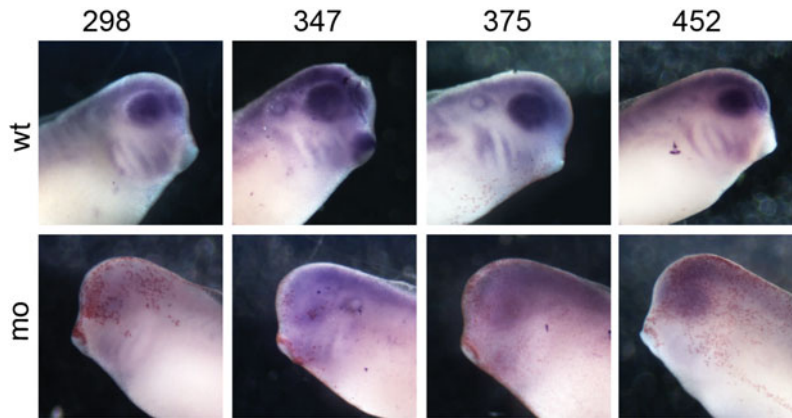


**SUPPLEMENTARY MATERIAL**

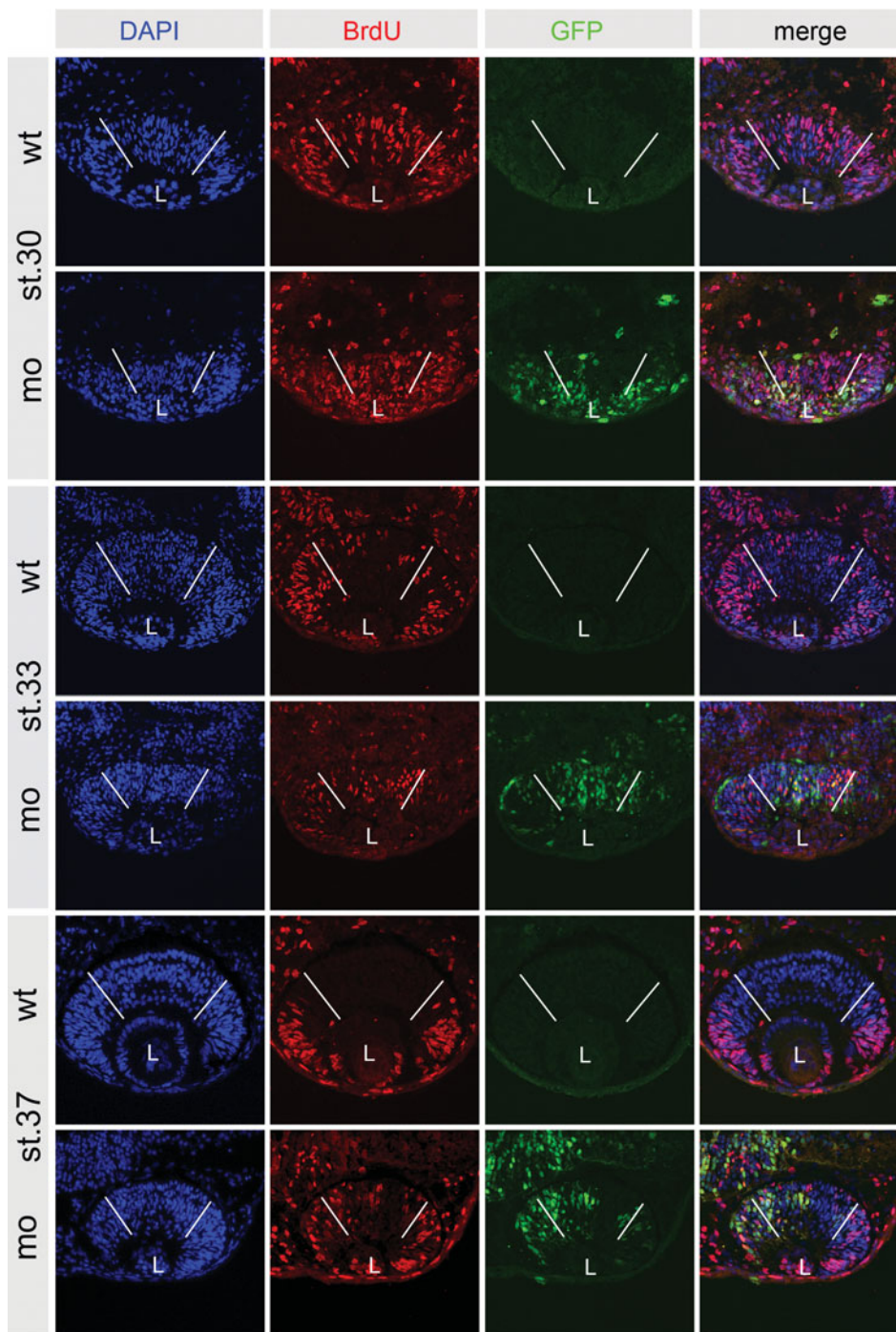
**corresponding to:**

**Dicer inactivation causes heterochronic retinogenesis  
in *Xenopus laevis***

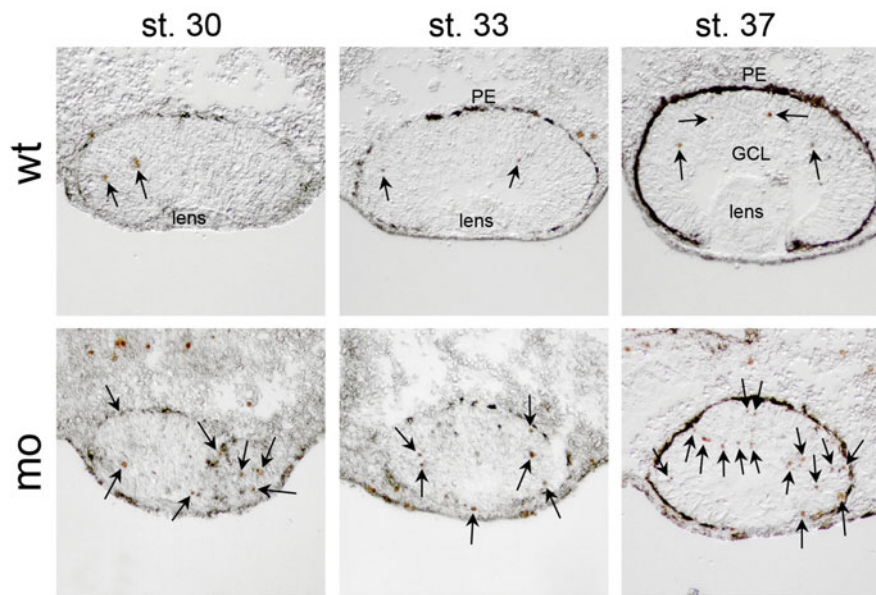
SARAH DECEMBRINI, MASSIMILIANO ANDREAZZOLI, GIUSEPPINA BARSACCHI and FEDERICO CREMISI\*



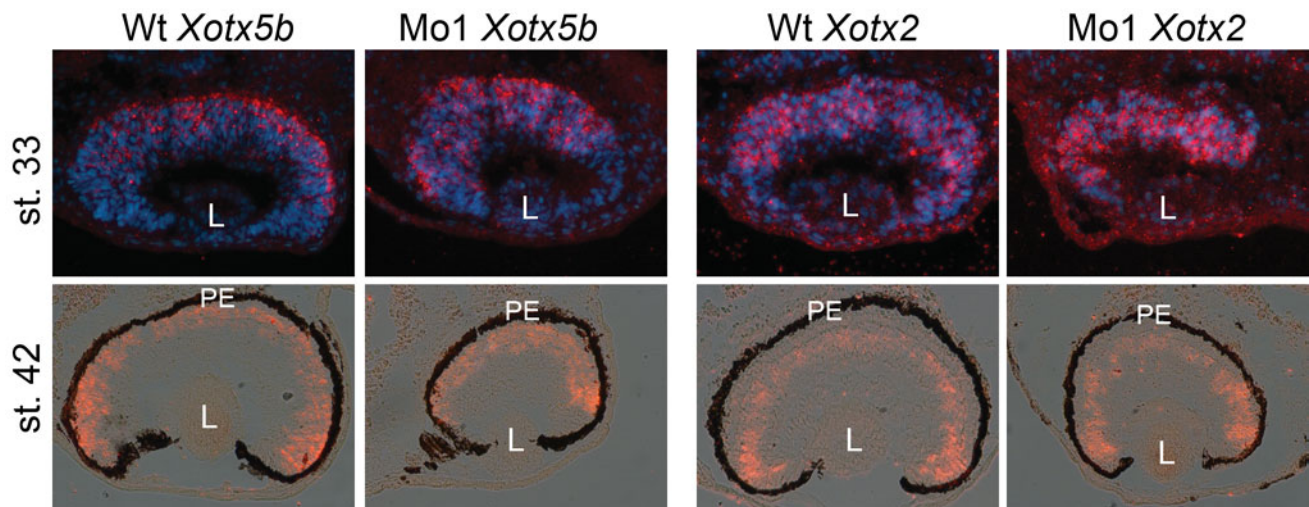
**Supplementary Fig. 1. Down-regulation of miRNA expression in morphants.** Examples of whole mount in situ hybridisation with 4 different miRNA probes on st. 33 *Xenopus* embryos that were unilaterally injected at 4-cell stage with *Xdcr-Mo1*. *wt* indicates control uninjected side, *mo* shows injected side, which was *lac-z* traced and detected by salmon-Gal staining (visible as a nuclear red staining). Numbers indicate the miRNA corresponding to the probe sequence. As in these examples, hybridisation signal was always lower in the injected side compared to the control side when using these and 2 additional probes (not shown), on embryos injected either with *Xdcr-Mo1* or *Xdcr-Mo2* in two independent experiments.



