SUPPORTING INFORMATION

DHHC7-mediated palmitoylation of barttin is critically involved in regulation of the ClC-K chloride channel functions

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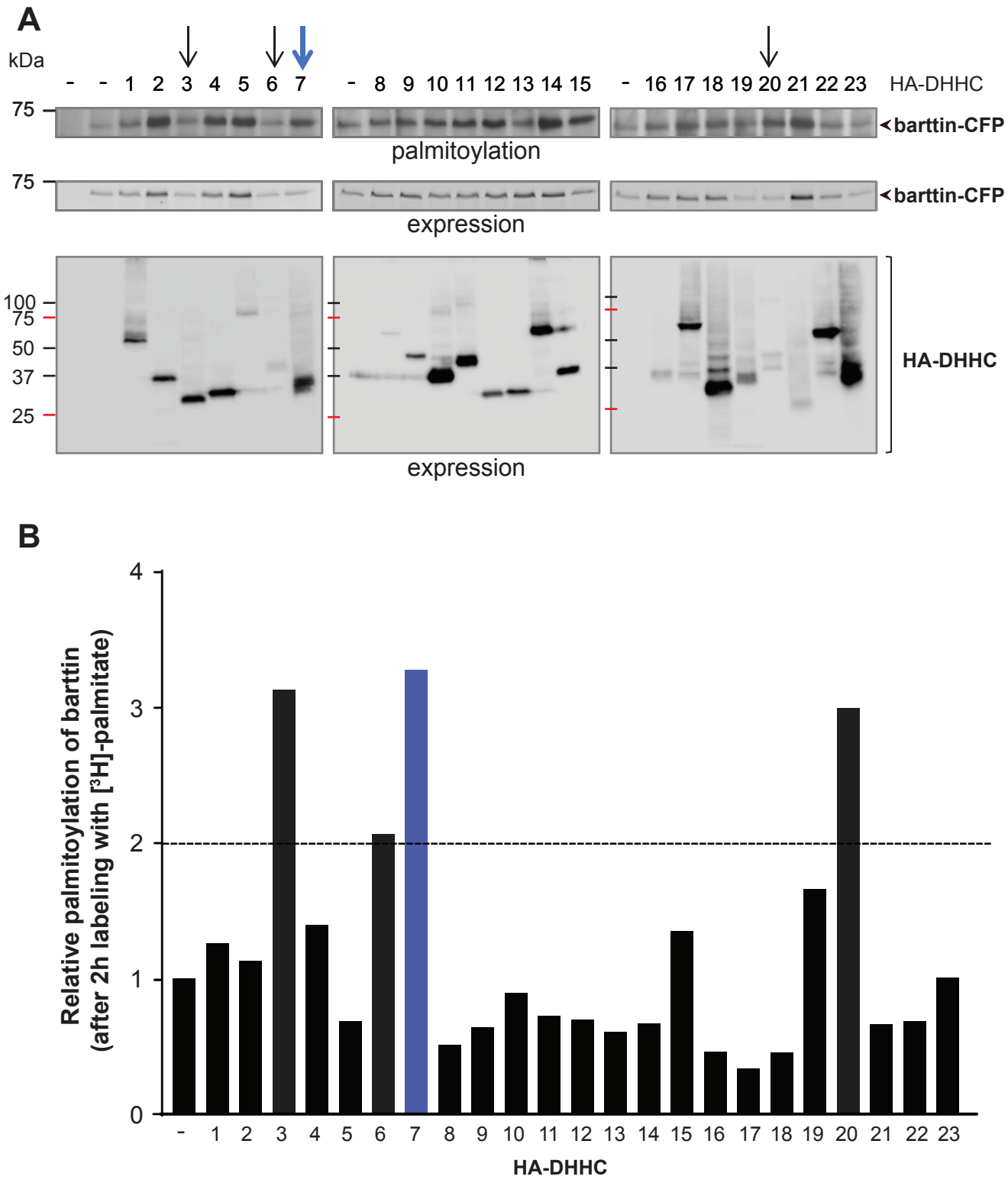

Figure S1:

(A) [^{3}H-]-palmitate incorporation into barttin-CFP after labelling with [9,10(n)^{3}H] palmitic acid for 2 h at 37°C in HEK293T cells co-transfected with barttin and corresponding DHHCs. **(B)** Relative changes in barttin palmitoylation levels.

