Supplementary methods

Design of self-deliverable RNAi (sdRNAi) compounds

The candidate sdRNAi compounds were designed specifically to target the mouse Panx1 gene (NM_019482) or the mouse Casp4(11) gene (NM_007619). The proprietary algorithm (Advirna, LLC) used for predictions was created on the basis of a functional screen of over 500 sdRNAi sequences. Regression analysis was used to establish a correlation between the frequency of occurrence of specific nucleotides and modification at any specific position in the sdRNAi duplex and its functionality in the gene suppression assay.

The best 10 sequences with the highest score were selected for each gene. Target sites were located in the open reading frame (ORF) and untranslated regions. Chemically modified oligonucleotides were synthetized as separate strands by TriLink sdRNAi was prepared by hybridization and analyzed in native TBE electrophoresis to ensure duplex formation (>90%)

Selection of functional sdRNAi

Luciferase reporter plasmids were constructed by inserting Panx1 or Casp4 targeted regions into psiCheck2 plasmids (Promega, C8021) following Renilla luciferase sequences. A previously validated MAP4K4 sdRNAi sequence was inserted into each construct as a positive control. The plasmids also contain a Firefly luciferase sequence under a separate promoter for cell number variability normalization. The obtained clones were verified by sequencing (Genewiz, South Plainfield, NJ, USA). Selected clones were amplified and palsmids were purified using a Plasmid Midi-prep kit (Macherey Nagel, 740412; Bethlehem, PA, USA).

HeLa cells were transfected with the cloned Panx1 or Casp4 plasmids using FuGENE® HD (Promega, E2311; Madison, WI, USA) according to the manufacturers instructions. Briefly, cells were seeded at 2.5 x 10^6 cells/ 10 cm^2 dishes in the EMEM (ATCC, 30-2003) medium without antibiotics. Six hours later cells were transfected with the Panx1 or Casp4 plasmids at a 2.5:1 FuGENE:DNA ratio. Cells were cultured for additional 16-18 h, washed 3 times with PBS, trypsinized and seeded into a 96-well plate with pre-diluted sdRNAi compounds at a final concentration of 1 μM sdRNAi/5,000 cells/100 μl EMEM with 3% FBS. Cells were treated with sdRNAi for 48 h to facilitate passive cellular uptake of compounds, lysed with Glo lysis buffer (Promega, E266A) and assayed for

Renilla and Firefly expression. For that, 20 µl aliquots of each lysate were added into duplicate opaque 96-well plates and mixed either with Matthews assay buffer (Renilla) or Firefly luciferase assay buffer (25 mM glycylglycine, 15 mM $MgSO_4$, 4 mM EGTA, 1 mM DTT, 2 mM ATP, 15 mM K_2PO_4, pH 7.8 and 1 mM D-Luciferin). The D-Luciferin was from Promega (Cat. No. E1605) and the h-Coelenterazine was from NanoLight Technology (Cat. # 301; Pinetop, AZ, USA). The substrates were added to the assay buffers just before use. Luminescence was measured on SpectraMax i3 (Molecular Devices, Sunnyvale, CA, USA), normalized and expressed as a percent untreated control.

Supplementary figure 1. Schematic diagram of sdRNAi structure. Self-deliverable RNAis are double-stranded asymmetric siRNA with a variety of backbones (2'OMe, 2'F and phosphorotioate) and hydrophobic modifications (i.e. 3'Cholesterol is attached to a passenger strand) allowing increased stability and passive cellular uptake[70].

Supplementary figure 2. Identification of functional sdRNAi compounds silencing Panx1 and Casp4 (11). Selected sdRNAi oligonucleotides targeting Panx1 (A) or Casp4 (11) (B) were tested in HeLa cells using dual luciferase reporter screening. Cells transfected with reporter construct were treated with 1 μM sdRNAi (also see Supplemental Table 1), positive control sdRNAi against MAP4K4, negative non-targeting control (NTC) or left untreated (NT) for 48 h. Results were normalized for control Firefly luciferase and expressed as percent of untreated control (n=3 wells, mean ± standard deviation). Px23 and Cs12 were selected for further experiments.

