Supplementary materials

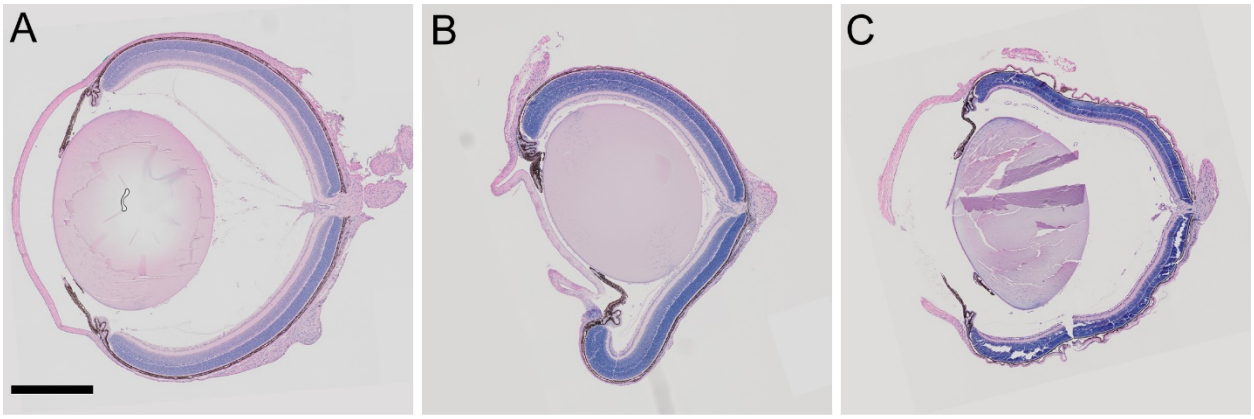

Figure S1. HE-stained eyeball paraffin sections from eye samples from 1-week-old mice. Related to Step 2, 7 and 8, and problem 1 and 2 in troubleshooting. (A) is an example of an eyeball that is processed by the method we proposed in this protocol. (B) an eyeball for which the window technique was not employed. (C) is from an eyeball fixed with 4%PFA for all 40 hours other than Hartman's fixative for the first 20 hours with window technique. Scale bar: 500 μm.