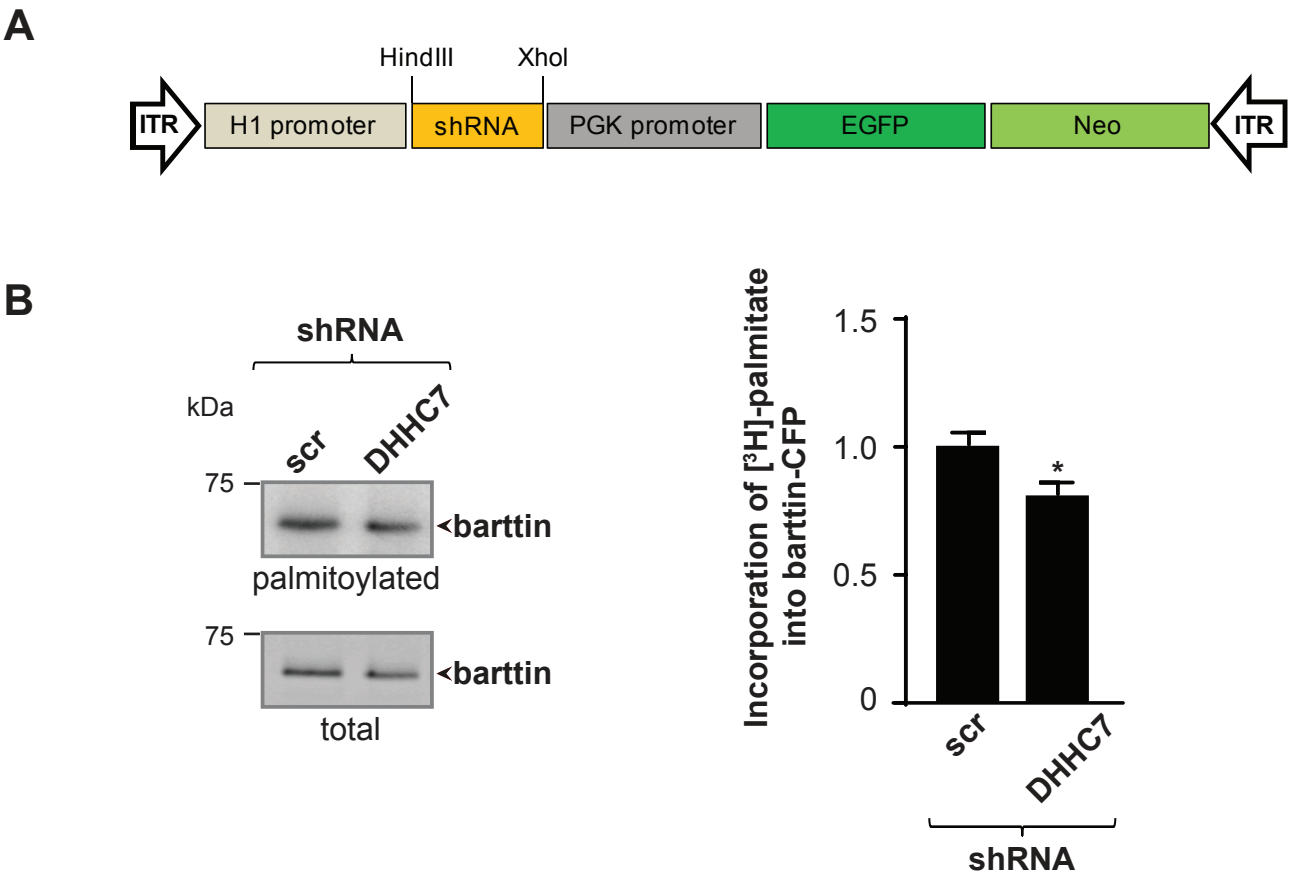

Figure S2:

(A) Schematic presentation of construct encoding for shRNA against DHHC7 together with the green fluorescent protein. **(B)** Autoradiographic analysis of barttin palmitoylation and expression in HEK293T cells labelled with [9,10(n)^{3}H] palmitic acid after knockdown of endogenous DHHC7 by shRNA (left panels). Quantification is shown on the right. *, $P < 0.05$. Student's *t*-test. All data are shown as mean + S.D.

