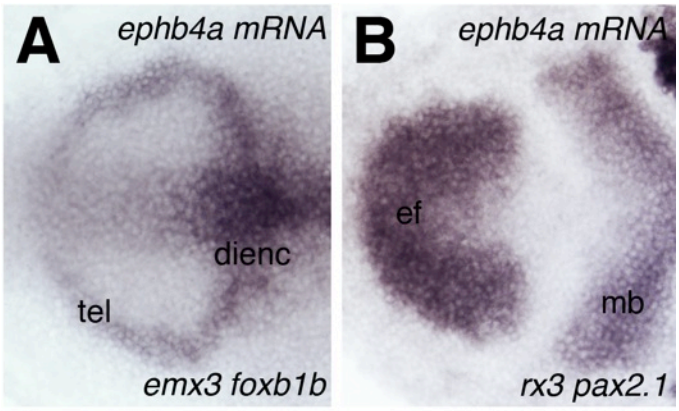
Supplementary figures



E

Controls at 2ss	rx3+pax2.1		emx3+foxb1b	
	N	% normal	N	% normal
efnB2a+efnB1 MOs	26	100	23	100
EphA4a mRNA	19	100	27	100
EphB4a mRNA	35	86	45	84
solefnB2a mRNA	74	88	83	90

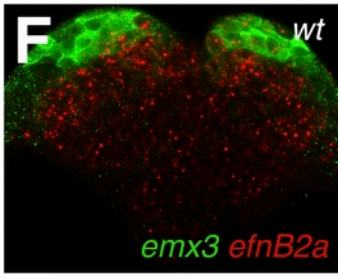


Figure S1: Eph/Ephrin manipulations do not perturb ANP patterning.
(A-D) Dorsal views showing ANP regionalisation at the end of gastrulation in embryos manipulated as labelled in the panels, revealed by expression of regional markers in the ANP (*emx3*/*foxb1b*, A,C and *rx3*/*pax2.1*, B,D). All expression domains are as in wildtype (not shown). (E) numbers of embryos analysed for each condition tested. (F) frontal view through the ANP of a 3ss stage embryo showing complementary expression of emx3 (green) and efnB2a (red). ef: eye field; tel: telencephalon; dienc: diencephalon; mb:midbrain.

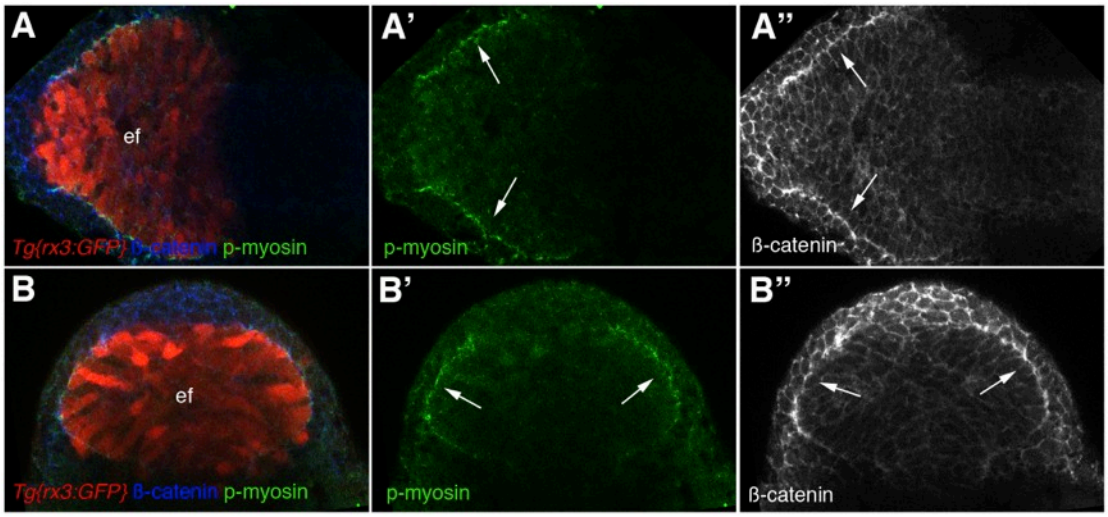


Figure S2: Phosphorylated light-chain-myosinII and ß-catenin co-localise at the edge of the eye field.

Dorsal (A-A") and frontal (B-B") views of neural plates immunostained as shown. Arrows point at the edge of the eye field. ef: eye field.

