

Supplemental Information

Supplemental Procedures

Auditory brain stem responses (ABR)

Auditory evoked brain stem responses (ABR) to clicks were recorded in anaesthetized animals (16 mg/kg xylazine hydrochloride, 60 mg/kg S-ketamine hydrochloride). Measurements were carried out in a sound-proof chamber and animals were kept warm on a heating pad during the experiment. Electric potentials were recorded by subdermal silver wire electrodes at the vertex (reference), forehead (ground) and at both mastoids. Acoustic click stimuli were generated by a Beyer DT-48 earphone fitted closed-field into the external auditory meatus with a conical earpiece. An integrated probe microphone (Brüel & Kjær 4135) in the earpiece served to monitor the actual sound signal. Calibration was performed in a 19 μ l volume using a second probe microphone (Brüel & Kjær 4135) with a sound level meter (Brüel & Kjær 2215). Acoustic click stimuli (~200 μ s duration) had a flat frequency spectrum (± 5 dB) with an upper corner frequency of 5.5 kHz. Alternating clicks were applied at a rate of 21/s and averaged 400 to 2000 times. Stimulus intensities were varied starting at 118 dB peak equivalent SPL [dB SPL pe] in increments of 20 dB except near threshold where 5 dB steps were used. The hearing threshold was defined as the lowest intensity to generate a reproducible ABR waveform.

Endocochlear potential and potassium concentration

Measurements of endocochlear potential (EP) and potassium concentration ($[K^+]$) were performed in anaesthetized mice (16 mg/kg xylazin hydrochloride, 40 mg/kg S-ketamin hydrochloride) on a temperature controlled surgical table. Additional doses (50% of the initial mixture) were administered when needed. The bullae were opened ventro-laterally, leaving the tympanic membrane intact. The bone over the first turn of the cochlea was thinned and opened apically to the stapedial artery.

The stria vascularis was penetrated with a double-barreled microelectrode and the potential and $[K^+]$ were measured in the scala media. An Ag/AgCl electrode in a salt

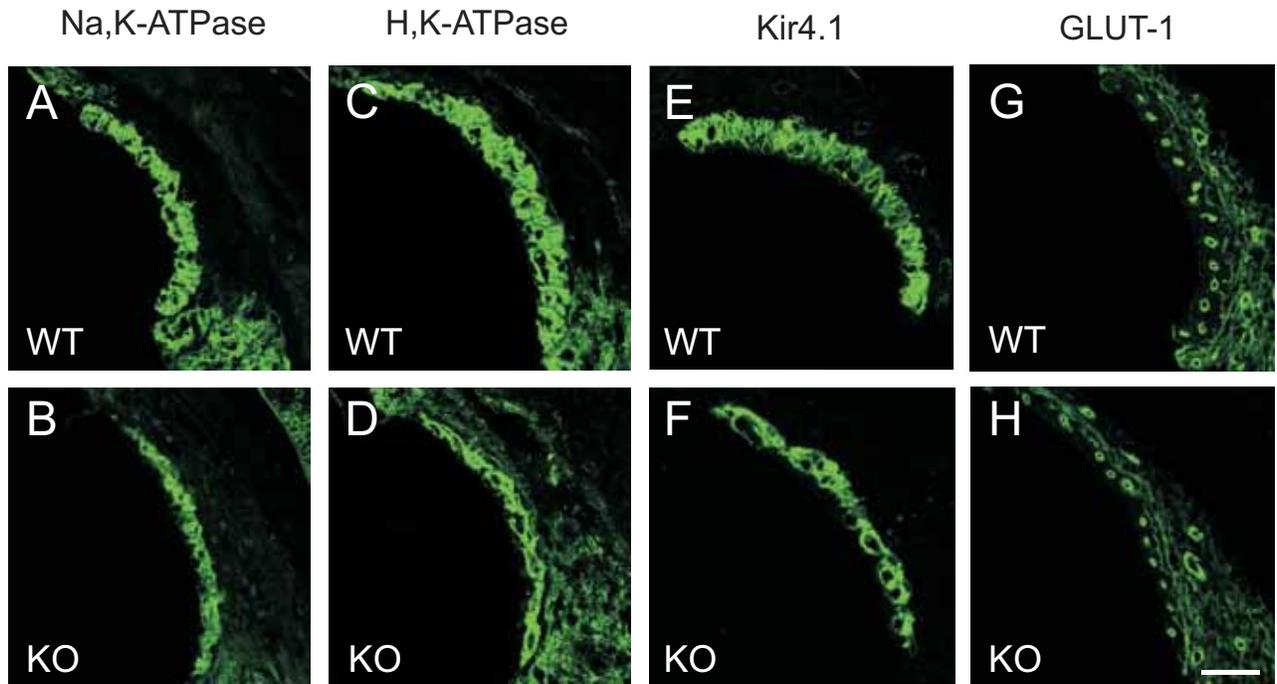
bridge (0.9% NaCl) was placed under the skin near the neck muscle, serving as reference electrode. Before penetration of the stria and after the experiment the electric potential measured with the double barreled electrode in the neck muscle served as DC reference potential for the EP. K^+ -selective barrels used liquid K^+ ionophore I, cocktail B (Fluka, #60398) and were calibrated before and after each experiment in standard solutions ranging from 1 – 200 mM $[K^+]$. Measurements exceeding predefined limits in pre/post DC potential shift (5mV/20 min) or differences in pre/post $[K^+]$ calibration were excluded from further analysis. After penetration of the stria vascularis the EP and $[K^+]$ were recorded for minimally 2 minutes before the animal was sacrificed by an intracardial injection of a barbiturate (T61). The measured potential decreases nearly instantly after active transport ceased and reaches a negative minimum several minutes (10-15) *post mortem*, determined by the steady state potential (SSP) due to the passive part of current components. Especially in KO animals where the obtained EP was low or zero this characteristic behavior served as a control that the measurement site was actually inside the scala media.

