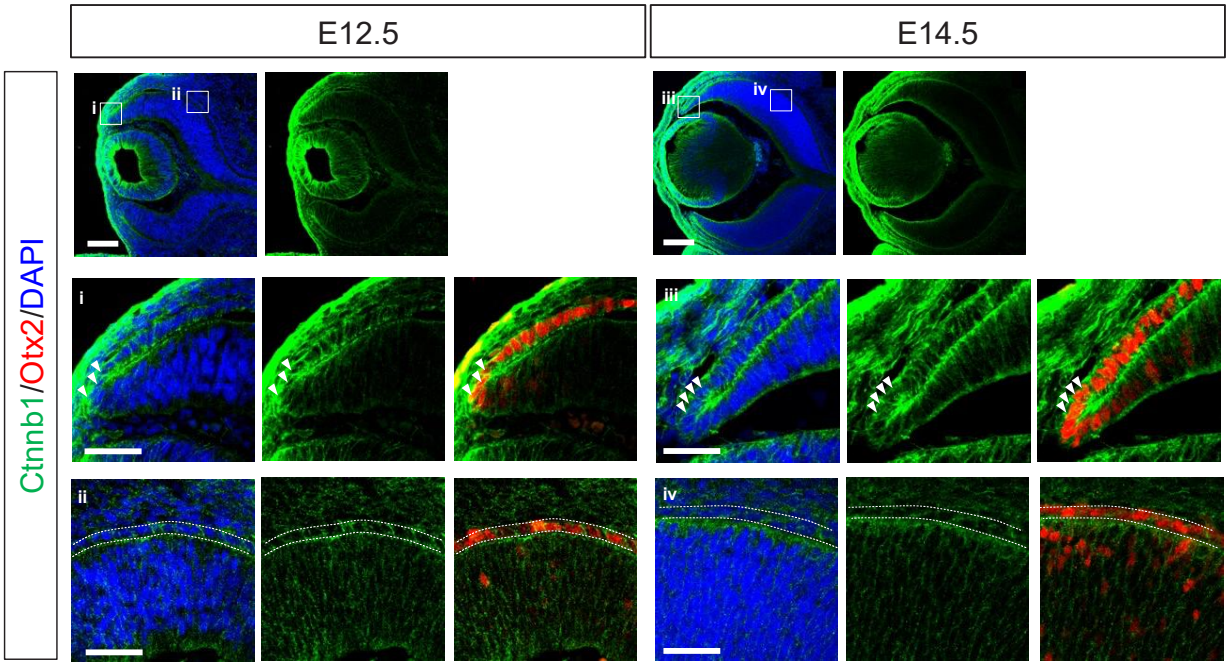
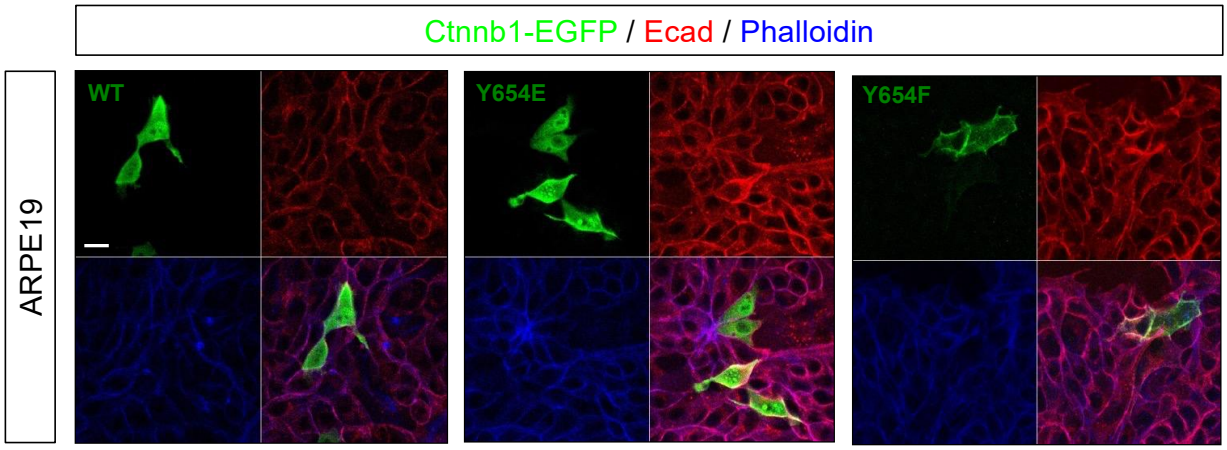
