Activation of renal ClC-K chloride channels depends on an intact N terminus of their accessory subunit barttin

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Supporting Information

including three figures: Fig. S1

Fig. S2

Fig. S3

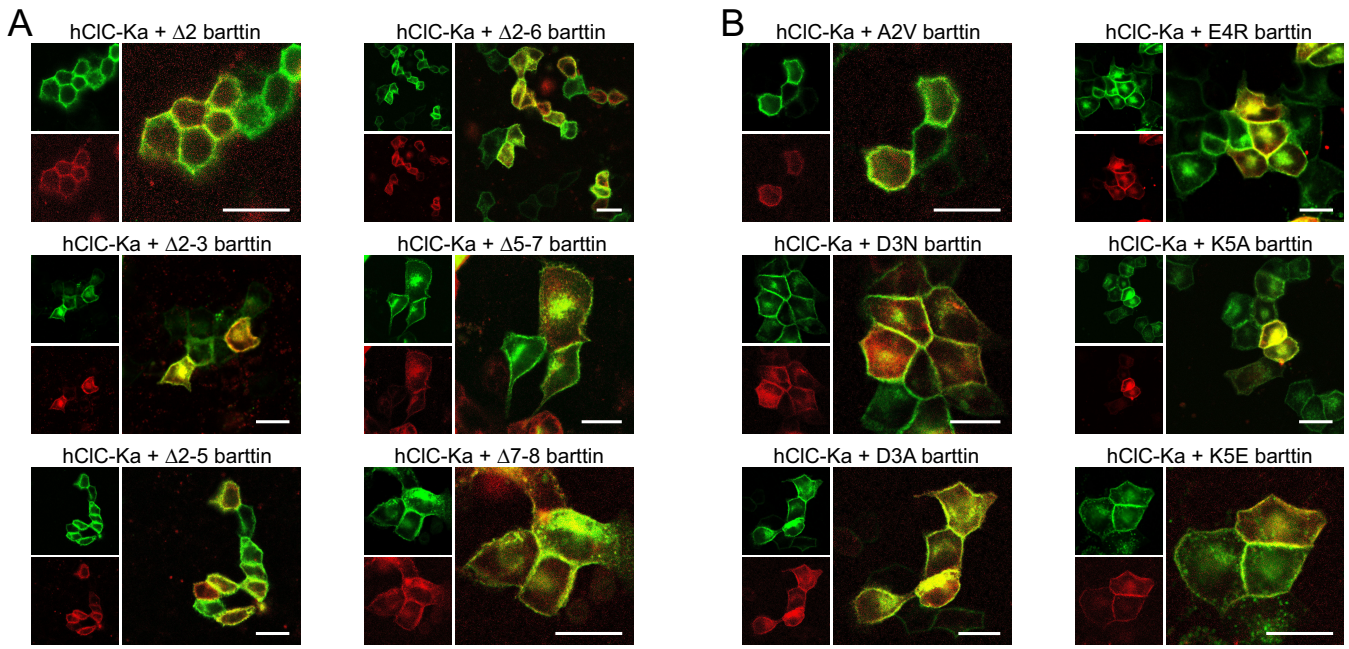
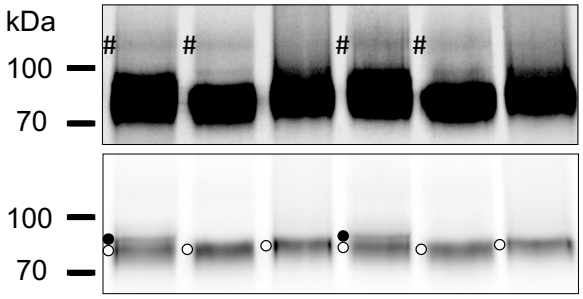


FIGURE S1. **Mutations of the barttin N terminus marginally affect subcellular distribution of barttin and hClC-Ka channels.** *A* and *B*, confocal images of MDCK-II cells displaying the subcellular localization of transiently expressed barttin-mCFP together with YFP-hClC-Ka channels. In *A,* different portions of the barttin N terminus were deleted as indicated. In *B*, single amino acids of the barttin N terminus were substituted. Mutant barttin promotes subcellular trafficking of co-expressed hClC-Ka channels like WT barttin. mCFP fluorescence is displayed in green, YFP fluorescence in red. Scale bar: 20 μm.

