Supplemental Fig. 1

A

endogenous Clcn7 locus

targeting insert

floxed Clcn7 locus with neo

floxed Clcn7 locus

B

17.1 kb (WT)

7.5 kb (lox)

C

8.2 kb (2-3)

7.6 kb (1-3)

6.5 kb (WT)
Supplemental Fig. 4

Cln7+/+;EMX1cre

Cln7lox/lox,EMX1cre

Cortex
Supplemental Fig. 5

A  
Clcn7lox/lox;EMX1cre  Clcn7+/+;EMX1cre

IBA1

B  
GSA  GFAP  GSA GFAP Topro

cortex  

*  *

cerebellum  

*  *
Supplemental Fig. 6

Clcn7\textsuperscript{lox/lox}

Clcn7\textsuperscript{lox/lox}, EMX1cre

Clcn7\textsuperscript{+/-}
Supplemental Fig. 8

A

CIC-7

\( \text{Clcn7}^{\text{lox/lox}}, \text{ApoEcre} \)

\( \text{Clcn7}^{\text{lox/lox}}, \text{ApoEcre} \)

WT

KO

\( \text{Clcn7}^{\text{lox/lox}}, \text{ApoEcre} \)

WT

KO

\( \text{Clcn7}^{\text{lox/lox}}, \text{ApoEcre} \)

WT

KO

\( \text{Clcn7}^{\text{lox/lox}}, \text{ApoEcre} \)

WT

KO

\( \text{Clcn7}^{\text{lox/lox}}, \text{ApoEcre} \)

WT

KO

\( \text{Clcn7}^{\text{lox/lox}}, \text{ApoEcre} \)

WT

KO

\( \text{Clcn7}^{\text{lox/lox}}, \text{ApoEcre} \)

WT

KO

\( \text{Clcn7}^{\text{lox/lox}}, \text{ApoEcre} \)

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WT

KO

\( \text{Clcn7}^{\text{lox/lox}}, \text{ApoEcre} \)

WT

KO

\( \text{Clcn7}^{\text{lox/lox}}, \text{ApoEcre} \)

WT

KO

\( \text{Clcn7}^{\text{lox/lox}}, \text{ApoEcre} \)
SUPPLEMENTAL INFORMATION

Supplemental Fig. 1. Generation of a ‘floxed’ CIC-7 mouse model. A) Targeting of the Clcn7 gene. The top shows a genomic clone encompassing all exons of the Clcn7 gene. The targeting vector and ‘floxed’ Clcn7 locus after homologous recombination are depicted below (dtA: diphtheria toxin A cassette). The ‘floxed’ Clcn7 locus was generated by removing the neomycin resistance (neo) cassette via cre-mediated recombination in embryonic stem (ES) cells. Important restriction sites (B=BsiWI; E=EcoRI; M=MfeI; N=NotI; X=XmnI), loxP-sites (red triangles) and probes used for Southern blot confirmation of recombination events are indicated. B) Southern blot and detection with probe 1 after EcoRI digest of ES cell DNA identified a homologous recombination event (WT: normal Clcn7 locus; lox: floxed Clcn7 locus). Lanes 1 and 3: WT clones, lane 2 heterozygous clone after integration of the targeting insert. C) After expression of cre recombinase in targeted ES cells, loss of the neo cassette was confirmed via Southern blot with probe 2 after digestion with XmnI.

Supplemental Fig. 2. Immunoblot analysis and morphological studies of ‘floxed’ CIC-7 mice. A) Immunoblot analysis of brain (left) and kidney (right) lysates showed no alterations in CIC-7 protein levels in heterozygous (+/lox) or homozygous (lox/lox) ‘floxed’ Clcn7 mice compared to WT littermates (+/+). No CIC-7 signal was present in Clcn7 KO mice (-/-). B) X-ray pictures revealed no changes in bone density of ‘floxed’ CIC-7 mice (lox/lox) or mice heterozygous for the ‘floxed’ and Clcn7 KO allele (lox/-) compared to control mice (+/lox). C) After crossing ‘floxed’ CIC-7 mice to deleter-cre mice, CIC-7 was absent from tissue lysates of offspring homozygous for the deleted ‘floxed’ Clcn7 allele (lox/lox;cre) as shown by immunoblotting with an antibody directed against the amino-terminus of CIC-7. X-ray analysis revealed the typical osteopetrotic increase in bone mass in Clcn7^{lox/lox;cre} mice. Scale bars: 2.5 cm.

Supplemental Fig. 3. Deletion of CIC-7 in forebrain-specific KO mice. A-C) Brain sections of 38-days old mice were co-stained for CIC-7 and the neuronal marker NeuN (A) or parvalbumin, a marker for interneurons (B,C). A) In WT (Clcn7^{+/+}) mice, CIC-7
(green) was highly expressed in neurons of the hippocampal CA3 region. NeuN staining (red) revealed the loss of neurons in the hippocampal CA3 region of a forebrain-specific ClC-7 KO mouse ($Clcn7^{lox/lox};EMX1cre$). No ClC-7-signal was detected in the majority of remaining hippocampal neurons. Apparent ClC-7 signal in KO mice is likely caused by autofluorescence of storage material (6). B) In the CA3 region, ClC-7 (green) was detected both in principal (pyramidal) cells, as well as in interneurons that were identified by parvalbumin-staining (red). In $Clcn7^{lox/lox};EMX1cre$ mice, ClC-7 was mainly expressed in parvalbumin-positive interneurons (arrow). C) As expected for the forebrain-specific KO, normal ClC-7 expression was observed in the cerebellum with strongest staining of Purkinje cells. Scale bars: 40 µm in A, and 20 µm in B and C.