Distortion product otoacoustic emission (DPOAE)

For recordings of distortion product otoacoustic emissions (DPOAE), mice aged 22-26 days were anaesthetized by intraperitoneal injection of ketamine (125 mg/kg) and xylazine (2.5 mg/kg) and placed on a rectal temperature-controlled heat blanket (Hugo Sachs Elektronik – Harvard Apparatus GmbH, March-Hugstetten, Germany) in a sound-attenuated chamber. Primary tones ($f_2 = 6,8,12,16$ and 20 kHz, $f_1 = f_2/1.2$) were coupled into the ear canal by a custom-made probe containing the ED1/EC1 speaker system (Tucker-Davis-Technologies, Ft Lauderdale, FL) and an MKE-2 microphone (Sennheiser, Hannover, Germany), and adjusted to an intensity of 20-80 dB sound pressure level at the position of the ear drum for f_1 and $f_2=f_1-10$ dB. The microphone signal was sampled and amplified using a 24-bit sound card (DMP3, MIDIMAN) and analyzed by fast Fourier transformation using MATLAB (Mathworks, Natick, MA). At the highest stimulus levels used (80/70 dB), a small distortion at $2f_1-f_2$ was detected in one third of measurements after the animals were sacrificed by intraperitoneal injection of T61. In alive Barttin KO mice, a similar distortion was present in two out of 15 recordings and was considered to be a physical distortion rather than an otoacoustic emission.

