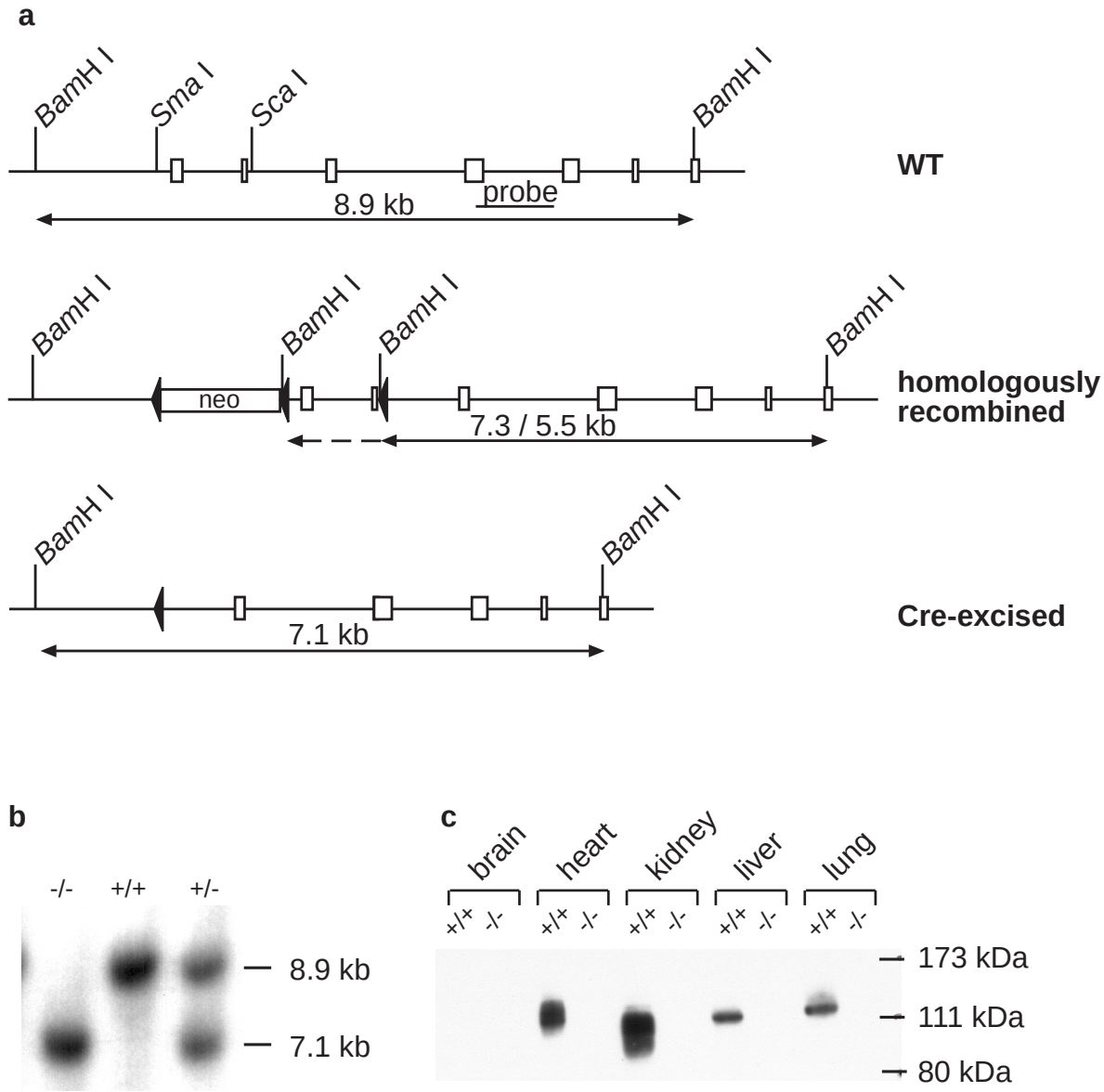

Supplementary Information 1

Disruption of the *Kcc4* gene in mice. **a**, KCC4 alleles: A neomycin resistance cassette flanked by loxP sites was inserted into the SmaI site in front of the exon coding for the first and a part of the second transmembrane span. A third loxP site was inserted into a ScaI site after the next exon that encodes the remainder of the second transmembrane span. A diphteria toxin A cassette was fused 5' to the homologous region. Recombinant clones were transfected with a plasmid expressing Cre-recombinase. Clones lacking the neomycin cassette and the two coding exons were injected into C57Bl6 blastocysts. Mouse lines were established trom two independent ES-clones. RT-PCR predicted a protein truncated before the first transmembrane span in *Kcc4*$^{-/-}$ mice. **b**, Southern blot of mouse genomic DNA using the probe shown in (a). A 7.1 kb band reveals disrupted *Kcc4* alleles. **c**, Western blot of membrane proteins from different WT and KO organs: KCC4 was detected in kidney, heart, liver, and lung, but not in brain. KCC4 was absent from KO mice. 25 µg protein per lane.

