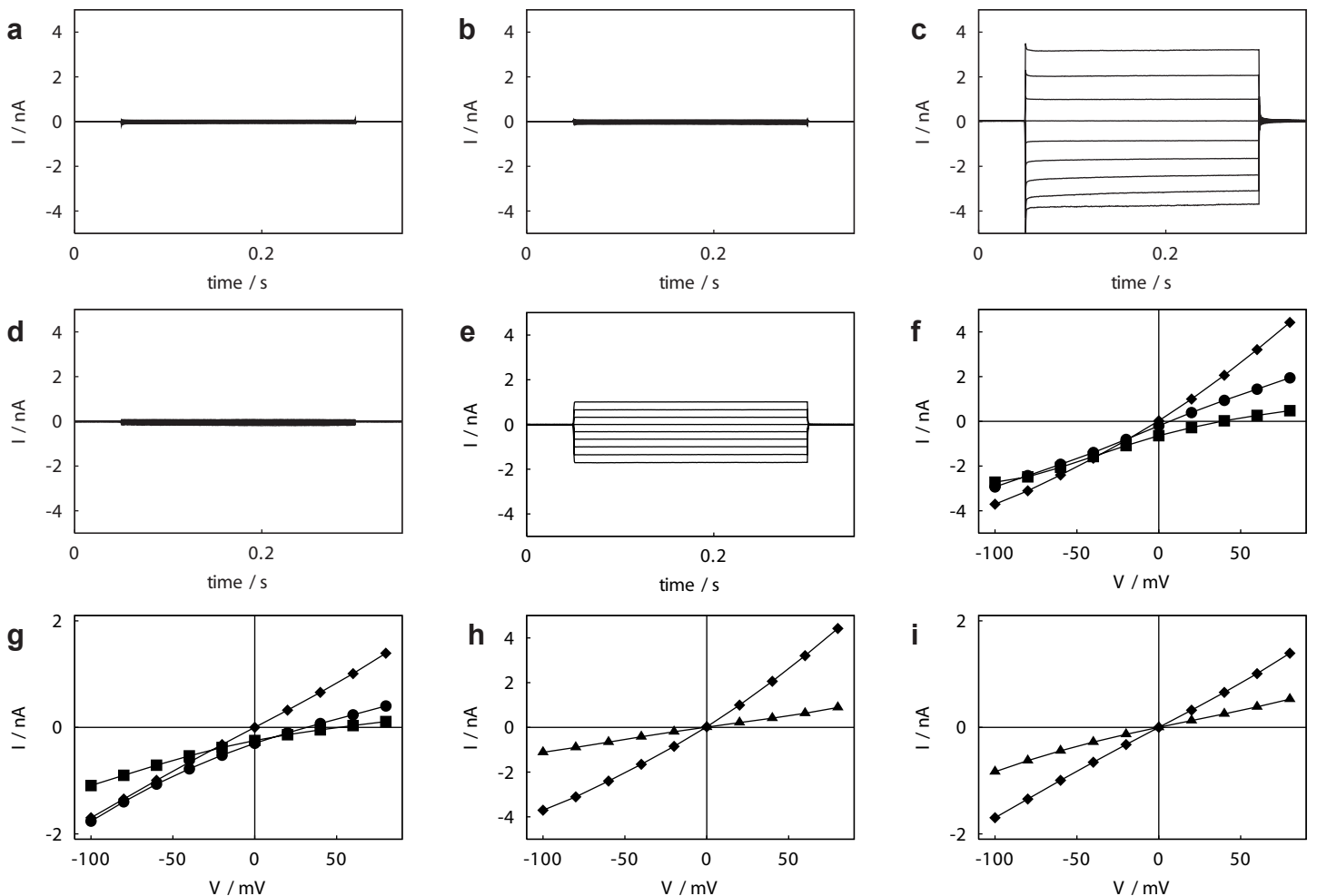


# Supplementary Information for "Barttin is a Cl<sup>-</sup> channel Beta-subunit crucial for renal Cl<sup>-</sup> reabsorption and inner ear K<sup>+</sup> secretion"

