Supporting Information

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SI Text

Gross Morphology and Cataract Detection. Slit-lamp examinations were performed using a Nikon FS3 Zoom Photo Slit Lamp without the use of anesthesia. Mouse lenses were examined after dilation with one drop each of 2% phenylephrine and 1% cyclopentolate. Digital images of the anterior surface of the eye were captured with a Pixelink PL-A662 megapixel camera at a magnification of ×32. Scheimpflug images were similarly captured, without anesthesia, using an EAS-1000 Scheimpflug Slit Imaging System (Nidek).

H&E Staining. Embryo heads (E9, E11, E14, E17, and P0) or dissected eyes (P6, P21, P60, P180 and older) were preserved in lens fixation buffer (65% ethanol, 4% formaldehyde, 5% acetic acid, 3% sucrose) at 4°C overnight, dehydrated the following day, and embedded in Paraplast (McCormick Scientific). Both longitudinal and transverse sections were prepared at 5 μm and stained with H&E (Sigma-Aldrich).

Immunohistochemistry. To examine the expression of crystallins and MIP-26 in the lens, Paraplast-embedded sections (prepared as described) of the adult lens were incubated with rabbit anti–α-crystallin (1:100), anti–β-crystallin (1:100), anti–γ-crystallin (1:100), or anti–MIP-26 (1:100) antibody followed by secondary labeling with biotinylated streptavidin–horseradish peroxidase (1:500) and treatment with 10 mg/ml of 3,3-diaminobenzidine.

For fluorescent staining of cytoarchitecture and cell junction proteins, P6 and P21 lenses were fixed in 4% formaldehyde for 10 min at room temperature, incubated in 10% sucrose in PBS overnight at 4°C, and cryosectioned at 10 μm. Cytoarchitectural changes were examined by staining with Alexa Fluor 546-phalloidin (1:25 for 1 h; Invitrogen). N-cadherin expression was detected with a rat monoclonal anti–N-cadherin antibody raised against amino acids 308–597, located in the extracellular domain of mouse N-cadherin (1) (1:1,000; Developmental Studies Hybridoma Bank, University of Iowa, #MNCD2-A2). A mouse anti–β-catenin antibody (1:3,000; BD Biosciences) was used to detect β-catenin. Alexa Fluor 488–conjugated anti-rat or anti-mouse IgG (1:200) were used as secondary antibodies. ZO-1 signals were detected with a rabbit anti–ZO-1 (1:200; Invitrogen) and a Cy3-conjugated anti-rabbit IgG (1:200) secondary antibody.

To show co-localization of gap junction proteins, ZO-1 was detected with a rat anti–ZO-1 antibody (Developmental Studies Hybridoma Bank, University of Iowa, Cat. #R26.4C; 1:200) and Cx46 was detected with a rabbit anti-Cx46 antibody (Zymed, Cat #38–8300; 1:200).

Image analyses were performed using Image-Pro Plus software (Media Cybernetics). Quantification of the fiber cell shape was performed using the "aspect" function to compare the width versus the length of the cells. To analyze N-cadherin membrane decoupling, cytoplasmic N-cadherin fluorescence signals were divided by the signals of the entire cell using bitmap analysis. Cell boundaries were defined by staining with Alexa Fluor 546-phalloidin.

For anti-EphA2 immunohistochemical staining, P21 WT and ephrin-A5$^{-/-}$ lenses were fixed in 4% formaldehyde for 10 min at room temperature, and then incubated in 10% sucrose in PBS solution overnight at 4°C. Ten-micrometer cryosections were then prepared and stained with goat anti-EphA2 antibody (1:200; R & D Systems). To detect ephrin-A ligand expression with EphA3-Fc (20 μg/ml), 10-μm fresh-frozen sections were prepared and dried onto Superfrost/Plus glass slides (Fisher Scientific). The sections were then fixed in methanol for 5 min, followed by acetone for 5 min, and then incubated with EphA3-Fc. EphA2 and ephrin-A signals were detected using Cy3-conjugated anti-goat (1:200) and anti-human IgG (1:200) secondary antibody, respectively.

N-Cadherin–β-Catenin Interaction. To examine the effects of Eph receptor activation on N-cadherin–β-catenin interaction, 293T cells were plated at 80% confluency and transfected using Lipofectamine 2000 (Invitrogen) with human EphA2 in the mammalian expression vector pRcCMV (gift from Dr. Bingcheng Wang, Cleveland, OH). Both transfected and untransfected 293T cells were stimulated with either 2 μg/ml ephrin-A5-Fc (cross-linked with anti-human Fc in a 5:1 ratio in μg) or vehicle for 45 min and then lysed in lysis buffer [50 mM Tris.Cl (pH 8.0), 150 mM NaCl, 1% Nonidet P-40, 1x protein inhibitor mixture (Roche Diagnostics), and 1 mM Na_3VO_4]. N-cadherin was immunoprecipitated from the cell lysates and fractionated by SDS/PAGE (Bio-Rad 10% Tris-HCl gel). The proteins were transferred to nitrocellulose membrane and analyzed for the presence of β-catenin. The Western blot membranes were re-probed with anti–N-cadherin antibody to assess the amount of N-cadherin proteins precipitated.