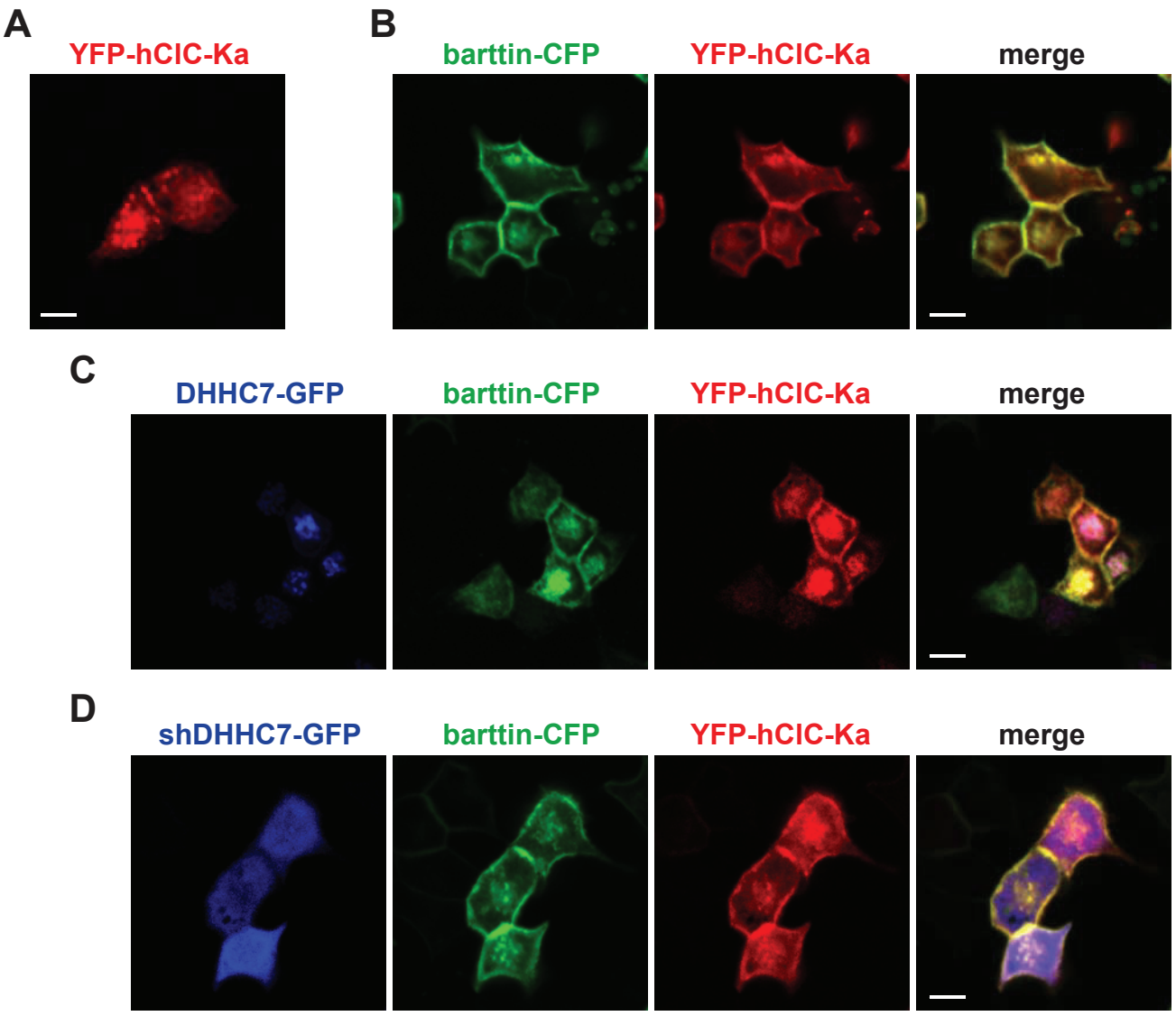

Figure S3:

(A, B) Representative confocal images of MDCK II cells expressing YFP-hClC-Ka alone (red) **(A)** or together with barttin-CFP (green) **(B)**. **(C, D)** Distribution of YFP-hClC-Ka (red) and barttin-CFP (green) in MDCK II cells after co-expression with DHHC7-GFP (blue) **(C)**, or with bi-cistronic plasmid encoding shRNA against DHHC7 and eGFP (blue) **(D)**. Scale bars represent 10 µm.