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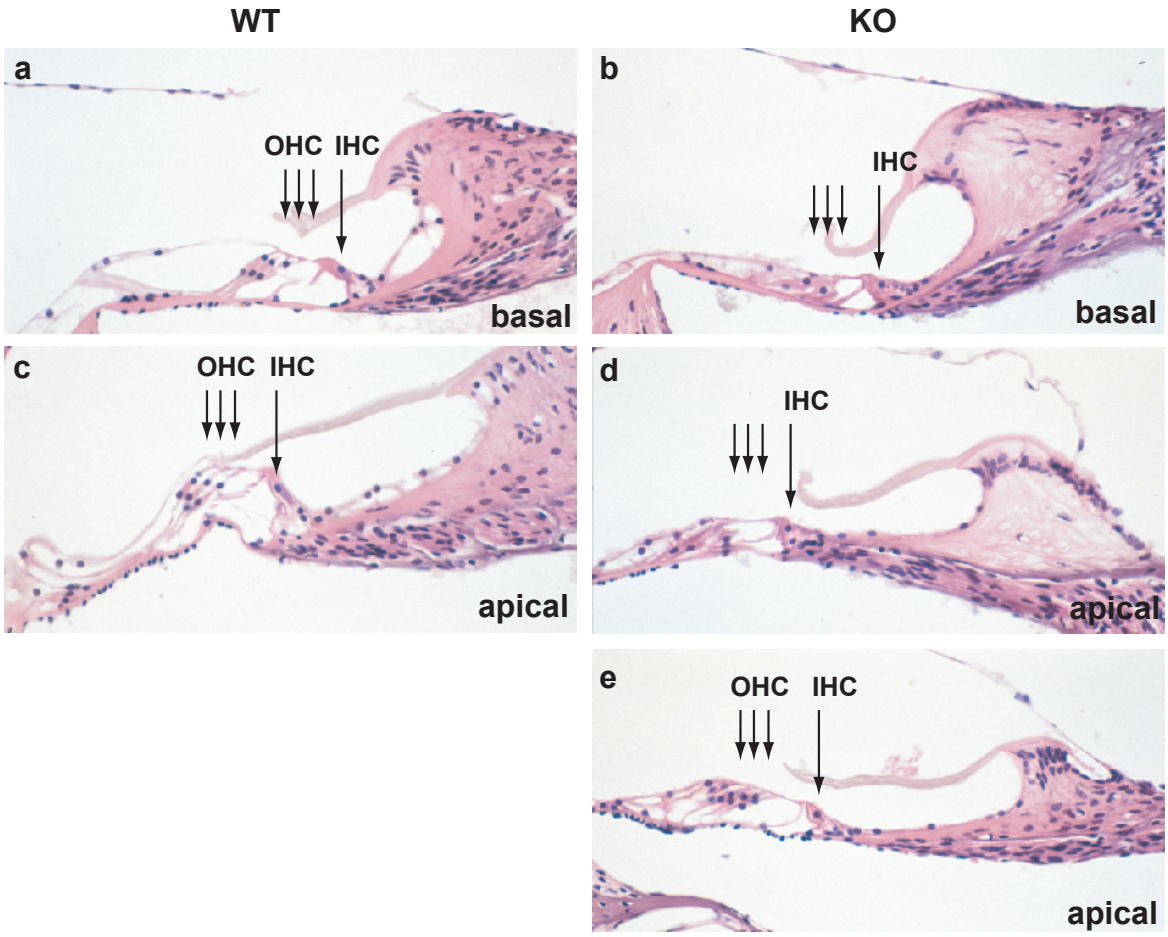
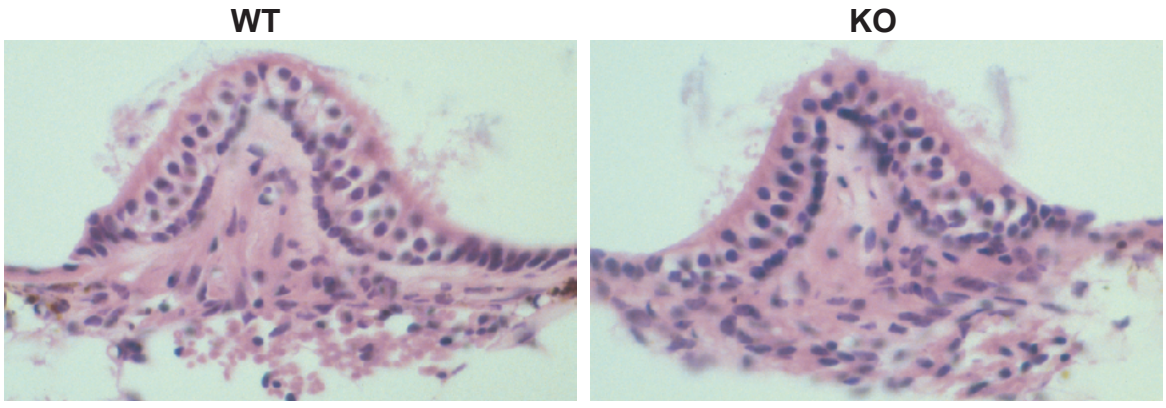
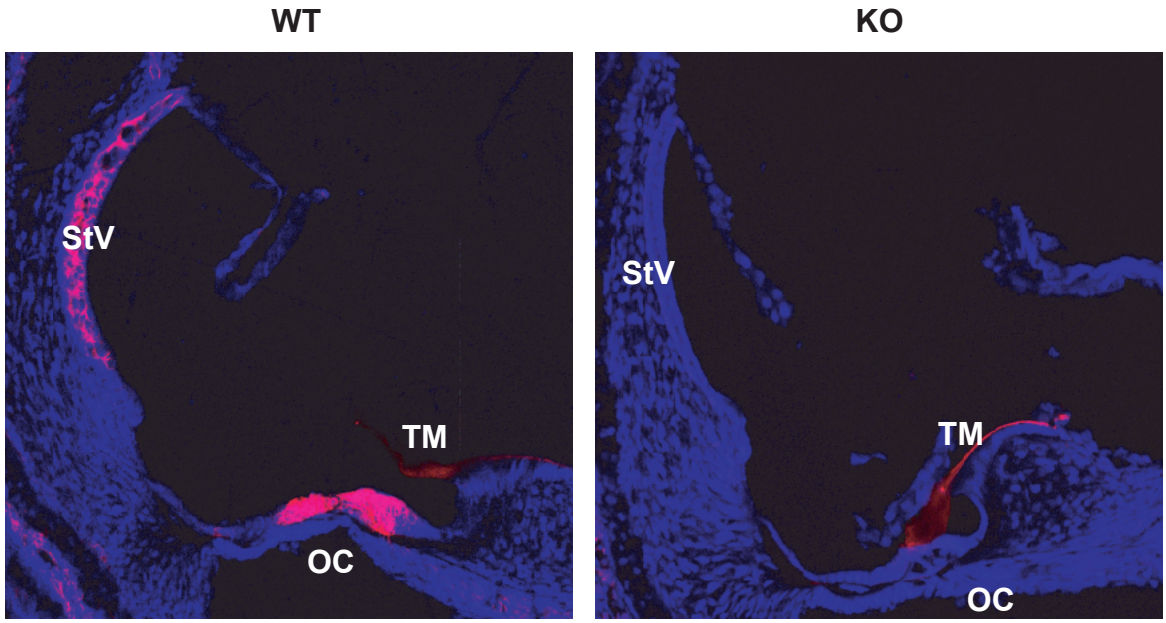


Supplementary Figure 2: Basal to apical gradient of outer hair cell degeneration
In a 4 weeks old WT animal the typical morphology of the organ of corti is seen in basal (**a**) and apical turns (**c**). In a 4 weeks old KO animal outer hair cells in the basal turn are completely degenerated (**b**). The inner hair cell is still present at this age, but also degenerates in later stages (10 weeks, Fig. 3 h). In the apical turn outer hair cells degenerate (**d**) but are still present in the most apical part of the cochlea (**e**). Neurons of the spiral ganglion are not degenerated at this age (not shown).



Supplementary Figure 3: The vestibular organ is not affected in KCC4 KO mice
Histological comparison between WT and KO vestibular organs shows no difference.



Supplementary Figure 4: Specificity of the KCC4 antiserum
Comparision between WT and KO cochlea at the postnatal day 8. Note the specific expression of KCC4 in the stria vascularis (**StV**) and the organ of Corti (**OC**) of the WT cochlea. An unspecific staining, also seen with other antisera, is present in the tectorial membrane (**TM**). No expression of KCC4 was detected in the adult vestibular organ.