

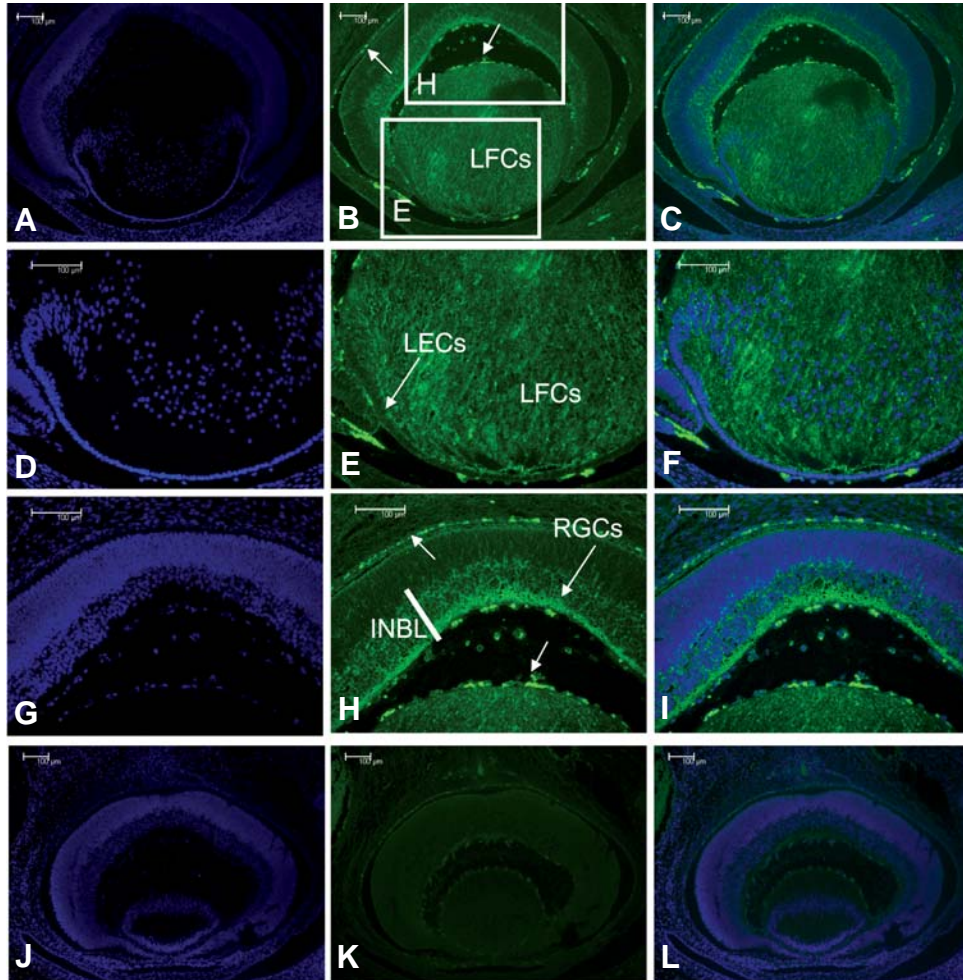
doi: 10.1387/ijdb.082597fm

SUPPLEMENTARY MATERIAL

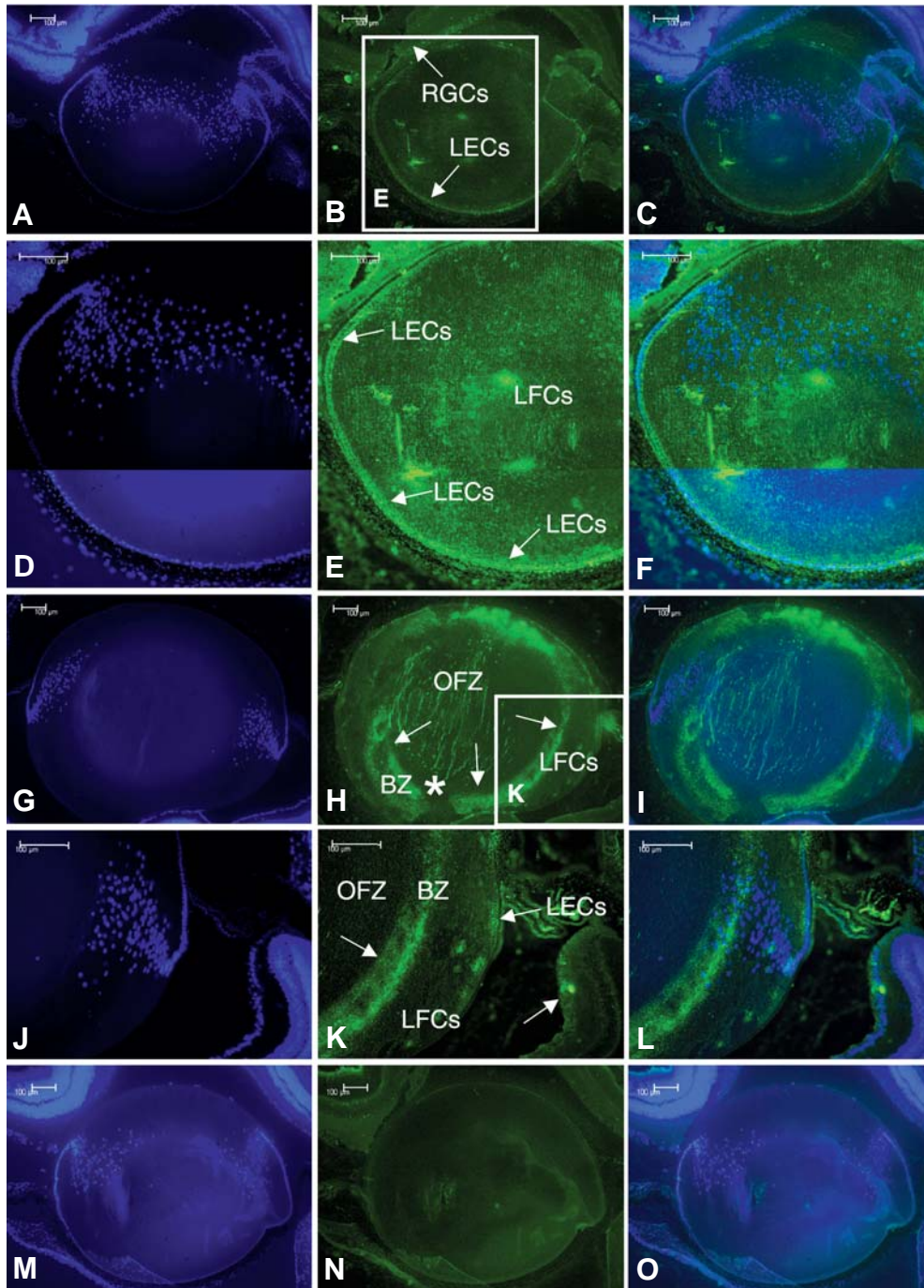
corresponding to:

**Developmentally regulated expression of
hemoglobin subunits in avascular tissues**

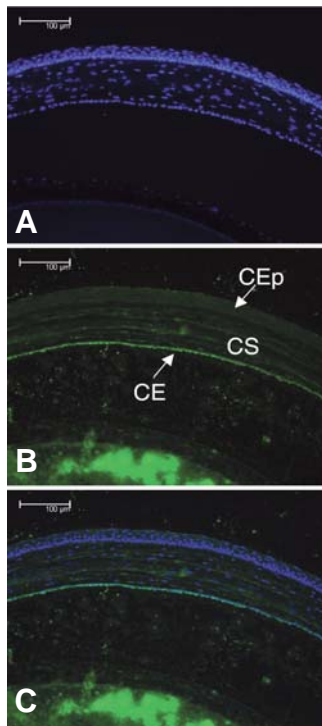
FIONA C. MANSERGH, SUSAN M. HUNTER, JENNY C. GEATRELL, MIGUEL JARRIN, KATE POWELL,
MARTIN J. EVANS and MICHAEL A. WRIDE*



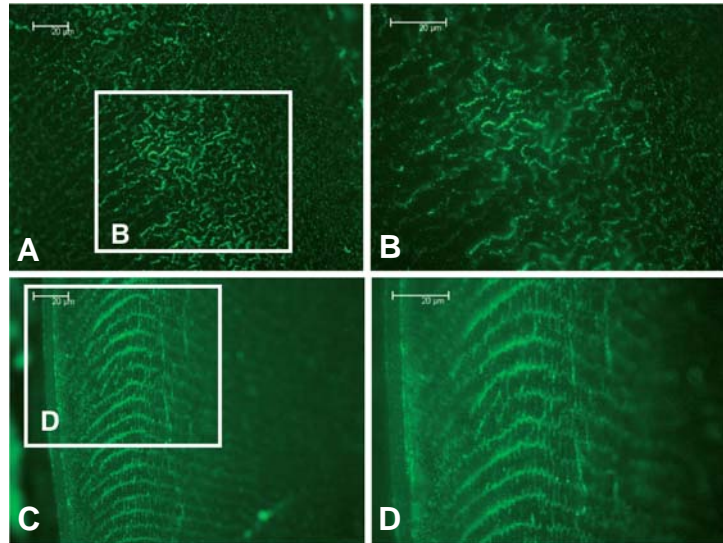
Supplementary Fig. 1. Immunofluorescence for hemoglobin, ED16.5 mouse eye. (A-J) DAPI labelling. **(B-K)** Hemoglobin immunofluorescence. **(C-L)** Merged images. Regions defined by white rectangles in (B) are those in (E) and (H). **(B,E)** Staining is present in the lens fiber cells (LFCs), but not in the lens epithelial cells (LECs), there is also some immunofluorescence for contaminating erythrocytes associated with the anterior lens capsule in the aqueous humor. **(H)** In the retina, staining is mainly associated with the developing retinal ganglion cell (RGC) layer, but also includes other cells in the inner neuroblast layer (INBL). **(J-L)** Sections stained with rabbit IgG at the same concentration as the hemoglobin antibody are negative for staining. Mag bars 100 μ M.