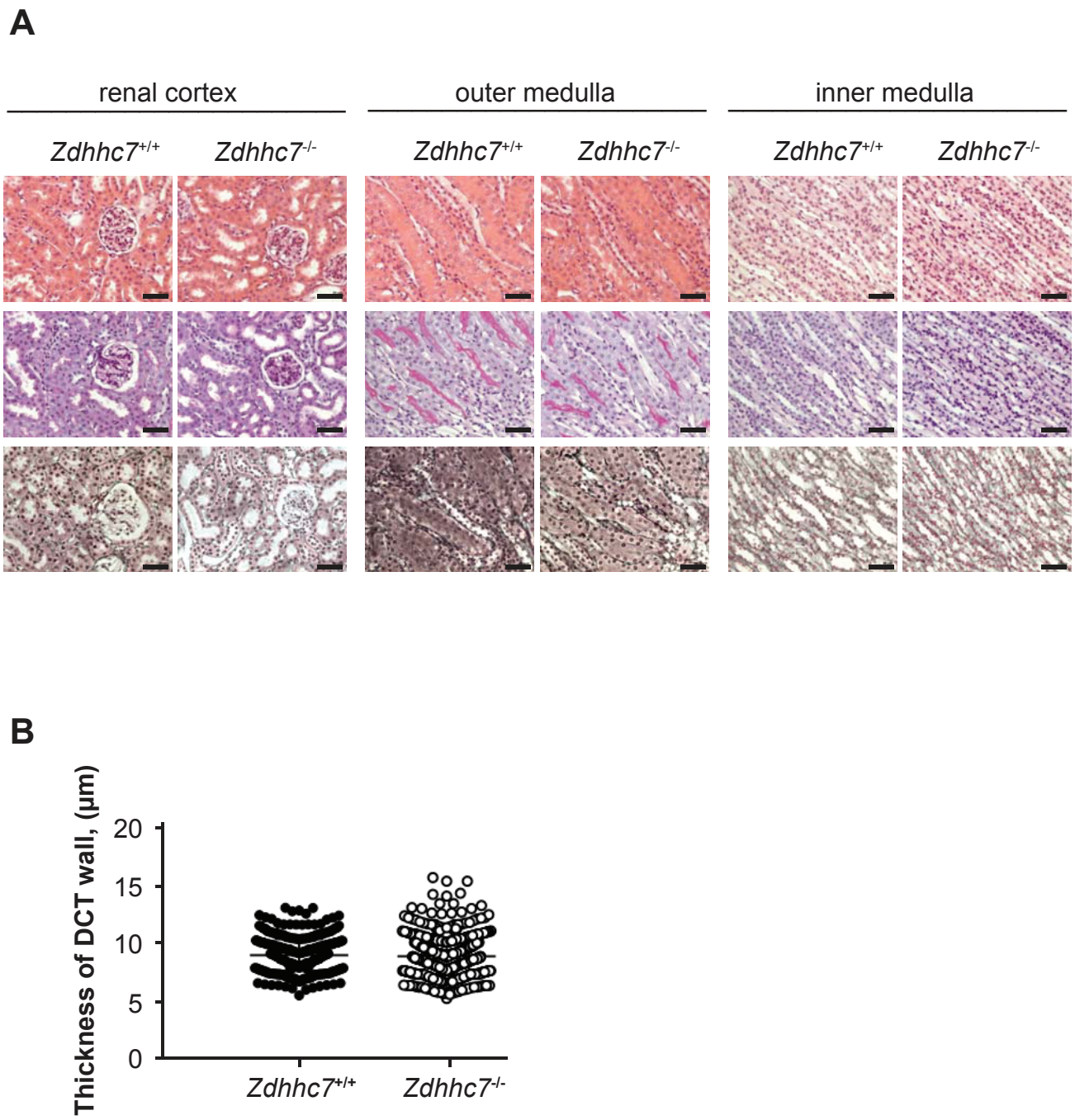

Figure S4:

(A) Histological comparison of different segments of nephrons from P90 male *Zdhhc7*$^{+/+}$ animals and *Zdhhc7*$^{-/-}$ littermates (n = 3). Histological sections were stained with H&E, PAS and silver (Jones Stain). Scale bars indicate 50 µm. **(B)** Quantification of tubule wall thickness from distal tubule in renal cortex from *Zdhhc7*$^{+/+}$ (n_{ROI} = 436) and *Zdhhc7*$^{-/-}$ mice (n_{ROI} = 618) is shown as dot plots. Student's *t*-test. All data are shown as mean ± S.D.