Fig.S1



YFP-hClC-Ka	+	+	+	+	+	+
WT barttin	-	-	-	+	+	+
Endo H_f	-	+	-	-	+	-
PNGase F	-	-	+	-	-	+

FIGURE S2. **Biochemical analysis of hClC-Ka channel glycosylation.** Cleared whole-cell lysates of HEK293T cells expressing YFP-hClC-Ka alone or in presence of WT barttin were electrophoresed. The same SDS-PAGE gel is displayed with two different intensities to visualize weak bands in the upper panel and prominent bands in the lower panel. To analyze the glycosylation state of YFP-hClC-Ka channels, samples of cell lysates were incubated for 1 h at 37°C with either endoglycosidase H (Endo H_f) or N-glycosidase F (PNGase F; New England Biolabs, Ipswich, USA). Endo H_f cleaves N-linked mannose rich oligosaccharides (core-glycosylation), but not the chemical bonds of complex oligosaccharides. PNGase F cleaves oligosaccharides of both core- and complex-glycosylated proteins. Supplement of 1% NP-40 prevented the inhibition of PNGase F by SDS. Three YFP-hClC-Ka bands are visible in absence of enzymatic digestion. The lower band of non-glycosylated protein (○) and the upper band of complex-glycosylated (#) protein remained visible under treatment with Endo H_f. With PNGase F, however, both bands of core-glycosylated (●) and complex-glycosylated (#) channel proteins are missing. Absence (-) and presence (+) of WT barttin and/or enzymes are indicated in the table.

Fig.S2

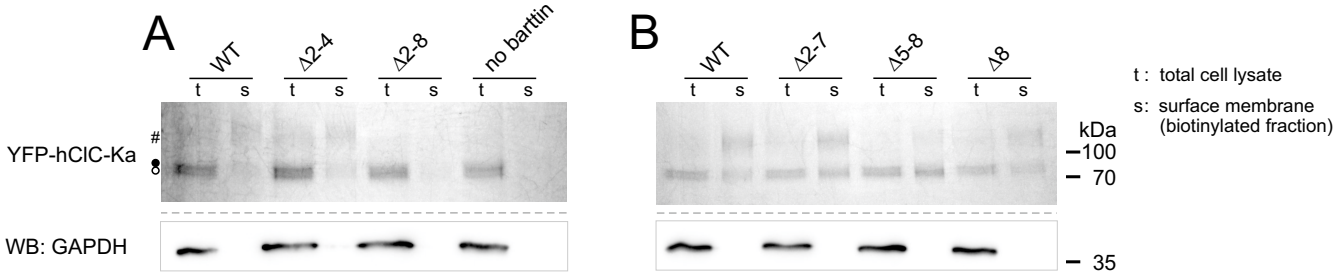


FIGURE S3. **WT and mutant barttin promote insertion of hClC-Ka channels in the surface membrane of MDCK-II cells.** *A* and *B*, fluorescence images of SDS-PAGE gels displaying YFP-hClC-Ka channels in total whole-cell lysates (t) and in the biotinylated protein fractions of the surface membrane (s) of MDCK-II cells that transiently co-expressed YFP-hClC-Ka and WT or mutant barttin-mCFP. Symbols indicate non-glycosylated (○), core-glycosylated (●) and complex-glycosylated (#) channel proteins. Membrane-integrated proteins were biotinylated from the extracellular side and purified after cell lysis using NeutrAvidin affinity chromatography. Contamination of the surface membrane fraction by cytosolic proteins was excluded by western blots using anti-GAPDH antibodies (WB) that exclusively revealed bands in the total cell lysates. - Notably, surface membrane insertion of YFP-hClC-Ka channels is close to the detection limit in presence of Δ2-8 barttin and in absence of barttin.

Fig.S3