Supplemental data for the manuscript by Haerteis et al.:

'The δ-subunit of the epithelial sodium channel (ENaC) enhances channel activity and alters proteolytic ENaC activation'

Generation and validation of rabbit anti-human β- and γ-ENaC antibodies

Subunit specific antibodies against human β- and γ-ENaC were obtained by immunizing rabbits (Pineda Antibody Service, Berlin, Germany) with keyhole limpet hemocyanin-coupled synthetic peptides. The immunizing peptides corresponded to the human peptide sequences homologous to the rat peptide sequences previously used to generate subunit specific antibodies against rat ENaC (Masilamani 1999, *J Clin Invest* **104**, 19-23). Peptides were synthesized corresponding to the amino acid sequence 619-640 of β-hENaC (NH_2-NYDSLRLQPLDVIESDSEGDAI-COOH) and to the amino acid sequence 628-649 of γ-hENaC (NH_2-NTLRLERAFSNQLTDTQMLDEL-COOH). The specificity of the affinity-purified antibodies for the β- and γ-subunits of hENaC was tested using the oocyte expression system and western blot analysis as illustrated in the figure below.

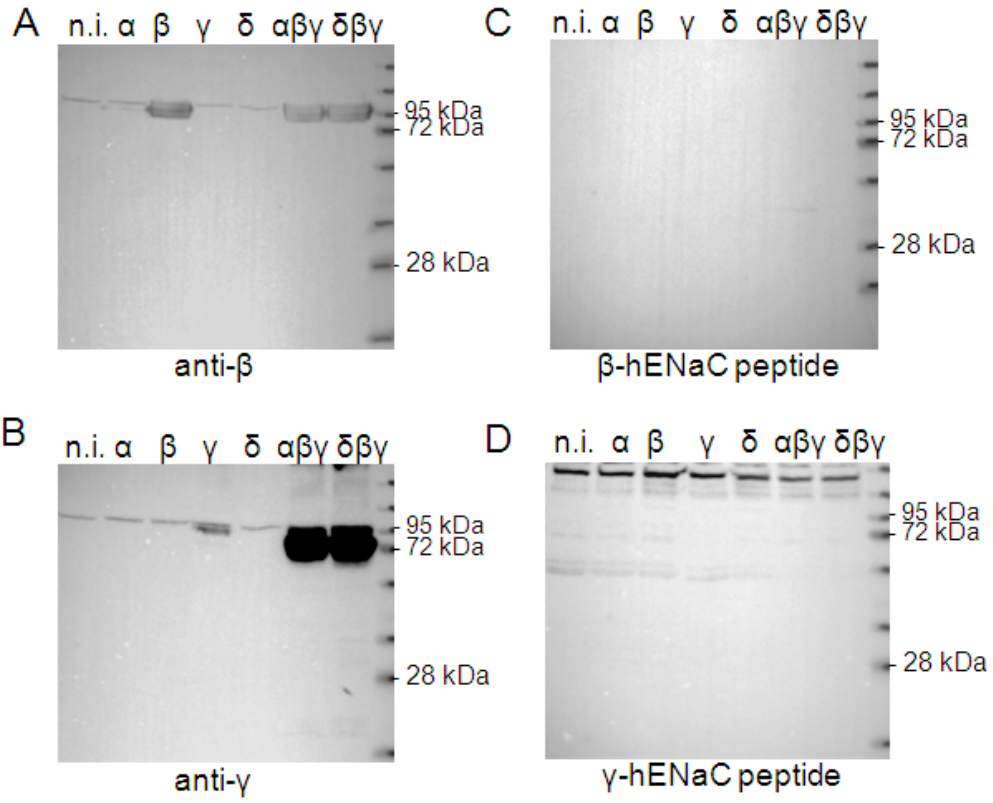

Figure legend:

To test the newly generated antibodies against β- (*A*) or γ-hENaC (*B*) by western blot analysis we used membrane-enriched fractions from whole-cell lysates of oocytes expressing an individual hENaC subunit (α, β, γ, or δ) or a combination of three subunits (αβγ-hENaC or δβγ-hENaC). *A*, The β-hENaC antibody detected a specific band of expected size (~95 kDa) in material from oocytes expressing β-

hENaC (lane 3) or all three subunits (αβγ in lane 6; δβγ in lane 7). In contrast, no specific band was detected in non injected oocytes (lane 1) or in oocytes expressing α-, γ-, or δ-hENaC alone (lanes 2, 4, and 5). *B*, Similarly, a specific signal for γ-hENaC was seen in oocytes expressing the γ-subunit alone (lane 4) or in combination with αβ-hENaC (lane 6) or δβ-hENaC (lane 7). *C-D*, As expected, pre-incubating the antibodies with the corresponding immunizing peptides largely prevented the detection of specific bands by the β-hENaC antibody (*C*) and the γ-hENaC antibody (*D*) in western blots. Antibodies were used in a dilution of 1:5,000 either with or without pre-incubation of the antibodies with the immunizing peptide (1:100) for one hour at 4 °C.

Evaluation of single channel P_O of δβγ-hENaC

Number of apparent channels in the patch (N)	Number of patches	NP_O	P_O
1	5	0.89 ± 0.04	0.89
2	1	1.77	0.89
3	2	2.84 ± 0.15	0.95
4	1	3.70	0.93
5	1	4.54	0.91

Table 1: P_O was estimated in outside-out patches of oocytes expressing δβγ-hENaC by dividing NP_O by the number of apparent channels (N) in the patch. NP_O was derived from amplitude histograms; N was determined by visual inspection of the recordings. Average NP_O values (±SEM) are given for groups of patches containing the same number of apparent channels.