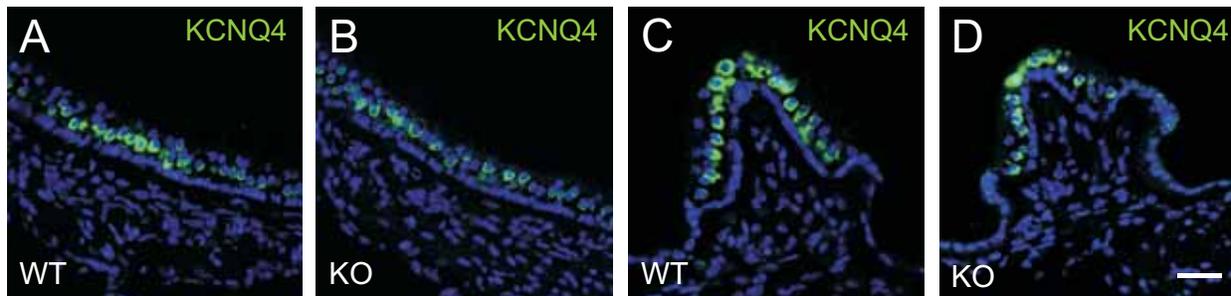
Vestibular tests

Three days before behavioral testing, a prefabricated piece equipped with two nuts was cemented to the skull in order to fixate the mouse's head in a restrainer device. The surgical procedures were performed under general anaesthesia using a mixture of isofluran (Isofloran 1-1.5%; Rhodia Organique Fine Ltd) and oxygen. During the experiment the mouse was placed in a restrainer, with the head fixed above the center of the turntable. A cylindrical screen (diameter 63 cm) with a random-dotted pattern (each element 2°) surrounded the turntable (diameter 60 cm), and both the screen and turntable were driven independently by AC servomotors (Harmonic Drive AC, the Netherlands). The table and screen position signal were measured by potentiometers, filtered (cut-off 20 Hz), digitized (CED Limited, UK) and stored on a computer. A CCD camera was fixed to the turntable to monitor the mouse's eye using an eye-tracking device (Iscan Inc.). Both video calibrations and subsequent eye movement computations were performed as described (48). Angular optokinetic reflex (OKR), angular vestibulo-ocular reflex (VOR) and visually enhanced angular vestibulo-ocular reflex (VVOR) were evoked by rotating the surrounding screen, the turntable in dark and the turntable in light, respectively (rotations of 0.1 - 1 Hz at 5° rendering a velocity of 3.1 – 31.4 deg/sec). Before measuring the VOR pilocarpine 4% (Laboratoires Chauvin, France) was used in order to limit the pupil dilatation in darkness. Data are presented as mean \pm SEM. For statistical comparisons we used the 2-way ANOVA with repeated measures (SPSS 15.0 Inc.).



