Supplementary Materials and Methods

Mice
For the generation of Clcn7<sup>−/−</sup> mice 8 kb of mouse genomic sequence extending from exon 6 to 16 of Clcn7 were amplified from R1 ES cells and cloned into pKO Scrambler plasmid 901 (Lexicon Genetics Incorporated) containing a dtA cassette (diphtheria toxin A cassette). A neomycin (neo) resistance cassette flanked by FRT sites was introduced between exon 7 and 8 to select for recombination in embryonic stem (ES) cells. Exon 11 was modified by insertion of the E312A mutation. The targeting construct was completely sequenced. Targeted R1 ES cells were screened by Southern blot analysis using BamHI and an external 1.0-kb probe. Correctly targeted ES cells were injected into C57Bl/6 blastocysts. Chimeric animals were crossed with FLPe-recombinase-expressing ‘deleter’ mice and resulting heterozygous animals (Clcn7<sup>+/−</sup>) were inbred to yield Clcn7<sup>−/−</sup>. Exon 11 of the genomic Clcn7<sup>−/−</sup> gene was amplified with intronic primers and sequenced. Experiments were performed with mice in a mixed C57Bl/6-129/Svj genetic background, always using littermates as controls.

Membrane preparation, tissue homogenates and immunoblot
For membrane preparation tissues were homogenized in PBS with protease inhibitors (Complete<sup>®</sup> protease inhibitor cocktail, Roche) and cleared two times by centrifugation at 1,000 x g for 10 min. Membranes were pelleted at 270,000 x g for 30 min and subsequently resuspended in PBS supplemented with protease inhibitors and 2% (w/v) SDS. For whole tissue homogenates organs were homogenized in PBS with 1% (v/v) NP-40 and protease inhibitors (Complete<sup>®</sup> protease inhibitor cocktail, Roche) and incubated for 30 min on ice. After centrifugation for 10 min at 20,800 x g the supernatant was used for SDS-PAGE. Equal amounts of protein were separated by SDS–PAGE and blotted onto nitrocellulose.

Histology and electron microscopy
Deeply anesthetized mice were perfused with 4% (w/v) PFA in PBS and isolated tissues were postfixed overnight at 4°C. Tibiae were decalcified for 4 days in 10% (w/v) EDTA in PBS and again postfixed. 3-µm paraffin sections of retina were used for H&E staining (old age), 8-µm paraffin sections of the brain were used for Nissl and periodic acid Schiff (PAS) staining and 8-µm cryosections for immunohistochemistry and lysosomal acid phosphatase assay. Lysosomal acid phosphatase activity <i>in situ</i> was determined using β-glycerophosphate as a substrate. Cryosections were incubated for 2 h at 37°C in 0.3% (w/v) sodium-β-glycerophosphate with 0.125% (w/v) lead nitrate in 50 mM acetate buffer pH 5.0. After washing, sections were stained with 0.1% (w/v) ammonium sulfide for 1 min. No staining was observed when substrate was omitted. For immunohistochemistry, sections were post fixed with 4% (w/v) PFA, permeabilized using 0.2% (v/v) Triton X-100 in PBS and blocked with 3% (w/v) BSA in PBS. Antibody incubation was in blocking buffer. For CIC-7, an antigen retrieval step (10 min in sodium citrate buffer, pH 6.0, at 95 °C) was included after fixation. For immunocytochemistry, fibroblasts were seeded onto glass coverslips, fixed for 12 min with 4% (w/v) PFA, treated with 30 mM glycine in PBS for 5 min, permeabilized with 0.1% (w/v) saponin and blocked with 3% (w/v) BSA in PBS. Confocal images were acquired with an LSM 510 (Zeiss) and ZEN software (Zeiss).
For electron microscopy, mice were perfused with 4% (w/v) PFA and 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were cut in 150-µm sagittal sections with a vibratome. The eyes were opened at the crystalline lens and the vitreous humour removed. Tibiae were decalcified with 10% (w/v) EDTA in PBS for 3 to 4 days. Thin slices were prepared and postfixed in 2% (v/v) OsO₄, dehydrated and embedded in epon. Semi-thin sections (0.5 µm) were labelled with toluidine blue. Ultrathin sections (60 nm) were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 902. Photographs were taken with a Megaview 3 Camera. Using the sealing zone as localization marker, the ruffled borders were graded in situ as absent, immature or mature with the experimenter blinded to the genotype.

