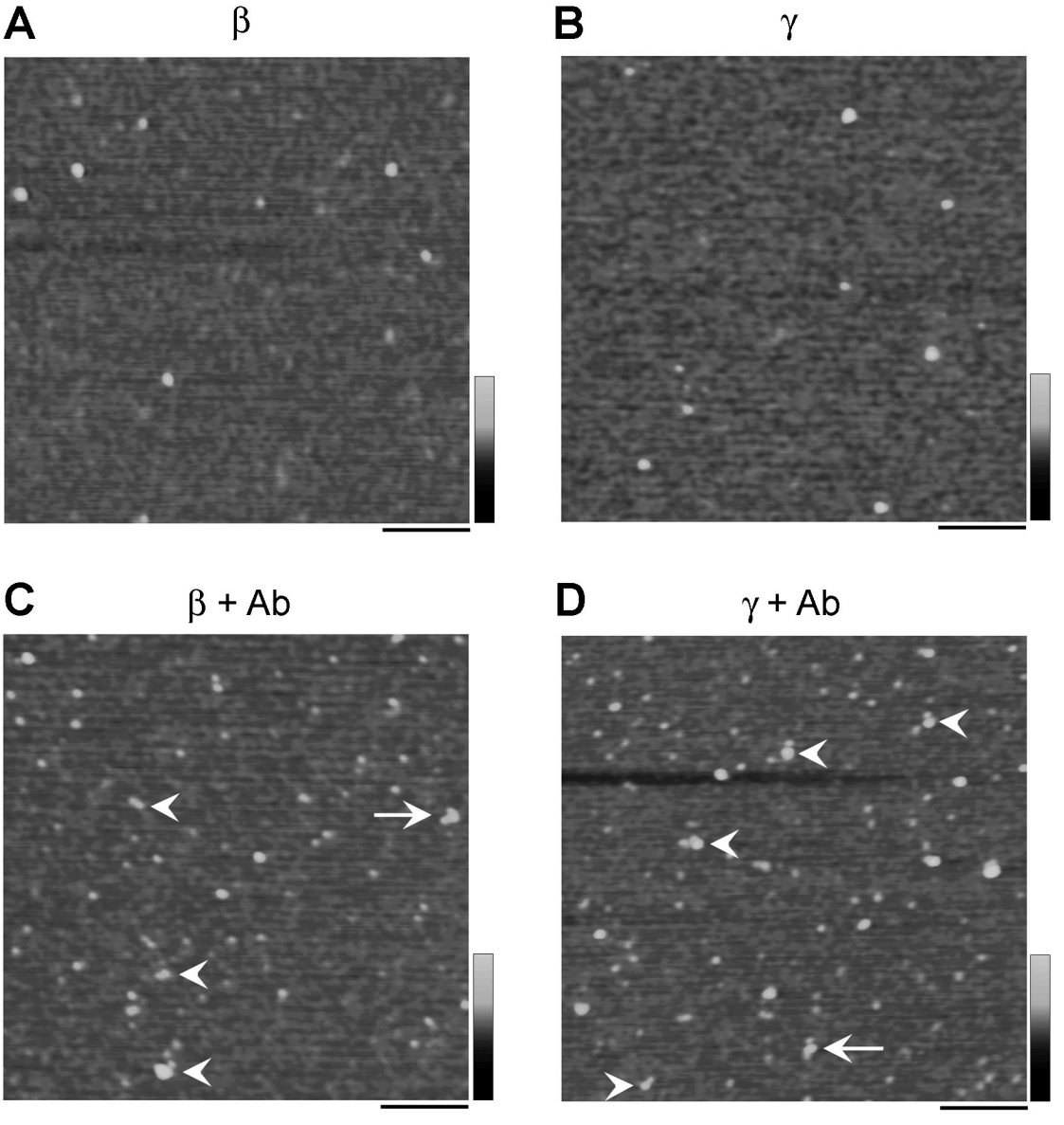

Figure S3