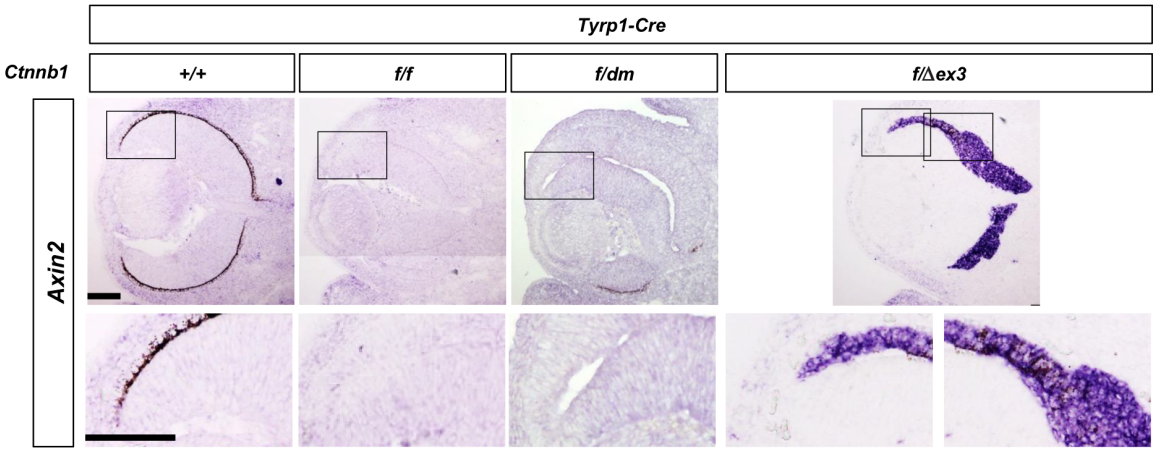