Supplemental Fig. 4. Neurodegeneration in forebrain-specific ClC-7 KO mice. Semi-thin sections of the brain of 1-year old KO ($Clcn7^{lox/lox};EMX1cre$) mice and controls ($Clcn7^{+/+};EMX1cre$) were stained with toluidine blue. In KO mice, the cortex was massively degenerated and no neuronal structures could be identified. In the midbrain (asterisk) of those mice, where ClC-7 was not deleted, structures were normal and no neuronal loss was observed. Scale bars: 20 µm (left) and 5 µm (right).

Supplemental Fig. 5. Microgliosis and astrogliosis in the cortex of forebrain-specific ClC-7 KO mice. Brain sections were stained with an antibody against the microglia marker protein IBA1 (A) or with the microglia-binding lectin (GSA) and an antibody against the glial fibrillary acidic protein (GFAP, staining astrocytes) (B). A) Intense IBA1 staining of microglia was present in the cortex (arrowhead) of 46-days old $Clcn7^{lox/lox};EMX1cre$ mice but not in $Clcn7^{+/+};EMX1cre$ controls. (B) Microgliosis and astrogliosis in the cortex of 20-weeks old $Clcn7^{lox/lox};EMX1cre$ mice (arrowhead). Activated astrocytes and microglia were never observed in those brain regions of 20-weeks old $Clcn7^{lox/lox};EMX1cre$ mice where ClC-7 was not deleted, such as the midbrain (asterisk in A and B) or the cerebellum (B, lower panel). Scale bars: 300 µm.
**Supplemental Fig. 6.** Lysosomal storage phenotype in forebrain-specific ClC-7 KO mice. Antibody staining identified altered lamp-1 distribution in hippocampal CA1 neurons of $Clcn7^{lox/lox};EMX1cre$ mice and constitutive ClC-7 KO mice ($Clcn7^{-/-}$) at the age of 38 days. DNA was stained with Topro. Scale bar: 25 µm.

**Supplemental Fig. 7.** ClC-7/Ostm1 in the kidney. A) Immunoblot on membrane preparation of kidneys reveals a drastic increase in the autophagic marker protein LC3-II in global ClC-7 KO mice (-/-) compared to wildtype controls (+/+). B) X-Gal staining of kidney sections of mice expressing a ClC-7/lacZ fusion protein (blue). ClC-7 was detected in all regions of the kidney. C) ClC-7 co-localizes sub-apically with its β-subunit Ostm1 in PT cells of WT mice. Villin stains brush-borders and is a marker of PTs. D) Altered lamp-1 distribution in PT cells of ClC-7 KO ($Clcn7^{-/-}$) and Ostm1-deficient grey lethal (gl/gl) mice but not in controls (WT). E) Immunoblot on kidney cortex membrane preparations or total kidney lysates did not show any consistent alteration in the amount of endosomal or late endosomal/lysosomal membrane proteins between wildtype (+/+) and ClC-7 KO (-/-) mice, including the related vesicular CLC proteins ClC-3 and ClC-5. Scale bars: 50 µm (left) and 14 µm (right) in B, and 20 µm in C and D. (Age: 3-4 weeks).

**Supplemental Fig. 8.** Loss of ClC-7 disrupts the degradation of endocytosed protein in PT cells. A,B) Kidney-specific ClC-7 KO mice ($Clcn7^{lox/lox};ApoEcre$) were injected intravenously with fluorescently labeled β-lactoglobulin. 1 h after injection, β-lactoglobulin fluorescence (red) was much more intense in ClC-7 KO than in WT cells. WT PT cells were identified by immunofluorescence staining for ClC-7 (green) (A) The β-subunit Ostm1 (green) was present in WT cells but undetectable in ClC-7 KO cells of chimeric tubules, identified by the stronger β-lactoglobulin fluorescence (B). C) Immunostaining on kidney sections shows normal expression and localization of ClC-7 and lamp-1 in PTs of ClC-5 KO mice ($Clcn5^{-/-}$). (D) 30 min after injection of the fluid-phase endocytosis substrate horseradish peroxidase (HRP), no major difference in HRP activity between WT ($Clcn7^{+/+}$) and global ClC-7 KO ($Clcn7^{-/-}$) was detected by direct 3,3’-diaminobenzidine (DAB) staining. After 80 min, a stronger HRP activity was observed in in PTs of $Clcn7^{-/-}$ compared to $Clcn7^{+/+}$ mice. Scale bars: 15 µm.