Supplementary figure 3. Testing the sdRNAi compounds targeting the Panx1 and Casp4(11) genes. (A-B) Treatment of Neuro2A cell cultures with Panx1 targeting sdRNAi (Px23, 2.5μM) (A) and Casp11-targeting sdRNAi (Cs12, 2.5μM) (B) compounds (red bar) showed approximately 10- and 4-fold fold silencing, respectively, relative to control (Ctl) and non-targeting (PxNT/scrambled, green bar) sdRNAi compounds. Cells were analyzed after 48hrs incubation with the compounds. Panx1-null Neuro2A cells Px1(-) were used as an additional control. Mean ± standard error of the mean; n=3 $P<0.01$ (C) Intravitreal injection of Panx1-targeting sdRNAi (Px23, 2.5μM) compound resulted in approximately 60% inhibition (red bar) relative to control (Ctl) retinas 48hrs post-injection. Mean ± standard error of the mean; n=4, $P<0.01$.

Supplementary figure 4. Low level of Panx1 expression in MECs. Whole mount immunostaining of LGs was performed using antibodies to Panx1 (green) and SMA

(red). Nuclei were stained with DAPI. Z-stacks were acquired from the base of the MEC cells to beyond the top with a Z step size at 0.31 μm and Z stacks were rendered for 3D reconstruction. Acinar cells expressed high level of Panx1, while MECs had low level of Panx expression. The scale bar is 5 μm.

Supplementary figure 5. Panx1 expression in human LGs. (A-I) Panx1 (brown) is specifically expressed in the lobes (labeled with white arrowheads), ducts (*duct*), acini (ac), blood vessels (bv) of the human LGs. Panx1 expression was visualized by different antibodies to Panx1. (A, B) Our homemade affinity purified rabbit polyclonal antibody against the carboxyl terminus of human PANX1; (C, D) Negative controls to Panx1 antibody shown in (A and B) in which primary antibodies were omitted (C) or substituted with preimmune serum (D). (E-G) Affinity purified rabbit polyclonal antibody to Panx1 CT-395 (Px-34, kind gift of Dr. D.W. Laird, University of Western Ontario, Canada). (H) Negative control to CT-395 antibody, in which the primary antibody was substituted with isotype-specific immunoglobulin (Normal Rabbit IgG, Sigma-Aldrich). (A-F) LG was obtained from an 84-year old female donor; (G, H) LG was obtained from a 62-year old female donor.

Supplementary movie 1. Panx1 expression in acinar and MEC cells. Z-stacks were acquired from the base of the MEC cells to beyond the top with a Z step size at 0.31 μm and Z stacks were rendered for 3D reconstruction. The movie was recorded using IMARIS animation software. MEC, which is located on the surface of the acinus, shows very little Panx1 labeling whereas acinar cells at the right upper corner show high levels of Panx1 expression.