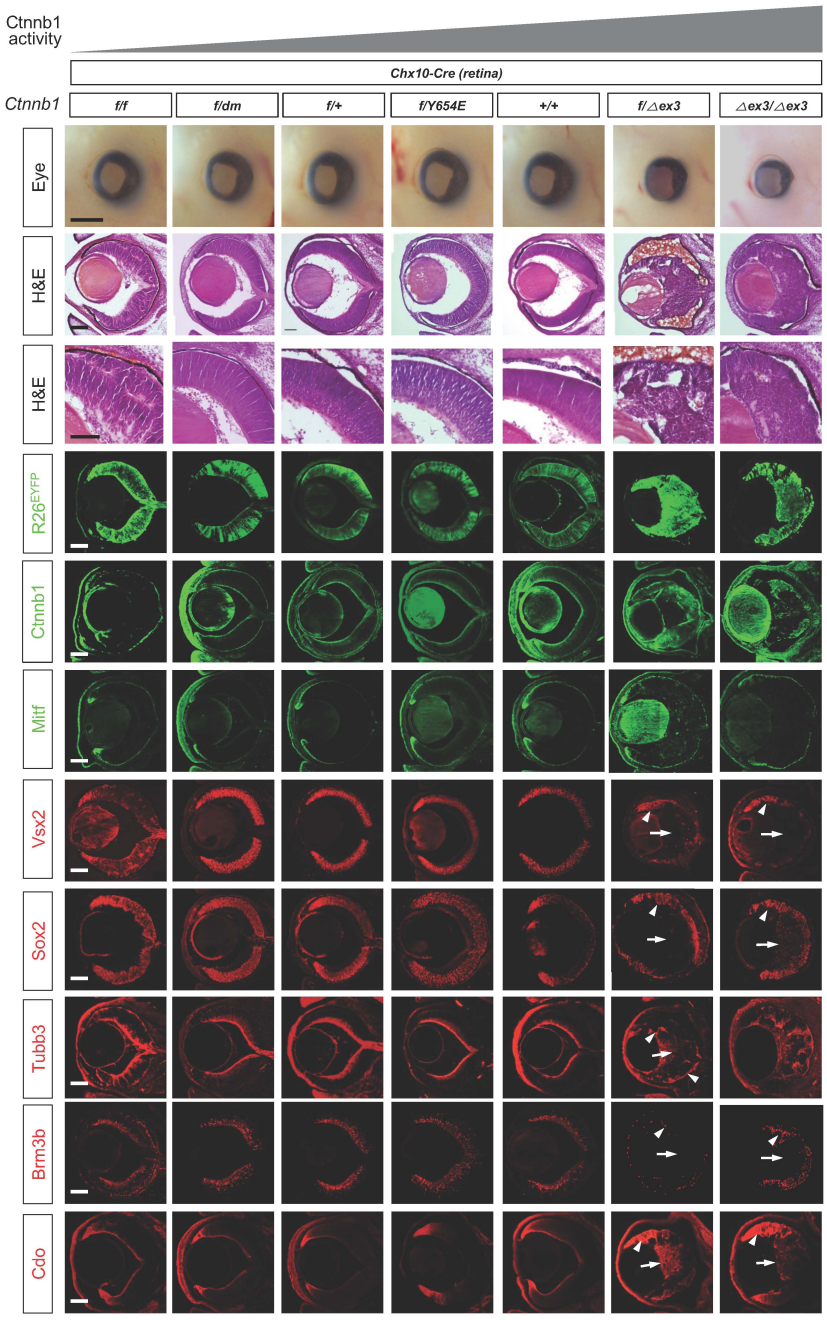

**Supplementary Fig. S1. Ctnnb1 is detected in the nucleus of OCM cells in the developing mouse eye.** Intracellular distribution of Ctnnb1 in RPE, CM, and retina was determined by co-immunostaining of Ctnnb1 with Otx2, a nuclear marker for RPE, OCM, and photoreceptors in the retina. Nuclei of the cells are also stained with DAPI. Images in the second and third rows show magnified version of the distal (i and iii) or central (ii and iv) regions in the first row. Arrowheads indicate the nuclear Ctnnb1 immunostaining signals. Scale bars = 100 μm (top row) and 50 μm (two bottom rows).



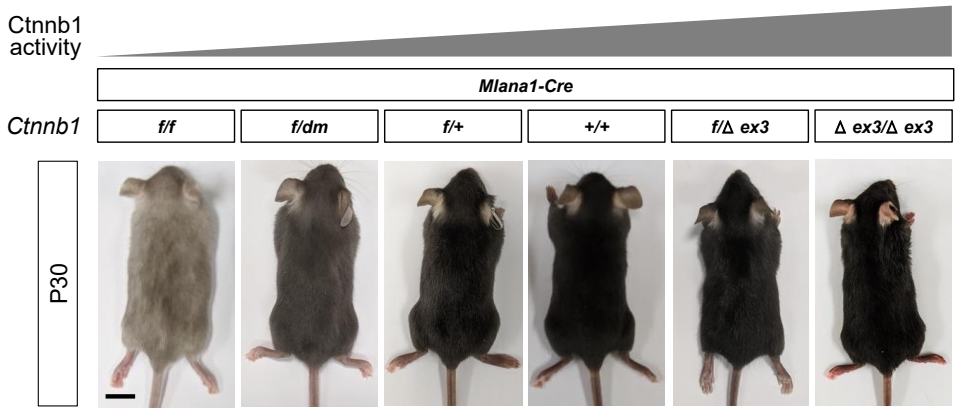

**Supplementary Fig. S2. Intracellular distribution of Ctnnb1$^{Y654E}$ mutant.** DNA constructs expressing EGFP-fused wild-type (WT), Y654E, and Y654F mutant versions of mouse Ctnnb1 cDNA were transfected into human ARPE-19 cell-line. The fluorescence of EGFP-fused Ctnnb1 proteins in the cells were visualized by examined by confocal microscopy. Distribution of AJs and actin filaments of the cells were determined by immunostaining and staining with Phalloidin-AlexaFluo647, respectively. Scale bar = 10 μm.



