

## Supplementary materials and methods

Four adult male C57BL/6J mice (age, 8 weeks; weight, 20±g) were obtained from GemPharmatech Co., Ltd. and maintained in the experimental environment for one week. Prior to sacrifice, the mice were anaesthetized with isoflurane (the induction concentration of the anesthetic was 5% and the maintenance concentration was 2-3%) and euthanized by cervical dislocation. The gallbladder was precisely removed and then tissue samples were either stored at 0-4°C, examined by transmission electron microscopy (TEM) or fixed in 4% paraformaldehyde and then embedded in paraffin for multiplexed immunohistochemistry (mIHC). All protocols were approved by the Animal Experimental Center of the East Hospital of Tongji University with license No. 2022-02-18.

Selected fresh gallbladder tissue (3x3 mm) blocks were transferred into a microcentrifuge tube with fresh 3% glutaraldehyde (Wuhan Servicebio Technology) for further fixation at 4°C for preservation and transportation. Subsequently, the samples were fixed with 1% osmium tetroxide in the dark (pH 7.4) for 2 h at room temperature. After removal of osmium tetroxide and gradient drying, the samples were embedded in resin. The resin blocks were cut into 60-80 nm sections on the ultra-microtome and the tissues were fished out onto 150-mesh cuprum grids with formvar film. Finally, the cuprum grids were observed under TEM and images were acquired (supported by Wuhan Servicebio Technology).

The mIHC was performed by staining 4-μm-thick formalin-fixed, paraffin-embedded whole tissue sections with standard, primary antibodies sequentially and paired with a Tyramide signal amplification 7-color kit (cat. no. abs50015-100T; Absinbio), followed by staining with DAPI. For instance, deparaffinized slides were incubated with anti-TMEM16A antibody (Ano1; 1:20 dilution; cat. no. ab64085; Abcam) for 30 min and then treated with anti-rabbit/mouse horseradish peroxidase-conjugated secondary antibody (cat. no. A10011-60; Absinbio) for 10 min. Labelling was then developed for 10 min using TSA 520 as per the manufacturer's protocol. Slides were washed in TBST buffer and then transferred to preheated citrate solution (90°C) prior to being heat-treated using a microwave set at 20% of maximum power for 15 min. Slides were cooled in the same solution to room temperature. Between all steps, the slides were washed with Tris buffer. The same process was repeated for the following antibodies/fluorescent dyes, in order: Anti-UCHL1 [PGP 9.5; 1:200 dilution; cat. no. 13179S; Cell Signaling Technology, Inc. (CST)]/TSA 570, anti-α-SMA (1:400 dilution; cat. no. 19245S; CST)/TSA 620, anti-PDGFRα (1:50 dilution, cat. no. 3174S; CST)/TSA 690. Each slide was then treated with 2 drops of DAPI (D1306; Thermo Fisher Scientific, Inc.), washed in distilled water and manually mounted with a cover slip. Slides were air-dried and images were acquired with an Aperio Versa 8 tissue imaging system (Leica Microsystems). Images were analyzed using HALO software (v3.5; Indica Labs).