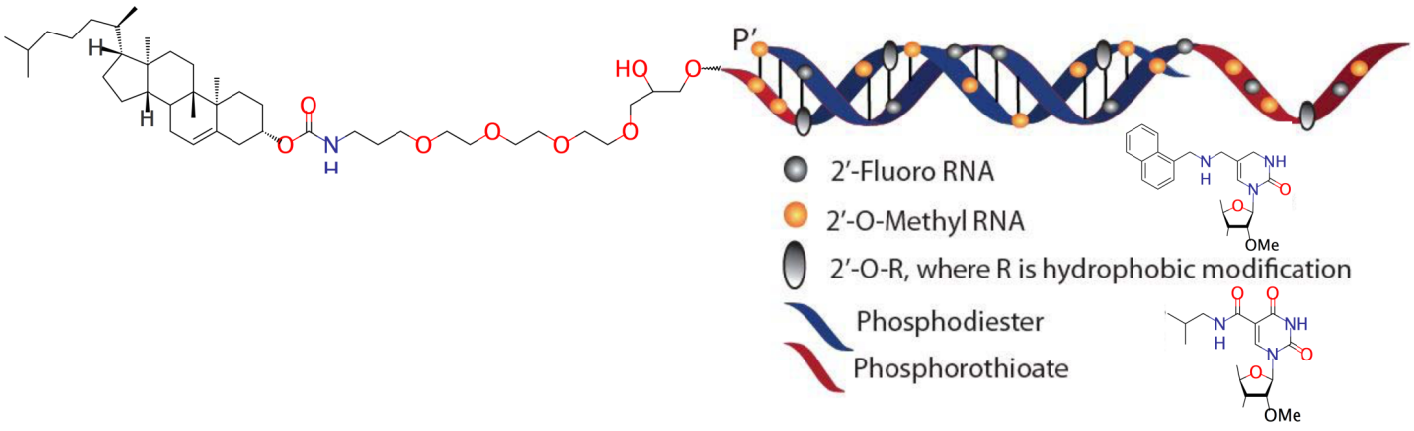
Targeting regions for Panx1

ID	20 mer Targeting region
Px21	CGAGATTTGGACCTAAGAGA
Px22	GGTTATACTGTTGGCATGTA
Px23	GTTGGCATGTATCTACTTGA
Px24	TCTGTGCAGCATCAAATCAG
Px25	CAAATCAGGCGTCCTGAAAA
Px26	AAGGTGCTGGAGAACATTAA
Px27	GTAGTGTGTGTCCTACCAAT
Px28	GATTAGAAACGTCCCACAAG
Px29	TGCTTTGTTTTGTGAGGTAA
Px30	TTGTTTTGTGAGGTAATAAA

Targeting regions for Casp4 (11)

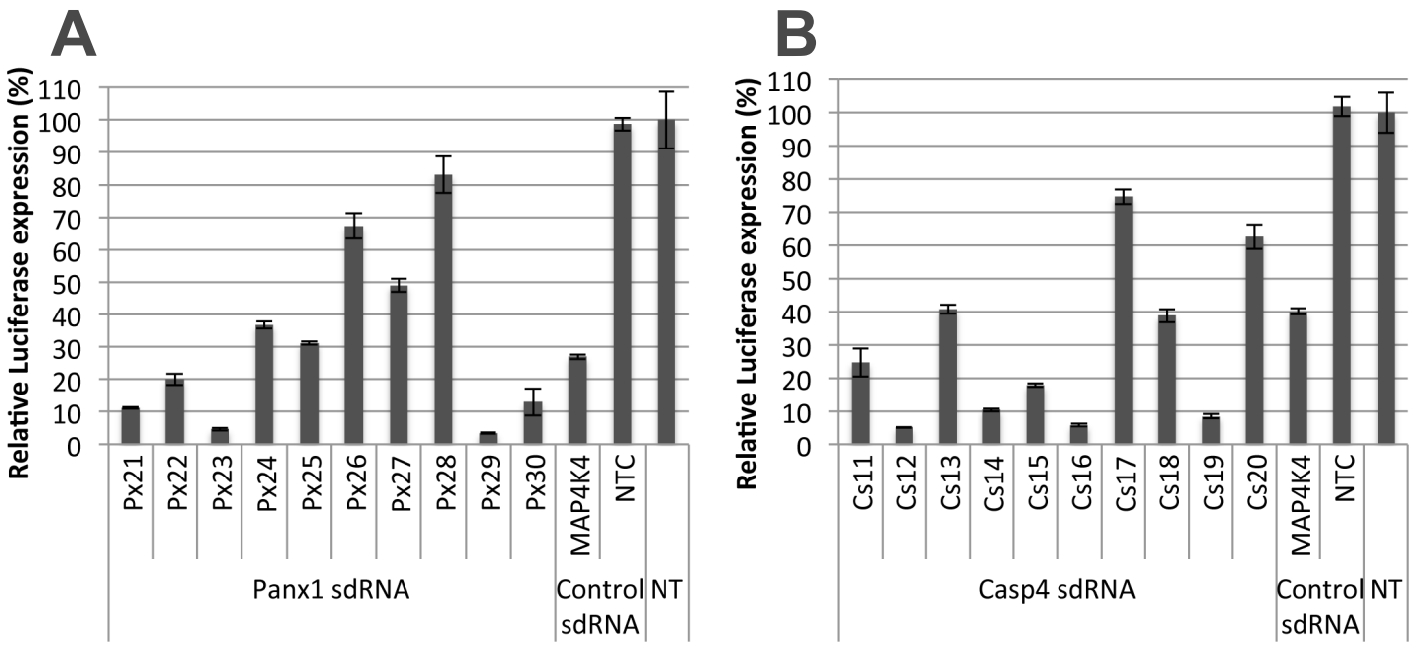
ID	20 mer Targeting region
Px21	CGAGATTTGGACCTAAGAGA
Px22	GGTTATACTGTTGGCATGTA
Px23	GTTGGCATGTATCTACTTGA
Px24	TCTGTGCAGCATCAAATCAG
Px25	CAAATCAGGCGTCCTGAAAA
Px26	AAGGTGCTGGAGAACATTAA
Px27	GTAGTGTGTGTCCTACCAAT
Px28	GATTAGAAACGTCCCACAAG
Px29	TGCTTTGTTTTGTGAGGTAA
Px30	TTGTTTTGTGAGGTAATAAA