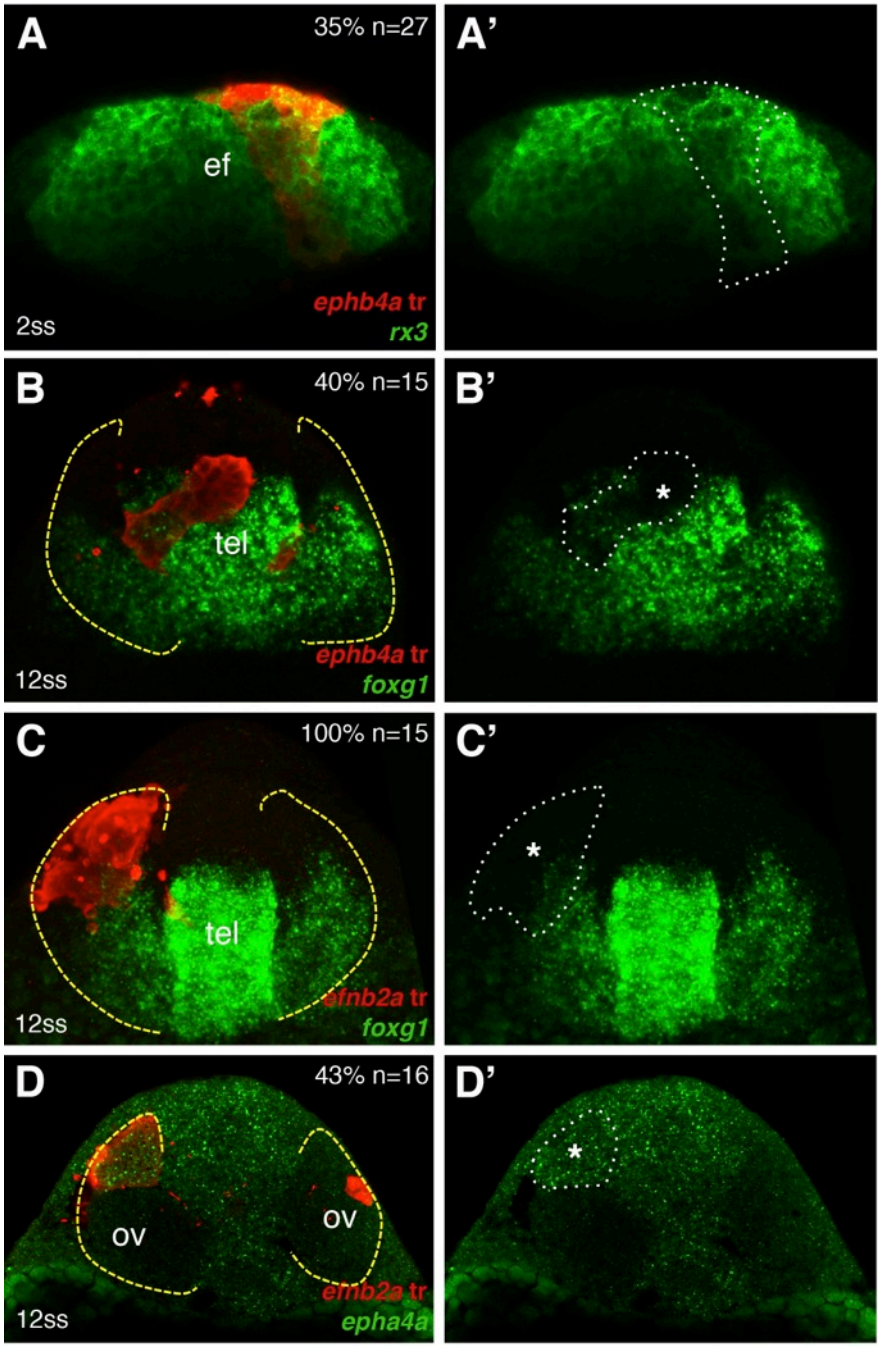
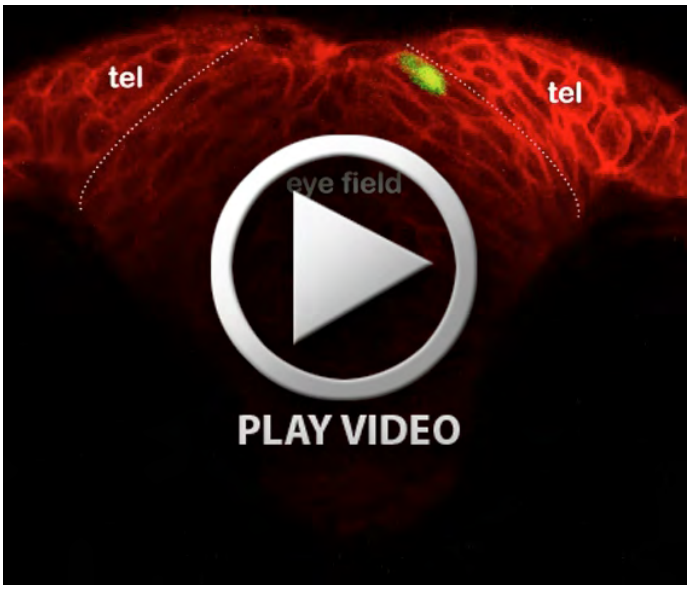
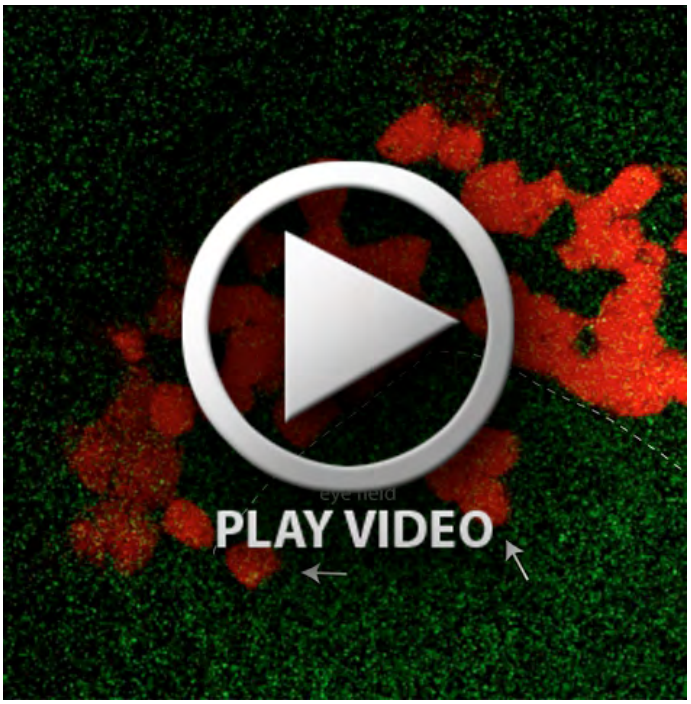


