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<th>Human CS 16</th>
<th>Mouse E11.5</th>
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**Legend:**
- **RPE:** Retinal pigment epithelium
- **NR:** Neural retina
- **L:** Lens
- **POM:** Pigmentary outer segment of the photoreceptors
**Figure S1.**

**A-F:** Serial sections through the optic fissure of a human embryonic eye at CS16 (Day 37) from anterior to posterior. **G-L:** Serial sections through the optic fissure of a mouse embryonic eye at E11.5 from anterior to posterior. The point of initiation of fissure closure is located immediately posterior to the developing lens in both human and mouse (D & I; arrowheads). At CS16 there remained a physical gap between the fissure margins except at the initiation point. **NR:** Neural retina (inner layer of optic cup); **RPE:** Retinal pigmented epithelium (outer layer of optic cup); **POM:** periocular mesenchyme; **L:** lens.
Figure S2.

A-C: Analysis of serial sections of developing mouse eyes at E11.5 (n=4) showed fissure closure had been initiated at the midpoint of the fissure at this developmental stage (B, arrow). Anterior and posterior to the point of closure, the margins were in contact but closure had not been initiated (A, C double arrows). D-G: Immunostaining for Laminin (red), a component of basement membrane shows the two fissure margins approach each other through their basal aspects. Discontinuous staining for laminin was observed at the point of closure (G, arrowhead) indicating a fragmentation of the basement membrane. H-J: Analysis of serial sections at E12.5 (n=3) showed that closure was complete along nearly the whole length of the fissure leaving a ventral indentation in the neural retina towards the posterior of the eye (G, arrow)
Figure S3. A: Schematic showing the process of Laser Capture Microdissection. B: Percentage of aligned RNA sequencing reads from human samples. C: Percentage of aligned RNA sequencing reads from mouse samples. The majority of reads in all samples aligned to coding and UTR regions. One sample CS17 (2) D showed some evidence of degradation. D, E: Principal component analysis of human and mouse RNA sequences respectively.
Figure S4.

A: Expression patterns of known coloboma genes (mouse orthologues of human coloboma disease genes and genes from animal models) in the E11.5 fissure margin and dorsal optic cup samples. B: Expression of known coloboma genes (mouse orthologues of human coloboma disease genes and genes from animal models) in the E12.5 fissure margin and dorsal optic cup samples. Black bars indicate groups of genes showing differential expression between the two regions, either enriched or suppressed in the fissure compared to the dorsal region; these groups are smaller at E12.5 than at E11.5. Black dots indicate genes that passed significance threshold in differential expression analysis.
Figure S5

A: Heat map showing the top 50 significantly fissure enriched and dorsal enriched genes in the human samples.
Figure S6

Cell roundness heatmaps of serial sections (anterior to posterior) of the optic fissure in a CS 17 (Day 41) human eye. Colours of the heatmap range from blue (elongated) to red (rounded). Cell membranes are labelled with WGA. A: Fissure margins are remodelling and cells at the margins have a rounded morphology. Cells distant from the fissure margin have elongated morphologies typical of a pseudo-stratified epithelium. B: At the point of closure rearranging cells have a rounded morphology. C: Closure is complete and cells have an elongated morphology, markedly in the presumptive NR. D: At the posterior closure point cells at the fissure margins show a rounded morphology. WGA: Wheat Germ Agglutinin, NR: Neural Retina, RPE: Retinal Pigmented Epithelium. Note: Sections in B and D are also shown in Figure 4F-G’ and in Figure 6 E-F with Phalloidin staining.
Figure S7. A, B: Histological sections showing the human optic fissure margins before and during closure at CS17. Selected nuclei are outlined to illustrate the change in morphology of the cells. C: Electron micrograph at the point of closure in a human eye showing both elongated (blue) and rounded (yellow) cells. D: High magnification electron micrograph showing cellular material being extruded at the fissure margins. Arrowheads indicate basement membrane. Red and blue indicate two selected cells.
**Movie 1:** 3D projection of a section of the fissure margins immediately anterior to the anterior point of closure of a CS17 human eye. Blue: DAPI, Green: Phalloidin, Magenta: WGA (Wheat Germ Agglutinin, labelling cell membranes). Cells at the fissure margins have a rounded morphology.

**Movie 2:** 3D projection of a section of the fissure margins at the anterior point of closure of a CS17 human eye. Blue: DAPI, Green: Phalloidin, Red: PAX6, Magenta: WGA (Wheat Germ Agglutinin, labelling cell membranes). Rearranging cells have a distinct rounded morphology.
**Movie 3:** 3D projection of a section of the fissure margins immediately posterior to the anterior point of closure of a CS17 human eye. Blue: DAPI, Green: Phalloidin, Red: PAX6, Magenta: WGA (Wheat Germ Agglutinin, labelling cell membranes). In this region closure is complete and cells have elongated morphologies.

**Movie 4:** 3D projection of a section of the fissure margins at the posterior point of closure of a CS17 human eye. Blue: DAPI, Green: Phalloidin, Red: PAX6, Magenta: WGA (Wheat Germ Agglutinin, labelling cell membranes). Puncta of strong phalloidin staining are visible in cells at the point of closure.
Table S1. Mouse fissure and dorsal enriched genes at E11.5

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Table S2. Mouse fissure and dorsal enriched genes at E12.5

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Table S3. Gene ontology terms represented by fissure and dorsal enriched genes in E11.5 mouse eyes

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Table S4. Gene ontology terms represented by fissure and dorsal enriched genes in E12.5 mouse eyes

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Table S5. Hallmark gene sets positively and negatively correlated with the fissure margins.

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Table S6. All significantly differentially expressed genes

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