Supplemental table 1. List of predicted target sites for sdRNAi in Panx1 and Casp4(11) genes. Targeting regions for Panx1 and Casp4(11) sdRNAi designed using the proprietary algorithm in Advirna, LLC. The best ten lead candidates with highest scores predicted to silence mRNA expression were selected for each gene to test in cell culture and *in vivo*.



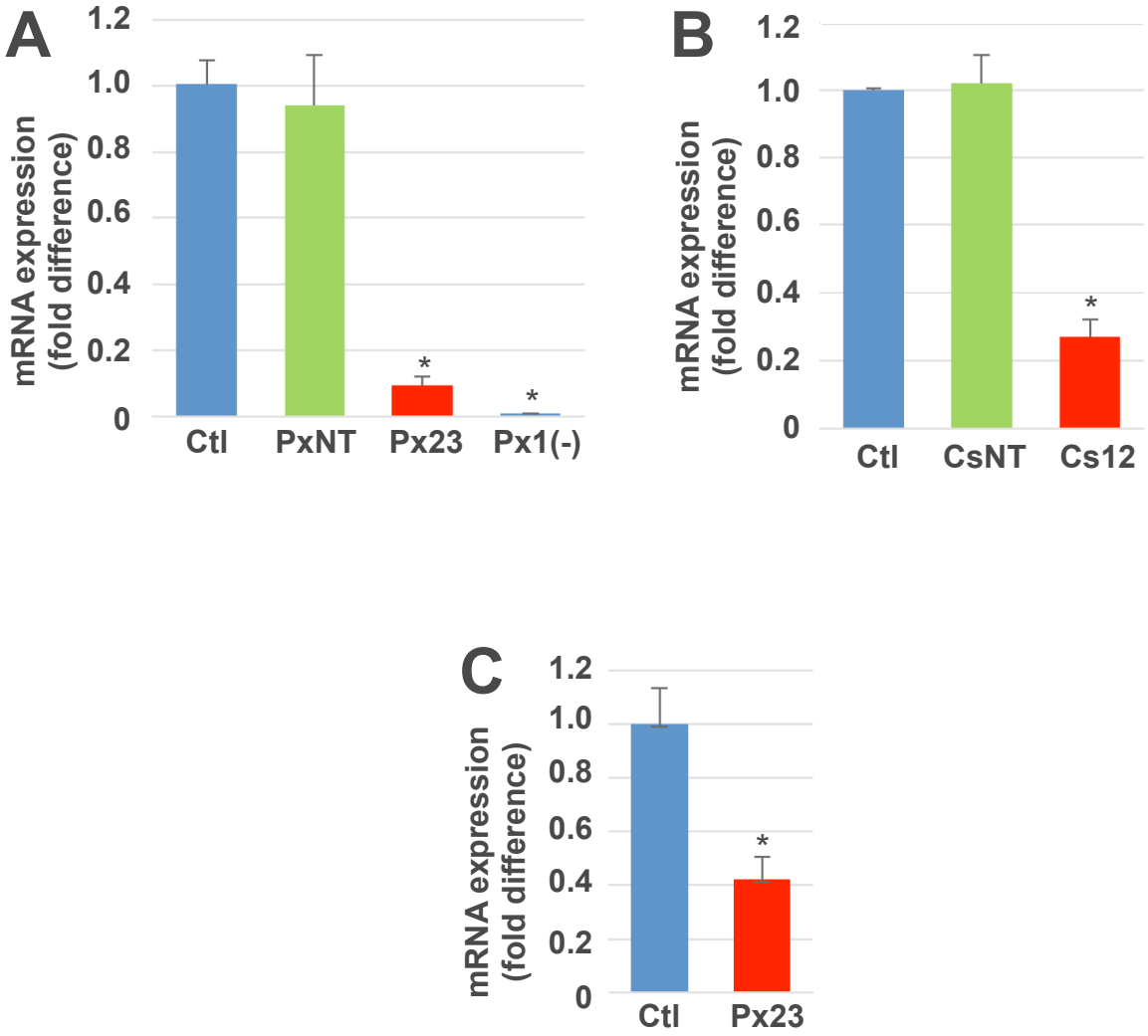
Supplementary figure 1

.