To examine EphA2 interaction with β-catenin, 293T cells, transfected with human EphA2, were lysed in lysis buffer, and EphA2 was immunoprecipitated from the cell lysates. The immunoprecipitates were then fractionated by SDS/PAGE. The proteins were transferred to a nitrocellulose membrane and analyzed for the presence of β-catenin using the anti–β-catenin antibody. In the reciprocal experiment, β-catenin was immunoprecipitated from the cell lysates and analyzed for the presence of EphA2. As a loading control, blots were re-probed for their respective immunoprecipitated proteins to determine total protein levels. To detect EphA2 activation, the protein was precipitated with anti-EphA2 antibody (rabbit anti-EphA2; Santa Cruz Biotechnology) followed by Western blot analysis using an anti-phosphotyrosine antibody (P-Tyr-100; Cell Signaling Technology). Peroxidase conjugated anti-rabbit IgG antibody (1:10,000; Sigma-Aldrich) was used as a secondary antibody. The proteins were detected with a chemiluminescence kit (Roche Diagnostics) according to the manufacturer's instructions.

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EphA2 Activation in WT and Ephrin-A5$^{-/-}$ Lenses. P6 WT and ephrin-A5$^{-/-}$ lenses were collected and homogenized in lysis buffer (50 mM Tris-HCl, pH 8.0; 150 mM NaCl, 1% Igepal and 1× protein inhibitor mixture). EphA2 was immunoprecipitated from 2.5 mg of lens lysate. The samples were fractionated by SDS/PAGE (10% Tris-HCl gel; Bio-Rad) and proteins were then transferred to nitrocellulose membrane and analyzed using an anti–P-Tyr antibody (Santa Cruz Biotechnology) by Western blot analysis. EphA2 protein levels were determined by re-probing the Western blot membrane with an anti-EphA2 antibody.

1. Matsunami H, Takeichi M (1995) Fetal brain subdivisions defined by R- and E-cadherin expressions: evidence for the role of cadherin activity in region-specific, cell-cell adhesion. *Dev Biol* 172:466–478.

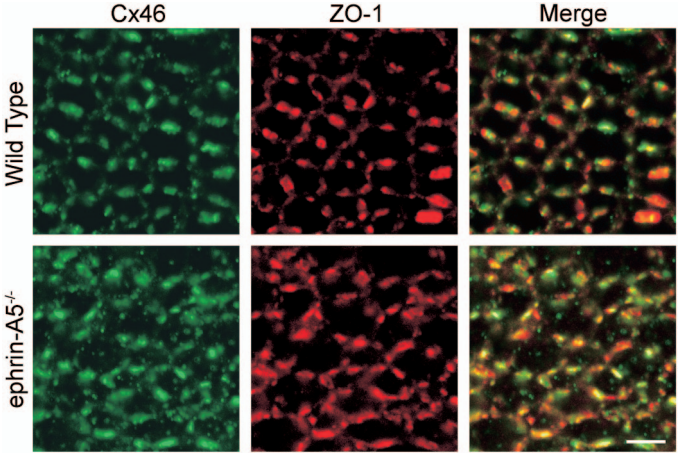

Fig. S1. Disorganization of gap junction proteins Cx46 and ZO-1 in ephrin-A5$^{-/-}$ mice. Transverse P7 lens sections show disrupted patterning in the mutant mouse, although Cx46 and ZO-1 remain co-localized and on the lens fiber cell membrane. (Scale bar, 5 μm.)

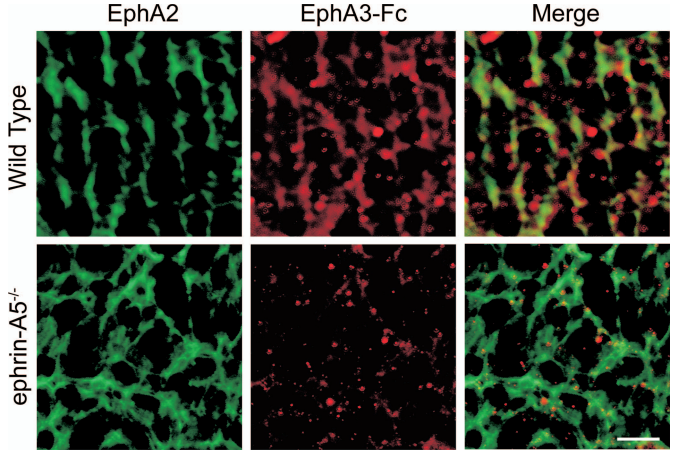

Fig. S2. EphA2 and ephrin-A ligands have overlapping expression in the lens. Anti-EphA2 antibody (green) and EphA3-Fc (red) both stained the lens most robustly in the cortical/subcortical regions. (Scale bar, 10 μm.)