## Nature V414, 558

**A: Functional characterisation of ClC-K/barttin channels expressed in transfected tsA201 cells.** Barttin (a), ClC-Ka (b), and ClC-Kb (d) did not yield significant currents, that were not larger than those from untransfected tsA201 cells. Large currents were seen upon ClC-Ka/barttin co-expression (c). Different from the oocyte system rather large currents were seen upon ClC-Kb/barttin expression (e). **f, g**, steady-state current-voltage relationships in tsA201 cells expressing ClC-Ka/barttin, and ClC-Kb/barttin, respectively, in the presence of different anions: diamond, Cl<sup>-</sup>; circle, Br<sup>-</sup>; square, I<sup>-</sup>. Cl<sup>-</sup> was totally replaced by the indicated anions. Similar to the oocyte system, in which ClC-Kb/barttin(Y98A) was investigated, Br<sup>-</sup> is less permeant in ClC-Kb/barttin than in ClC-Ka/barttin. **h, i**, currents at pH 7.4 (diamonds) and pH 6.5 (triangles) in Cl<sup>-</sup> solution for ClC-Ka/barttin and ClC-Kb/barttin, respectively. Pulse protocol: from -100 mV to +80 mV in +20 mV steps lasting 0.25 s.



### B: Topology of barttin

The first hydrophobic region of barttin may span the membrane or may act as a cleavable signal peptide. The latter possibility is suggested by the program SMART (<http://smart.embl-heidelberg.de>). To distinguish between these possibilities, we epitope-tagged barttin at either end. This did not affect its ability to elicit currents with CIC-Ka. When these constructs were expressed in COS cells with or without CIC-Ka (Ka) and analysed by Western blotting using an antibody against the epitope, bands corresponding in size to barttin were stained with comparable intensities irrespective of the position of the epitope (Fig. B). Mock-transfected COS cells (Ctrl) and CIC-Ka transfected COS cells were used as a controls (left two lanes). This argues against an amino-terminal cleavage, and supports a model in which barttin has two transmembrane spanning segments in its amino-terminal end.