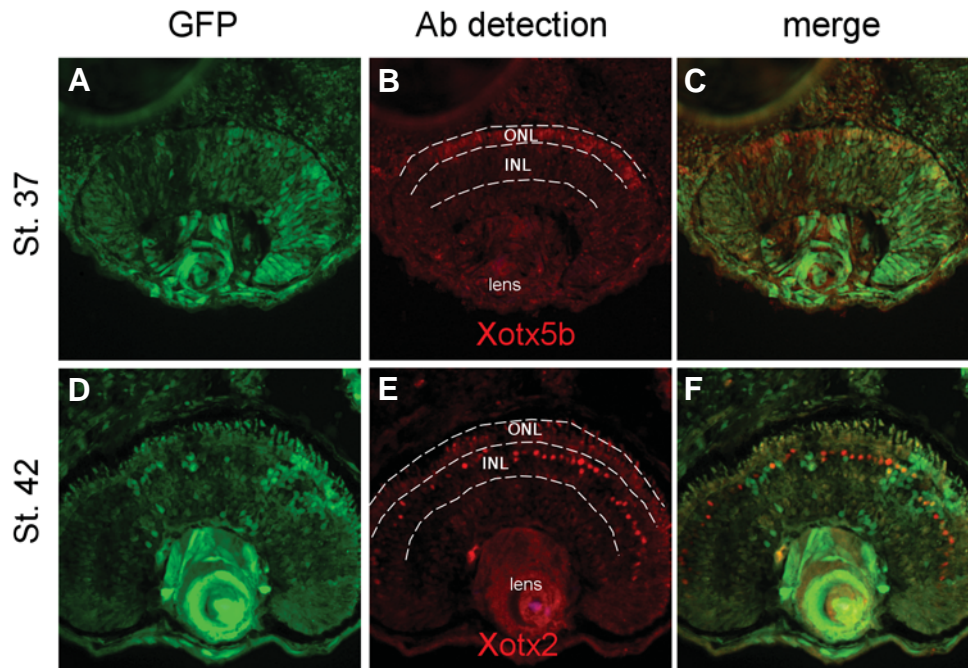
**Supplementary Fig. 2. BrdU labelling index analysis.** *BrdU* immunodetection on sections of *wt* and *Xdcr-Mo1*-injected (*mo*) retinas at different developmental stages. DAPI nuclei staining in blue; *BrdU* and *GFP* immunodetection in red and green, respectively; merge of the three markers. Lines indicate the central aspect of the retina in which *GFP* positive cells were counted. L, lens.



**Supplementary Fig. 3. TUNEL analysis.** Panels show examples of retinal section at different stages of wt and morphants (mo), after TUNEL assay to identify apoptotic cells. Arrows point to positive nuclei labelled in brown, PE, pigmented epithelium; GCL, ganglion cell layer.



**Supplementary Fig. 4. Xotx2 and Xotx5 mRNA expression in wt and morphants.** In situ hybridisation with Xotx5b and Xotx2 probes on retinal sections of either wt (Wt) or morphants (Mo1) at st. 33 and st. 42. Hybridisation signal was detected by fast red and visualised under epifluorescence as described (Decembrini et al., 2006). St. 33 retinal sections were counterstained with DAPI (blue nuclei). In the pictures of st. 42 sections, fast red epifluorescence was merged to the corresponding bright field view. Notably, Xotx5b and Xotx2 mRNA detection in morphants is comparable to wt. L, lens; PE, pigmented epithelium.



**Supplementary Fig. 5. Control morpholino does not affect Xotx5b and Xotx2 expression.** Antibody (Ab) immunodetection (red fluorescence) of Xotx5b at st. 37 (**B**) and Xotx2 at st. 42 (**E**) in retinal sections of embryos co-injected with control morpholino and GFP as tracer (green fluorescence in A,D). (**C,F**) Merge. 10 nl of 125 nM control morpholino (supplied by Gene Tools) were microinjected at 4-cell stage into one dorsal blastomere. ONL, outer nuclear layer. INL, inner nuclear layer. Control morpholino injection did not affect the expression of the two proteins.