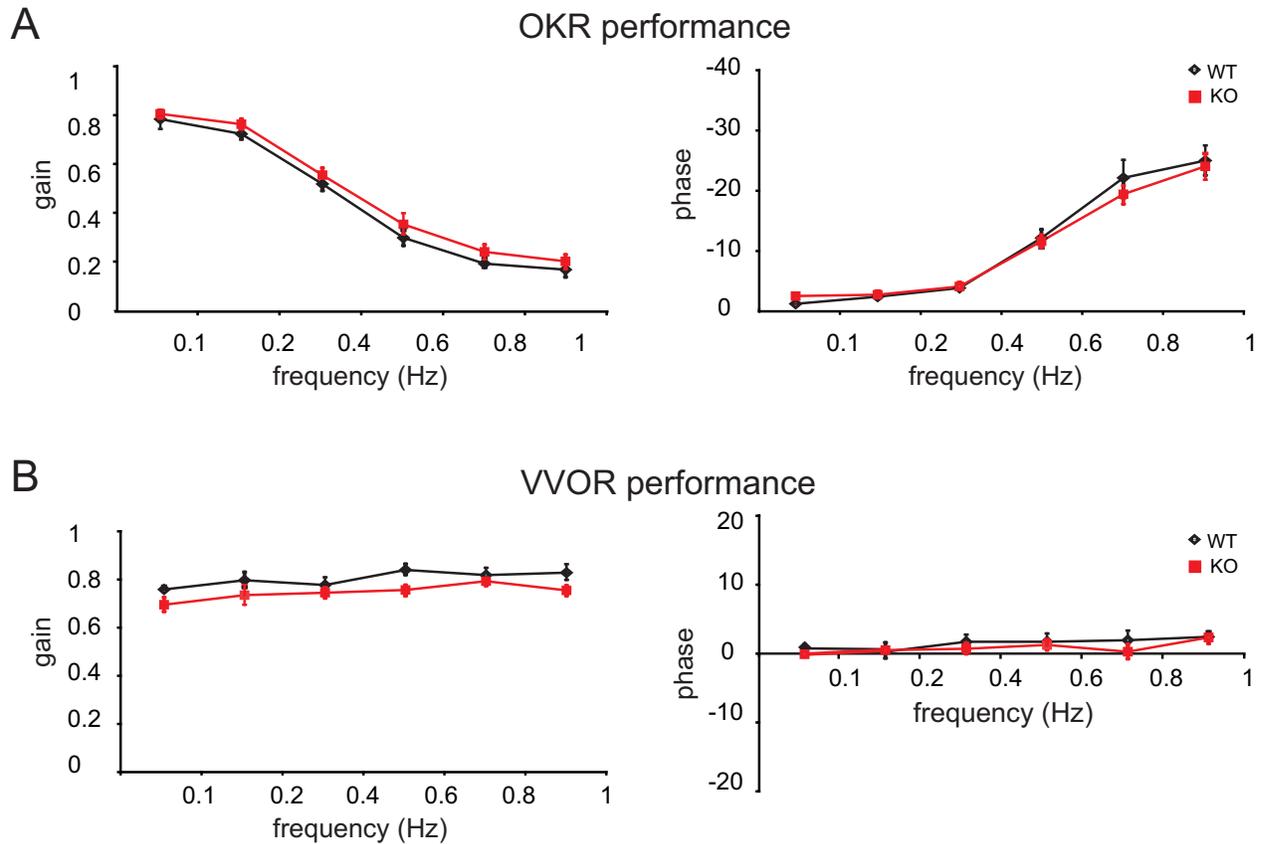
Suppl. Figure S1

All cell types of the stria vascularis are retained in *Bsnd*^{lox/lox} *Sox10::Cre* mice, although their numbers decline. Immunofluorescent detection of Na,K-ATPase (α 1 subunit) (**A, B**), H,K-ATPase (α subunit) (**C, D**) (both expressed in marginal cells), the K^+ -channel Kir4.1 (**E, F**) (of intermediate cells) and the glucose transporter GLUT-1 (**G, H**) (expressed in capillaries) in the stria vascularis of WT (**A, C, E, G**) and KO (*Bsnd*^{lox/lox} *Sox10::Cre*) (**B, D, F, H**) mice. Scale bar in (**H**): 40 μ m for all panels. Age: 4 weeks or more.



Suppl. Figure S2

Expression of the K⁺-channel KCNQ4 in vestibular hair cells is not changed by loss of barttin. Vestibular hair cells of one year-old WT (**A, C**) and *Bsnd*^{lox/lox} Sox10::Cre (KO) (**B, D**) mice were stained with an antibody against KCNQ4. (A,B) utriculus, (C,D) crista ampullaris. Note that KCNQ4 is only expressed in a subset of vestibular hair cells. Scale bar in (D): 20 μ m for all panels.



Suppl. Figure S3

Optokinetic reflex (OKR) performance and visually enhanced vestibulo ocular reflex (VVOR) are normal in *Bsnd^{lox/lox} Sox10::Cre* mice. The OKR (A) and VVOR (B) showed no significant differences in gain or phase between the genotypes over the entire frequency range (0.1 - 1 Hz) ($p > 0.3$ for all gain and phase comparisons; 2-way ANOVA). OKR gain values of *Barttin* mice ranged from 0.82 ± 0.02 at 0.1 Hz to 0.21 ± 0.03 at 1 Hz, while those of wild type littermates ranged from 0.80 ± 0.04 to 0.17 ± 0.03 . Following this visual stimulation at 0.1 Hz to 1 Hz phase leads of *Barttin* mice increased from $2.6 \pm 1.8^\circ$ at 0.1 Hz to 24.1 ± 2.2 at 1 Hz, while those of their wild type littermates varied from $1.2 \pm 1.6^\circ$ to 24.7 ± 2.5 degrees. Black symbols and curves, WT; red symbols and curves, *Bsnd^{lox/lox} Sox10::Cre* mice.