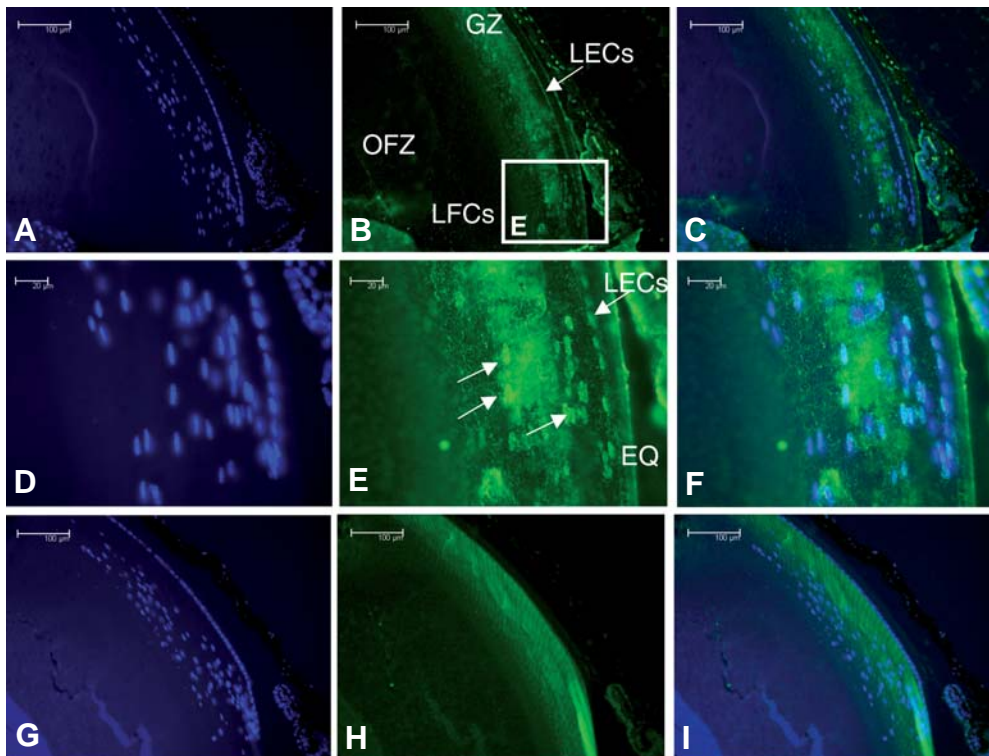
Supplementary Fig. 2. Immunofluorescence for hemoglobin, p14 mouse lens. (A-M) DAPI labelling. (B-N) Hemoglobin immunofluorescence. (C-O) Merged images. Regions defined by white rectangles in (B,H) are those in (E,K). (A-F) Sections through the lens at the margin of the organelle free zone (OFZ). (D-L) Sections through the centre of the lens. (D-F) Composite images. (B,E,H,K) Staining is associated with the cytoplasm of lens epithelial cells (LECs) and with the surfaces of lens fiber cells (LFCs). Staining is present in the LFCs specifically at the boundary zone (BZ) between the cortical LFCs and the organelle free zone (OFZ) (arrows; H, K) excluding the central posterior part of the lens where the posterior lens sutures are (*). There is very little staining in the retinal ganglion cells (RGCs) at this stage (K). (M-O) Sections stained with rabbit IgG at the same concentration as the hemoglobin antibody are negative for staining. Bars, 100 μ M.