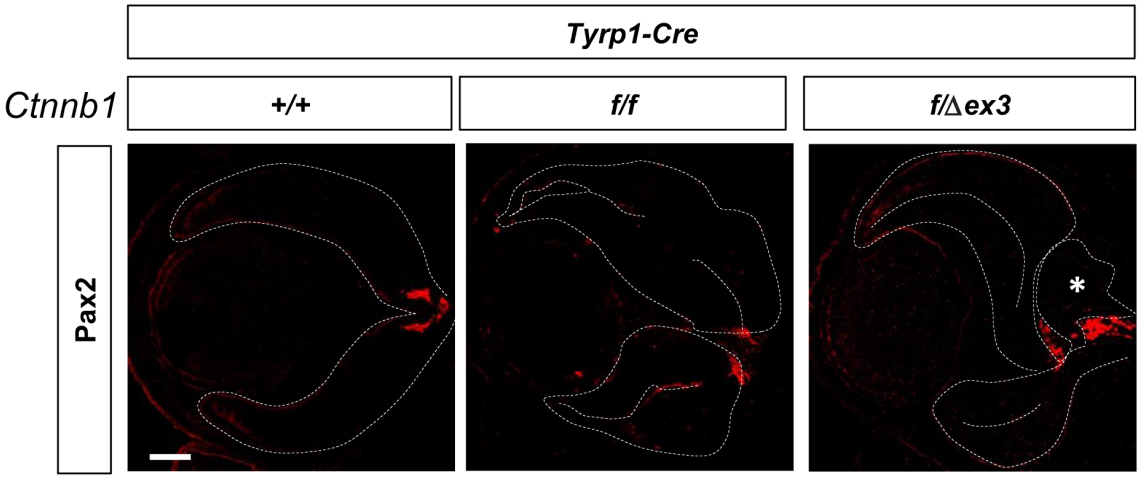

**Supplementary Fig. S3. The Wnt-Ctnnb1 targets, Axin2 and Lef1, are increased in the cells expressing constitutively active Ctnnb1.** Distribution of Axin2, a target of Wnt-Ctnnb1 signaling pathway, was investigated by ISH of E13.5 mouse eye sections, respectively. The images in the bottom row are the magnified version the boxed areas in top row. Scale bars = 100 μm.




**Supplementary Fig. S4. Ctnnb1 is dispensable for retinal cell fate acquisition.** Pictures of the eyes and H&E staining images of the eye sections of E14.5 mice, which express wild-type and functional variants of Ctnnb1, are provided in the first and second rows, respectively. Expression of $R26^{EYFP}$ Cre recombinase reporter (third row) and Ctnnb1 (fourth row) in the mouse eyes were examined by immunostaining of EYFP and Ctnnb1, respectively. The eye sections were also immunostained with the antibodies against specific markers to identify the distribution of corresponding cell types. Scale bars = 500 μm (first row), 50 μm (fifth row), and 100 μm (the rest rows).




**Supplementary Fig. S5. Coat color weakening of *Ctnnb1*$^{f/f}$*;Mlana1-Cre* and *Ctnnb1*$^{f/dm}$*; Mlana1-Cre* mice.** Pictures show the coat color changes in P30 mice, which express wild-type and functional variants of Ctnnb1 in *Mlana1-Cre*-affected RPE and melanocytes. Scale bar = 1 cm.




**Supplementary Fig. S6. Ectopic ICM populations formed by constitutively active Ctnnb1 at the RPE-OS border are not OS cells.** Sections of E13.5 *Ctnnb1*$^{+/+}$*;Tyrp1-Cre*, *Ctnnb1*$^{f/f}$*;Tyrp1-Cre*, and *Ctnnb1*$^{f/\Delta ex3}$*;Tyrp1-Cre* mouse retina were stained with rabbit polyclonal antibody against Pax2, which is expressed in the OS. Scale bar = 100 μm.

**Supplementary Table S1.** Antibodies used in this study

| Antibody name | Source | Cat. No. | Concentration |
|---|---|---|---|
| Anti-beta-catenin (Rabbit polyclonal) | Cell Signaling Technology | 9562 | 1:200 |
| Anti-Brn3b (Rabbit polyclonal) | Santa Cruz Biotechnology | sc-31989 | 1:200 |
| Anti-Cdo (Goat polyclonal) | R&D Systems | AF2429 | 1:200 |
| Anti-Ezrin (Mouse monoclonal) | Invitrogen Biotechnology | 35–7300 | 1:200 |
| Anti-Otx2 (Goat polyclonal) | R&D Systems | AF1979-SP | 1:200 |
| Anti-Pax6 (Rabbit polyclonal) | Covance | PRB-278P | 1:200 |
| Anti-Tubulin-ßIII (Tuj1; Mouse monoclonal) | Covance | MMS-435P | 1:200 |
| Anti-Vsx2 (Sheep polyclonal) | Abcam | AB_302279 | 1:100 |
| Anti-Mitf (Rabbit polyclonal) | Abcam | ab122982 | 1:100 |
| Anti-Msx1 (Goat polyclonal) | R&D Systems | AF5045 | 1:100 |
| Anti-Sox2 (Rabbit polyclonal) | Abcam | ab97959 | 1:100 |
| anti-P/E-cadherin (mouse monoclonal) | BD | 610181 | 1:200 |
| anti-ZO-1 (mouse monoclonal) | BD | 610966 | 1:200 |
| anti-Pax2( Rabbit polyclonal) | Biolegend | 901001 | 1:200 |