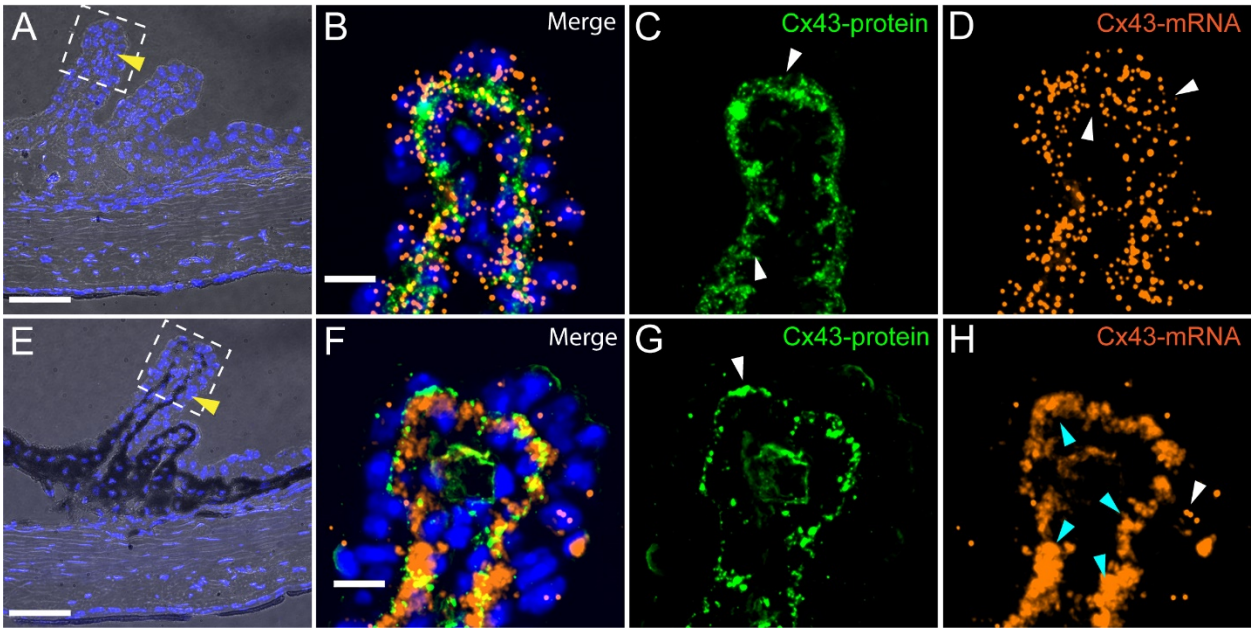

Figure S2. The outcome of de-pigmentation pretreatment. Related to Step 49, and problem 6 in troubleshooting. (A) is a merged image of fluorescent DAPI signal with a transmitted light image showing the ciliary body whose pigment is bleached by the bleaching method proposed in this protocol. Yellow arrowhead points to the apical side of OCE, where pigment granules were aggregated. (B) is a magnification of the region highlighted by the dashed white square in (A) and is a merged image of Cx43 protein (C) and mRNA (D) with DAPI. Cx43 proteins (C) at the apical side of ICE and OCE, and at the lateral side of OCE, which is pointed to by the white arrowheads, are clearly labeled by the immunostaining. Cx43 mRNA at both the ICE and OCE, as pointed to by the white arrowheads, are also clearly labeled by the smFISH processing (D). (E) is a merged image of fluorescent DAPI signal with a transmitted light image showing the ciliary body without pigment bleaching. (F) is a magnification of the region highlighted by the dashed white square in (E) and is a merged image of Cx43 protein (G) and mRNA (H) with DAPI. Cyan arrowheads indicate the autofluorescence of the pigmented region. Scale bar: 50 µm for (A, E) and 10 µm for (B, F).