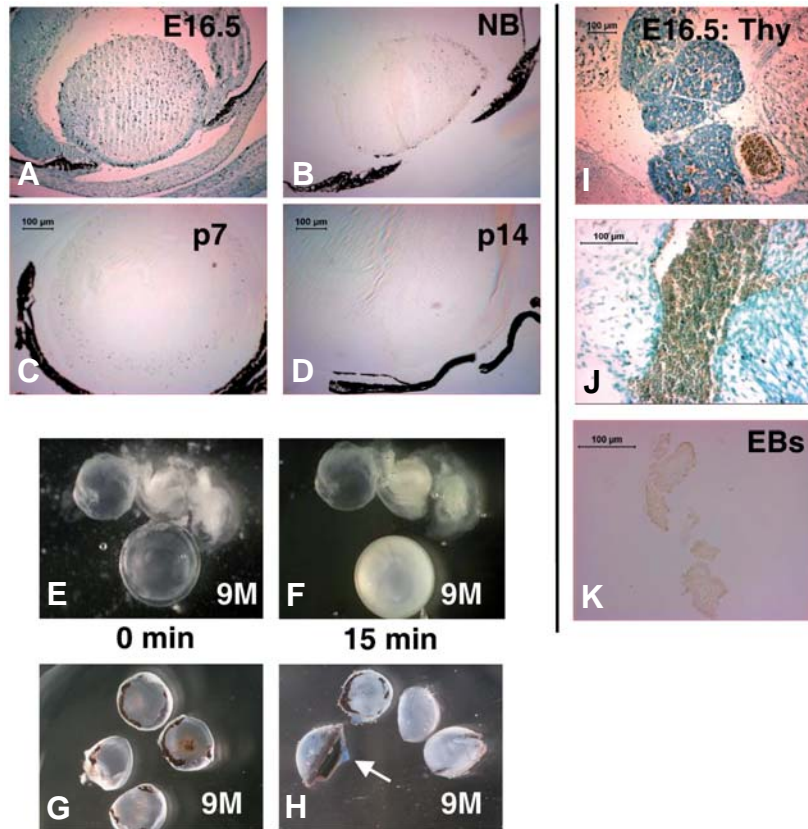
Supplementary Fig. 3 (Left). Immunofluorescence for hemoglobin in the p14 mouse cornea. (A) DAPI labelling. **(B)** Hemoglobin immunofluorescence. **(C)** Merged images. Staining is associated with the corneal endothelium (CE). There is some staining in the corneal stroma (CS), but none in the corneal epithelium (CEp). Bars, 100 μ M.



Supplementary Fig. 4 (Right). Immunofluorescence for hemoglobin proteins associated with the surfaces of posterior cortical lens fiber cells (LFCs) in the mouse lens. (A, B) 4 wks. **(C, D)** 9 wks. Regions defined by white rectangles in (A, C) are those in (B, D). Patterns of staining reflect the changing shapes of LFCs at these stages of LFC maturation. Bars, 20 μ M.



Supplementary Fig. 5. Immunofluorescence for hemoglobin, 9 mo old mouse lens. (A-G) DAPI labelling. **(B-H)** Hemoglobin immunofluorescence. **(C-I)** Merged images/ Region defined by white rectangle in (B) is that in (E). The core lens fiber cells (LFCs) in the organelle free zone (OFZ) have little or no staining. Immunofluorescence is associated with the cortical LFCs **(B-F)**. In particular, immunofluorescence is associated with the nuclei of cortical lens fiber cells (arrows, E). It is not clear if staining is associated with a nuclear-associated sub-compartment at this stage (compare with staining at 9 wks; Figure 8). Lens epithelial cell (LEC) staining is associated with the cytoplasm in the germinative zone (GZ), but staining becomes progressively more associated with nuclei approaching the equator of the lens (EQ) at which point LECs are differentiating into LFCs. **(G-I)** Sections stained with rabbit IgG at the same concentration as the hemoglobin antibody are negative for staining. Bars, 100 μ M.



Supplementary. Fig. 6. Histochemical staining for heme using difluoroacetate assay. (A-D) Lens sections. (A) ED16.5 eye. (B) Newborn (NB) lens. (C) p7 lens. (D) p14 lens. There is staining for heme in the ED16.5 eye and lens. (E-F) 9 mo whole lenses incubated in reaction mixture. Initially (0 mins; E) lenses are clear, while after 15 mins (F) lenses have become opaque, but have not changed color, implying the absence of the heme moiety in lenses. (G-H) 9 mo corneas incubated in reaction mixture. Because of attached iris material containing red blood cells, after 15 mins, a blue color has developed (arrow in G), confirming that the reaction is working. (I,J) Section of ED16.5 thymus (Thy; I) and a blood vessel (BV; J) are positive for heme staining. (K) A section of EBs shows a low-level of heme staining. Bars, 100 μM.