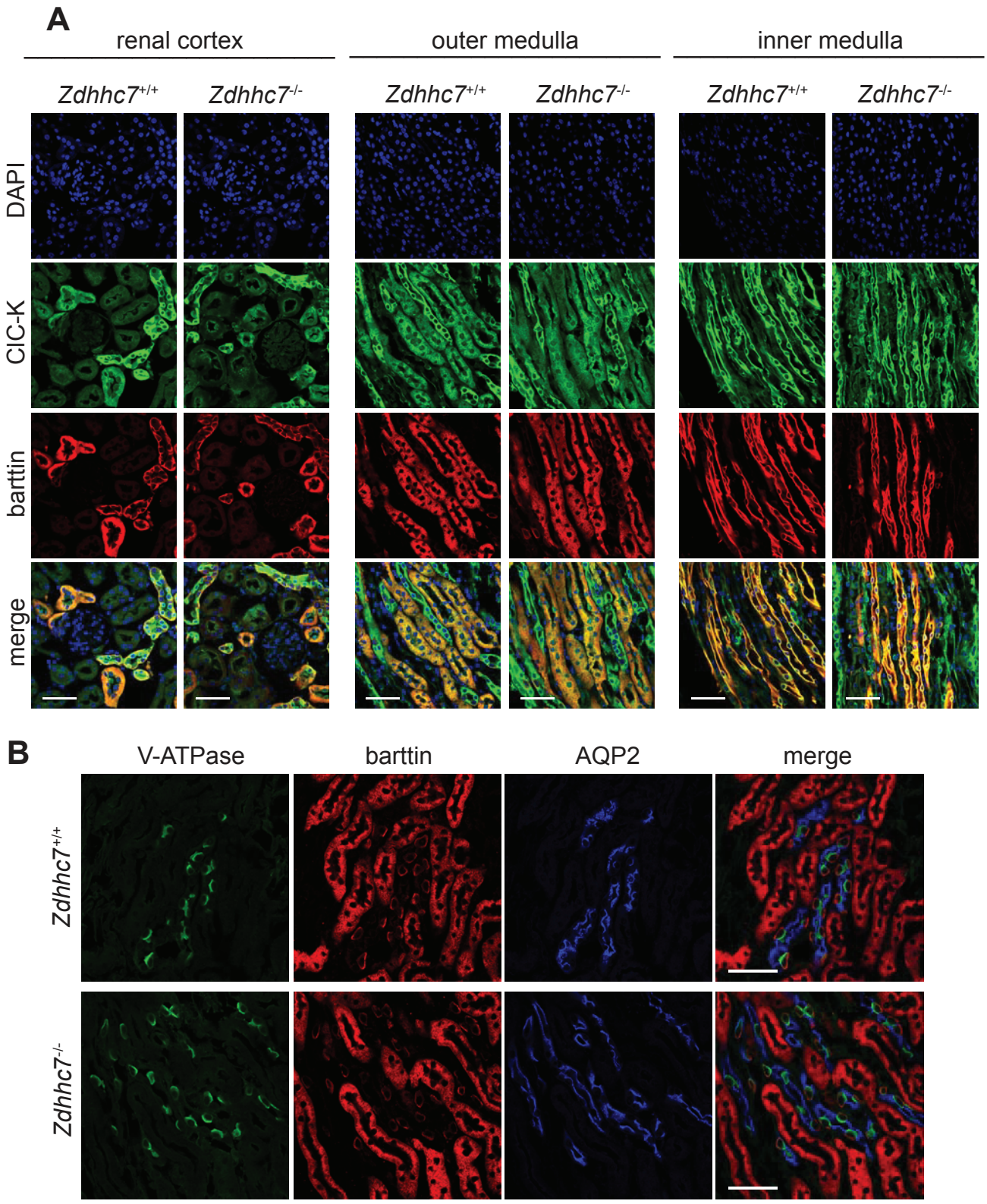

Figure S5:

(A) Representative images demonstrating distribution of ClC-K (green) and barttin (red)

in renal cortex, outer medulla and inner medulla of WT and *Zdhhc7*$^{-/-}$ mice (P90, males, littermates). DAPI staining (blue) visualizes nuclei. Scale bars represent 50 µm. **(B)** Distribution of barttin (red) in α-intercalated cells of collecting duct in *Zdhhc7*$^{+/+}$ and *Zdhhc7*$^{-/-}$ mice (P90, male, littermates). Aquaporin-2 (AQP2, blue) identifies principal cells of collecting duct, and V-ATPase (green) on apical side identifies α-intercalated cells. Scale bars represent 50 µm.