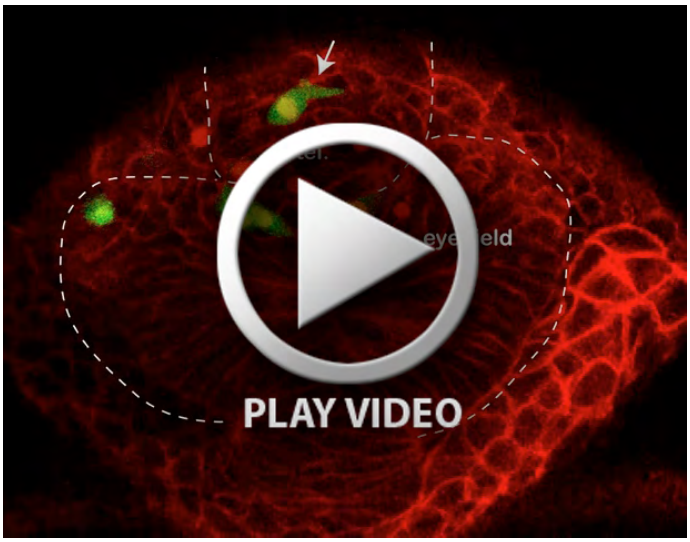
Figure S3: Telencephalic markers in *eph*/*ephrin*-expressing transplants.
(A-A') Some *ephb4a*+ transplants (red) remain embedded in the eye field (green). (B-B') *ephb4a*+ transplants in the telencephalon (red) do not express *foxg1* (green). (C-C'-D-D') *efnb2a*+ transplants in the eye (red) do not express *foxg1* (green, C-C') but express *epha4a* (green, D-D'). Dashed white lines demarcate the transplants; dashed yellow lines outline the optic vesicles. ef: eye field; tel: telencephalon; ov: optic vesicle. All panels show frontal views through the forebrain.



Movie 1. *rx3*:GFP+ cells at the edge of the eye field respect the border with the telencephalon throughout ANP morphogenesis. Time-lapse sequence starting at 1 ss of an embryo in which membranes are labelled with Lyn-cherry bearing a transplant of *rx3*:GFP^{+} cells. Transplanted cells placed in the eye field express the transgene. One cell located at the boundary between eye field and telencephalon closely respects this boundary as morphogenesis progresses. The movie shows one *z*-section through the ANP; images were acquired every 5 minutes/46 seconds for 2.4 hours.



Movie 2. Transplanted cells expressing exogenous *ephb4a* are actively excluded from the eye field. Time-lapse sequence starting at around 80% epiboly of a *Tg{rx3:GFP}* embryo bearing a transplant of *ephb4a*$^{+}$ cells (red). A few cells initially located in the future eye field (arrows) are actively excluded from the eye field as development proceeds. The eye field can be detected by GFP expression at the end of the time-lapse (green, white outline). The movie is a projection of four *z*-slices covering around 25 microns through the ANP; images were acquired every 10 minutes for 2.3 hours. Cells displaying this behaviour were seen in all the movies analysed (n=7).



Movie 3. Eye field cells can migrate into the optic vesicles at late stages. Time-lapse sequence starting at around 6ss of an embryo in which cell membranes are labelled with Lyn-cherry bearing a transplant of *rx3*:GFP^{+} cells. One GFP+ cell mislocated in the telencephalon (arrow) is followed as it migrates into the evaginating optic vesicles. The transplanted cells are labelled by the expression of the *rx3*:GFP transgene (green) and the nuclear accumulation of H2bRFP (red). The movie is a projection of two *z*-slices covering around 4.18 microns through the ANP; images were acquired every 6 minutes/25 seconds minutes for 2 hours. Only four cells in eight embryos analysed (showing an average of 20 cells per embryo) show this behaviour.