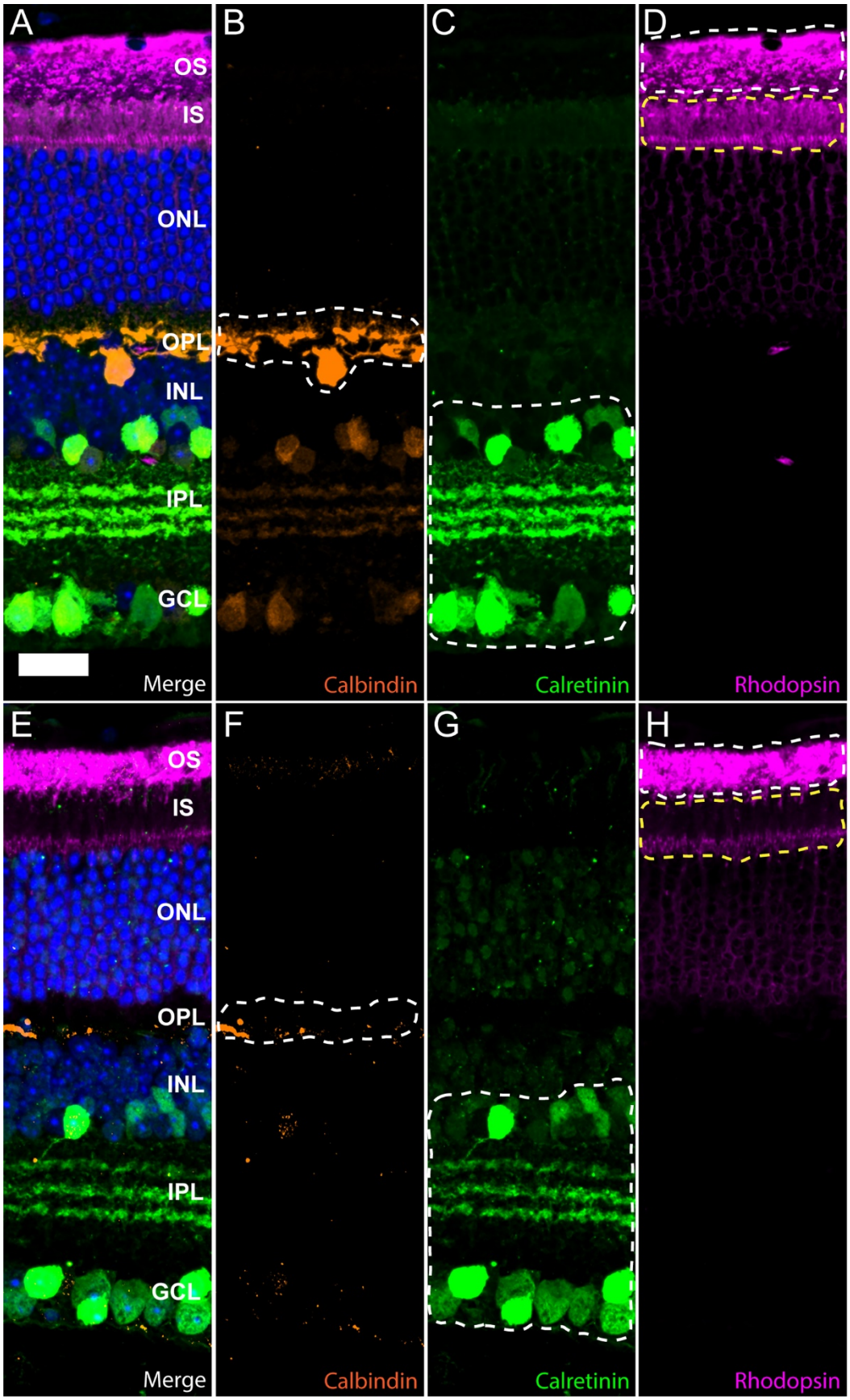
A
OS
IS
ONL
OPL
INL
IPL
GCL
Merge
B
Calbindin
C
Calretinin
D
Rhodopsin
E
OS
IS
ONL
OPL
INL
IPL
GCL
Merge
F
Calbindin
G
Calretinin
H
Rhodopsin

Figure S3. The outcome of immunofluorescence assay with different antigen retrieval pretreatments. Related to Step 44 and 45, and problem 7 in troubleshooting. A-D demonstrate the indicated signals from sections that were treated with 10 minutes antigen retrieval at 95 ℃ in citrate buffer, and E-H shows the same immunofluorescence assay from sections that were treated with 30 minutes antigen retrieval at 70 ℃. (A) is the merged image of (B)-(D) with DAPI. (E) is the merged image of (F)-(H) with DAPI. Calbindin (Orange, B and F) labels horizontal cells at the outer plexiform layer, which is highlighted by white polygons in (B) and (F). The low signal in (F), compared with (B), shows that the IF assay on Calbindin requires 95 ℃, 10 minutes AR condition. Calretinin (Green, C and G) labels amacrine cells and ganglion cells at the inner nuclei, inner plexiform and ganglion cell layer, which is highlighted by white polygons in (C) and (G). The IF assay on Calretinin is compatible with 70 ℃, 30 minutes AR condition. Rhodopsin (Magenta, D and H) labels rod cells at the outer segment layer, which is highlighted by white polygons in (D) and (H). The yellow polygons in (D) and (H) show the inner segment layer, where rhodopsin is lowly expressed. Images shown here are auto contrasted using ImageJ software rather than applying the same parameters throughout all images in this figure. Scale bar: 20 μm. OS: outer segment; IS: inner segment; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.