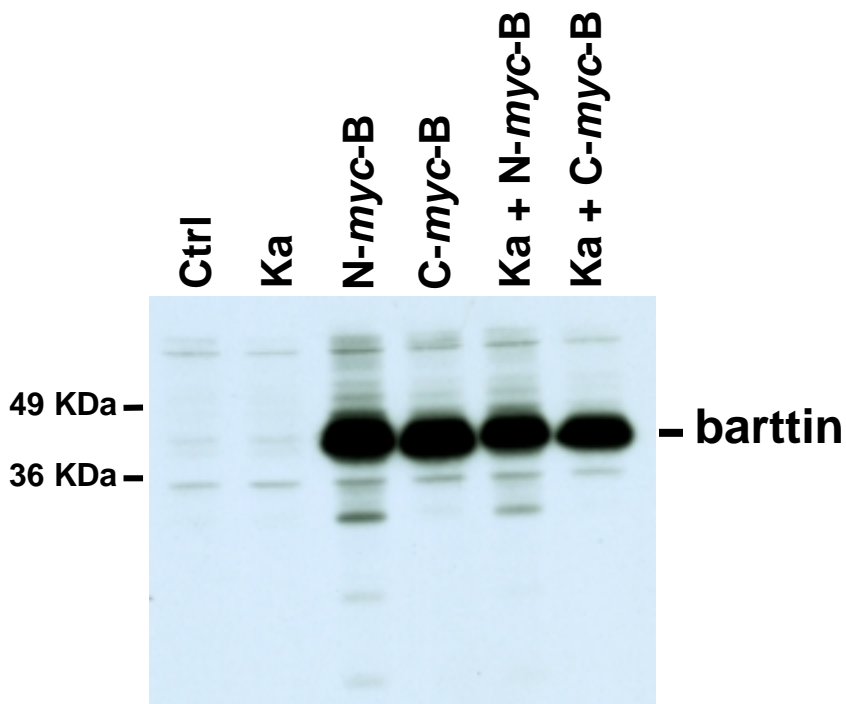
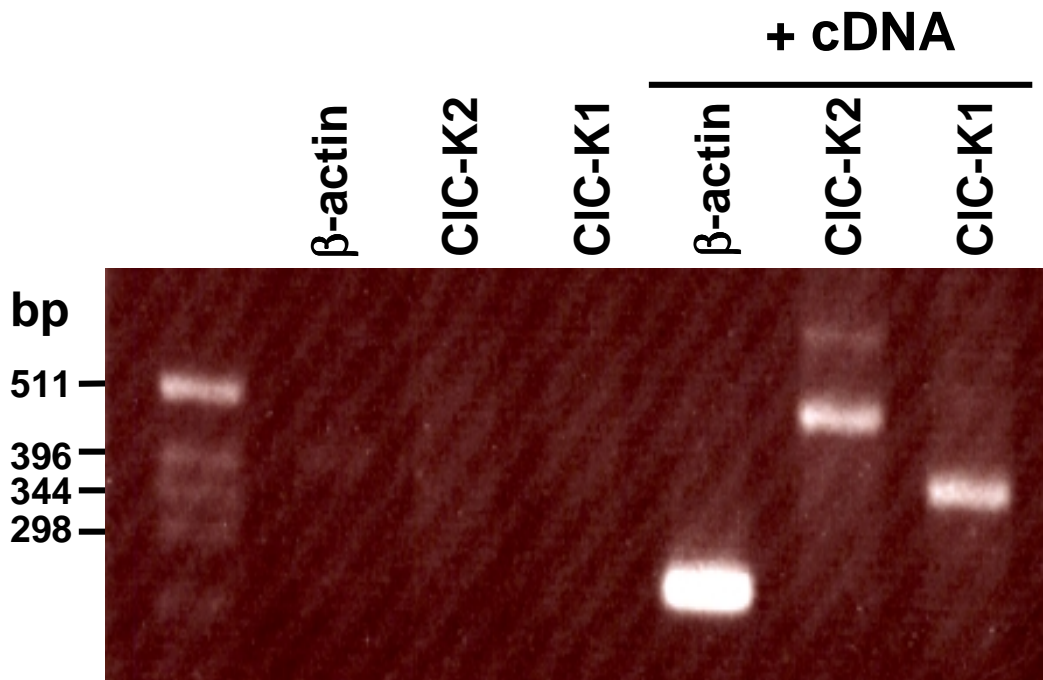


Figure B

**C: RT-PCR detection of CIC-K1 and CIC-K2 in the cochlea**

To examine whether CIC-K1 and CIC-K2 is expressed in the cochlea, we performed RT-PCR experiments on RNA obtained from mouse cochlea. The primers used could differentiate between the highly homologous (~90% identity) CIC-K1 and CIC-K2 mRNAs. Using PCR conditions and primers described in reference 24, we observed bands of the correct sizes in samples containing cochlear RNA, but not in controls (Fig. C). Thus, both CIC-K1 and CIC-K2 are expressed in the cochlea. Because immunofluorescence indicates that CIC-K channels are only present in the stria vascularis, this strongly suggests that these cells express both isoforms.



**Figure C**

### D: Antibodies against barttin:

Polyclonal antisera against barttin were raised in rabbits against the peptide VPADSDFQGILSPKA (residues 63-77) and in guinea-pigs against a mixture of the peptides PEQEEEDLYYGLPD (residues 288-301) and LLPDKELGFEPDIQG (residues 306-320). They were affinity-purified against the corresponding peptides. They were specific as they recognised a band of the expected size only in cells transfected with barttin, but not in non-transfected control cells, as shown for the guinea-pig antibody in Fig. Da: PPI: preimmuneserum, AB: antiserum. In basolateral kidney membranes, only a band of the correct size was detected (Fig. Db). Both antibodies, directed against non-overlapping epitopes, detected identical structures in the kidney (not shown) and the inner ear. In Fig. Dc a specific staining of the P0 stria vascularis is shown using the rabbit antibody. Compare this to Fig. De and Fig 5, which shows staining of the stria vascularis with the guinea-pig antibody. Fig D includes appropriate controls (pre-immune serum (Dd) and pre-incubation with the cognate peptides (Df).