**Primary cell culture**

Mouse adult fibroblasts from Clcn7<sup>+/+</sup>, Clcn7<sup>−/−</sup> and WT mice were prepared by dissociation of tail biopsies with 0.2% (v/v) collagenase / 2U/ml dispase in PBS and cultured in DMEM containing 10% (v/v) fetal calf serum. Three independent cell lines from different animal were generated. For immunocytochemistry, fibroblasts were seeded onto glass coverslips and fixed. Melanocytes were isolated from dorsal skin of new-born pups by overnight dissociation of the epidermis in 0.25% Trypsin/EDTA solution (Life Technologies) at 4°C and cultured in melanocyte growth medium based on Ham’s F12 (Life technologies) containing 20% (v/v) FBS, 1% (v/v) penicillin/streptomycin (both PAN-Biotech), 0.1 mM isobutylmethyl xanthine (Sigma-Aldrich), 10 µg/ml bovine pituitary extract (Life Technologies) and 48 nM 12-O-tetradecanoyl-phorbol-13-acetate (TPA, Sigma-Aldrich)

**Determination of lysosomal pH**

Lysosomal pH was measured by ratiometric fluorescence imaging of the pH sensor Oregon Green dextran 488 (Invitrogen) as described before. Primary cultures of fibroblasts were plated onto glass bottom life-cell dishes (MatTek) and loaded overnight with 0.5 mg/ml pH dye in growth medium. Cells were washed and Oregon Green dextran was chased into lysosomes for 2 h at 37°C in serum-supplemented growth medium. Ratiometric fluorescence images were acquired using an inverted microscope (Zeiss Axiovert 200 equipped with a 100x 1.30 NA oil immersion lens) connected to a Polychrom II monochromator (TILL photonics) at excitation wavelengths of 440 and 488 nm, respectively. The emitted light was filtered with a 535 ± 20-nm filter and captured with a Sensicam CCD camera (PCO). For each genotype, at least 3 different cells from 3 independent cell lines were measured in Ringer solution (in mM: 140 NaCl, 3 KCl, 2 K2HPO₄, 1 CaCl₂, 1 MgSO₄, 5 HEPES, 10 glucose, pH 7.4). Image analysis was performed using a Fiji plug-in, in which regions of interest (ROI) were defined as areas above a defined fluorescence threshold in the acquired images at 488-nm excitation. The mean intensity ratio between 488- and 440-nm excitation was calculated for each ROI. At the end of each experiment, in situ pH calibration curves were obtained after treatment in isotonic K⁺-based solutions (in mM: 5 NaCl, 115 KCl, 1.2 MgSO₄, 10 glucose, 25 of either HEPES or MES, ranging in pH from 3.9 through 6.45) supplemented with 10 µM of both nigericin and monensin (both Sigma-Aldrich). Cells were equilibrated for at least 2 min for each pH value. The resulting fluorescence intensity ratio (488/440) as a function of pH was fit to a sigmoid and used to interpolate pH values from the experimental ratio data.
**Determination of relative lysosomal chloride concentrations**

Lysosomal chloride was measured by ratiometric fluorescence live cell imaging of MEQ/TMR-dextran (6-methoxy-N-ethylquinolinium iodide / tetramethylrhodamine-dextran), that was targeted to lysosomes using a standard pulse-chase protocol. Primary cultures of fibroblasts were plated onto glass-bottom culture dishes (MatTek) and loaded for 1h with 20 mg/ml dye in growth medium. Cells were washed and MEQ/TMR-dextran chased into lysosomes for 2 h at 37°C in NaCl-reduced IMDM (Iscove’s Modified Dulbecco’s Medium; PAN-Biotech) with remaining [Cl\_] of 7 mM with NaCl substituted by Na-gluconate. Ratiometric fluorescence images at excitation wavelengths of 360 (MEQ) and 524 nm (TMR), respectively, were acquired using an inverted microscope (Zeiss Axiovert 200 equipped with a 100x 1.30 NA oil immersion lens) and an HC Tripleband Beamsplitter 403 497 574 (Semrock) connected to a Polychrom II monochromator (TILL Photonics) and a Lambda 10-2 emission filter wheel (Sutter Instruments). The emitted light was filtered with a 440 ± 20 nm (for MEQ) or 580 ± 20 nm filter (for TMR) and captured with a Sensicam CCD camera (PCO).

For each genotype, 12 (+/+; td/td) or 7 (−/−) dishes with 10 different cells from 5 (+/+), 6 (td/td) or 2 (−/−) independent primary cell lines were measured in imaging buffer (in mM: Na-gluconate 135 mM Na-gluconate 5 KCl, 1 CaCl2, 1 MgCl2, 10 HEPES, 10 glucose, pH 7.4). Image analysis was performed with the Vision software package (TILL Photonics). For each cell, 10 lysosomes were chosen as regions of interest (ROI). The mean fluorescence intensity ratio between 360- and 524-nm excitation was calculated for each ROI after background subtraction.

**Isolation of RNA and qRT-PCR**

RNA was isolated from primary cells using the RNeasy mini kit (Qiagen) and transcribed to cDNA using SuperscriptII reverse transcriptase and random primers (Life technologies). qRT-PCR for axin2 was performed using RT² SYBR Green ROX qPCR Mastermix (Qiagen) with axin2-specific primers (forward: AGTCAGCAGAGGGACAGGAA; reverse: CTTCGTACATGGGGAGCACT) in a StepOne Plus Cycler (Applied Biosystems). β-Actin served as housekeeping gene (forward: tgtgatgggtggaatgggtcagaa; reverse: tgtgggcagcatcttccatgt). The ΔΔCT method was used for analysis.