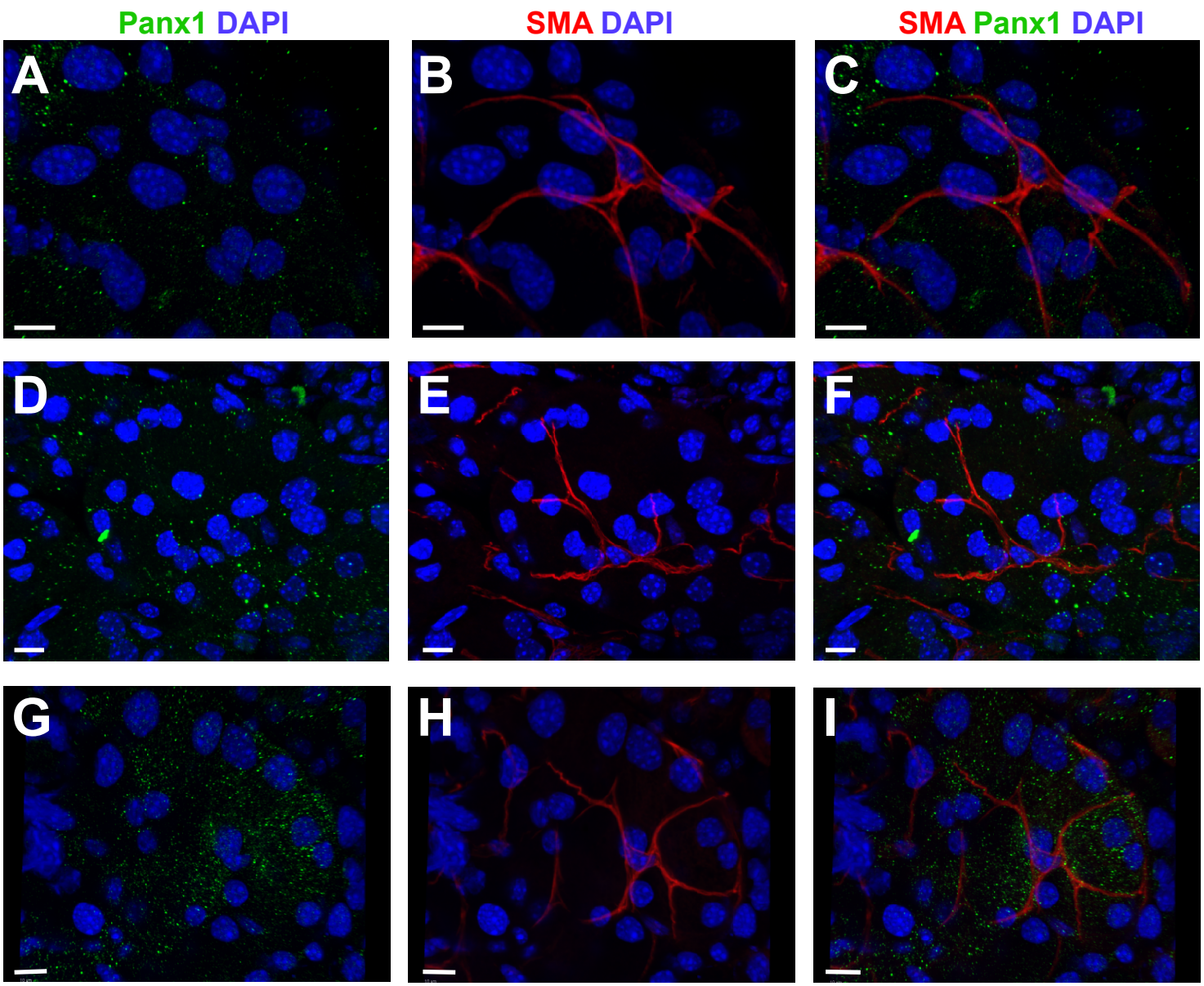


Supplementary figure 2

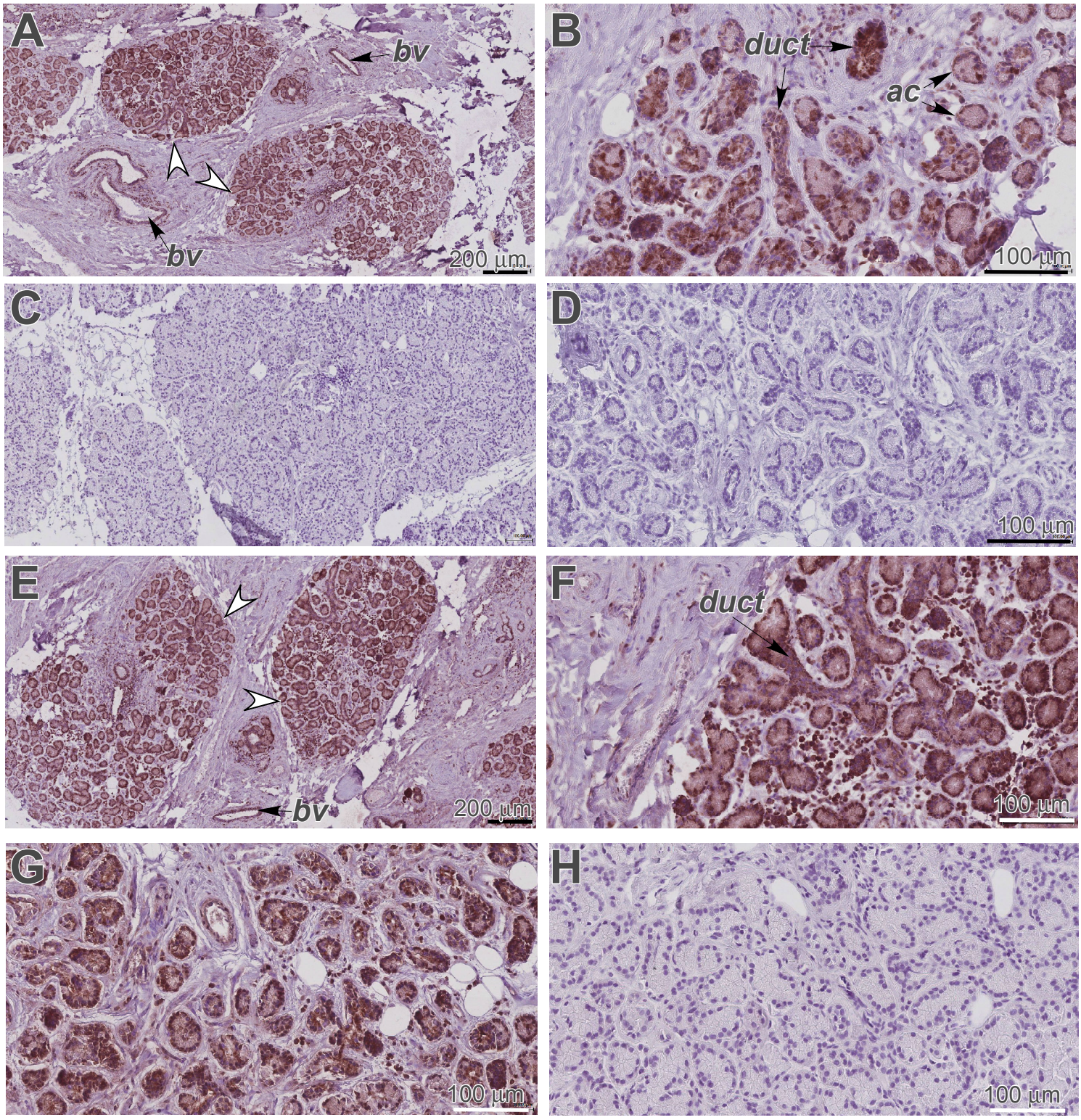
.



Supplementary figure 3.



Supplementary figure 4



Supplementary figure 5.