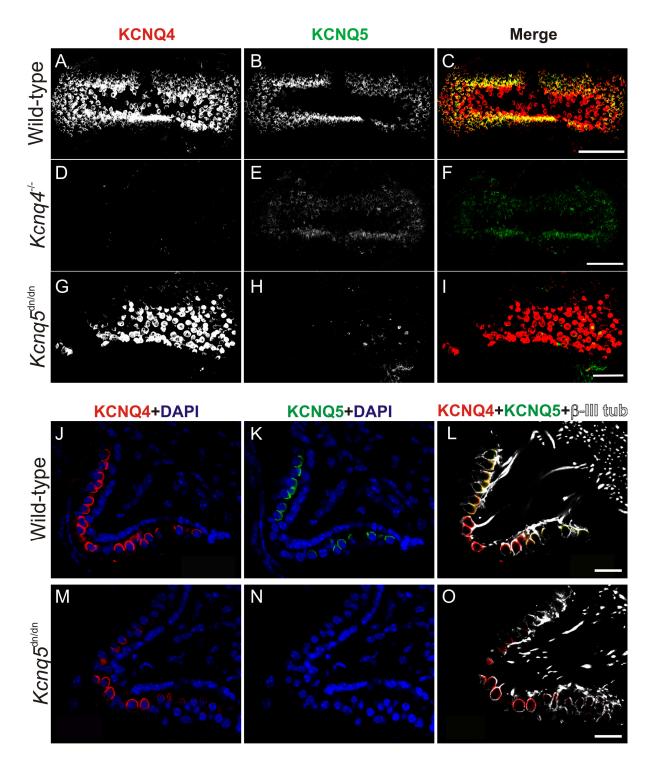
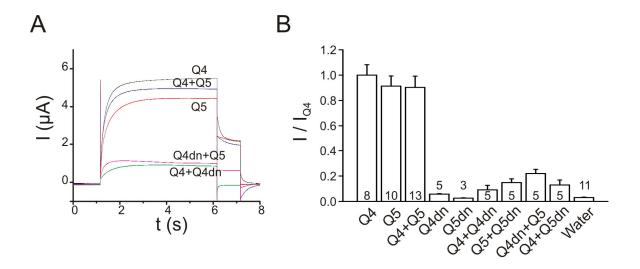
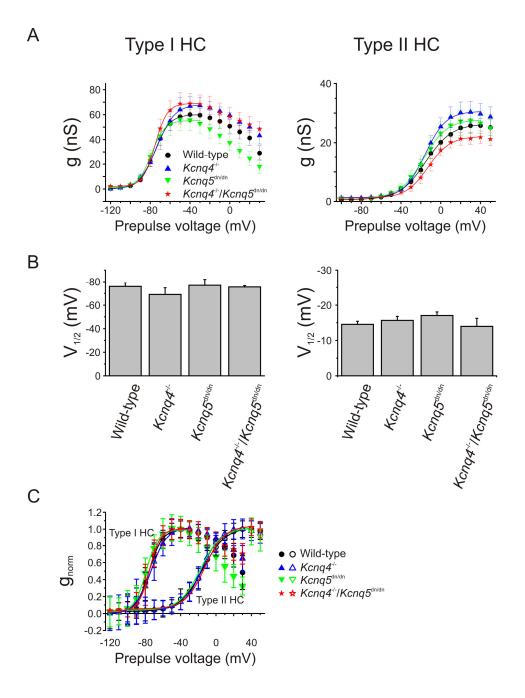
Suppl. Figures for Spitzmaul et al. 'Vestibular role of KCNQ4 and KCNQ5 K⁺ channels....'



Supplementary Figure S1. KCNQ4 and KCNQ5 in mouse cristae ampullaris. A-I, whole-mount preparations of cristae ampullares from WT (A-C), $Kcnq4^{-/-}$ (D-F) and $Kcnq5^{dn/dn}$ (G-I) mice stained with antibodies against KCNQ4 (left panel) and KCNQ5 (middle panel). Merged pictures are shown in the right panel C,F,I (KCNQ4, red; KCNQ5, green). Scale bars, 100 µm for A-F and 40 µm for G-I. Several confocal layers were superimposed to obtain images of the whole organs. J-O, slice preparation of cristae ampullares from WT (J-L) and $Kcnq5^{dn/dn}$ (M-O) mice. 8 µm sections were stained with antibodies against KCNQ4 (red), KCNQ5 (green) and β-III tubulin (grey). Note more restricted KCNQ4 expression pattern in $Kcnq5^{dn/dn}$ cristae. Nuclei were stained with DAPI (blue). Scale bars for J-O 20 µm.



Supplementary Figure S2. KCNQ4 and KCNQ5 form heteromers *in vitro*. A. Typical currents of *Xenopus* oocytes previously injected with RNA encoding KCNQ4 (Q4), KCNQ5 (Q5), KCNQ4(G285S) (Q4dn) and KCNQ5(G278S) (Q5dn). The total amount of injected cRNA was 20 ng. Mixtures were injected at 1:1 ratios. Oocytes were clamped to -80 mV and then depolarized to +20 mV for 5 seconds, followed by a 1-sec step to -20 mV and a return to -80 mV. B. Averaged currents (at the end of the +20 mV voltage step) of experiments like in (A), but including also expression of the dominant negative KCNQ5(G278S) mutant. Note that dominant negative mutants not only suppressed currents from the same subunit, but also from the other one, demonstrating formation of KCNQ4-KCNQ5 heteromers. 1:1 KCNQ4/KCNQ5 co-injection did not increase current amplitudes. Currents from two batches of oocytes were normalized to the respective mean KCNQ4 current and averaged. Error bars, SEM. Numbers in or above columns indicate the number of measured oocytes.



Supplementary Figure S3. Activation curves obtained from tail currents from type I and type II HCs of WT and the 3 mouse models. **A.** Activation curves for type I (left) and type II (right) HCs for WT (black circles) and $Kcnq4^{-/-}$ (Upper blue triangle), $Kcnq5^{dn/dn}$ (lower green triangle) and $Kcnq4^{-/-}/Kcnq5^{dn/dn}$ (red star) mice. The tail currents at the step to -30 mV was calculated and converted to conductance (g) by dividing the driving force (55 mV and 43 mV for type I and type II HCs, respectively) and then plotted versus the previous step potentials. Data were fit with the Boltzmann equation. Bars indicate SEM. **B**. Half-maximal voltage of activation (V_{1/2}) obtained from Boltzman fits for WT, $Kcnq4^{-/-}$, $Kcnq5^{dn/dn}$ and $Kcnq4^{-/-}/Kcnq5^{dn/dn}$ mice for type I (left) and type II (right) HCs. Fits were done individually for each experiment and then averaged. Differences in V_{1/2} were statistically not significant with respect to WT. **C**. Normalized conductance (g_{norm}) for the curves plotted in A for type I (closed symbols) and type II (open symbols) HCs.