Fig. S1. The PRC2 core components are expressed in dividing retinal progenitors. (A-D) Expression of the PRC2 core subunits Ezh2, Suz12, Eed and Rbbp4 in the developing eye by whole-mount in situ hybridization of stage 22/23 embryos. (E-J) Expression of Suz12 and Rbbp4/7 (by in situ hybridization) largely coincides with BrdU immunostaining in the CMZ of the *Xenopus* retina at stage 41. (K-P) Injection of GFP mRNA together with 5 ng control MO or Ezh2 ATG MO at the eight-cell stage, followed by immunostaining for EZH2 on stage 41 retinal sections. Control MO does not reduce the level of EZH2 immunolabeling in the GFP-marked clones (see yellow cells in M), whereas EZH2 immunostaining in significantly reduced by Ezh2 ATG MO (GFP-positive cells do not significantly co-label with EZH2 immunostain in P).
Fig. S2. Ezh2 lacking the SET domain functions as a dominant negative and prevents H3K27me3 labeling in *Xenopus* retina. (A-D) Immunostaining of a retinal section of stage 41 embryo with antibody against H3K27me3 (red) after co-injection of GFP mRNA with Ezh2 ΔSET mRNA (green) at the eight-cell stage. Hoechst labels nuclei (blue). Arrows show GFP-labeled cells with reduced H3K27me3 levels. INL, inner nuclear layer; GCL, ganglion cell layer. Scale bar: 20 µm.

Fig. S3. Suz12 is required for H3K27me3 in *Xenopus* retina. (A-D) Knockdown of Suz12 by injection of translation blocking morpholino (Suz12 MO) together with GFP mRNA results in decreased H3K27me3 staining in the retina in GFP-labeled cells (arrowheads). ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Scale bar: 10 µm.
Fig. S4. Blocking cell death or knockdown of p15<sup>ink4b</sup> does not rescue eye size after knockdown of Ezh2. (A) Ezh2 ATG MO or pan-caspase inhibitor, or both, were injected into eight-cell embryos and number of embryos that showed reduced eye size was counted at stage 41. No significant difference in the percent of embryos with microphthalmia was observed between injection of Ezh2 ATG MO alone (n=55) or in combination with pan-caspase inhibitor (n=46; Student’s t-test P=0.55). Injection of pan-caspase inhibitor alone has minimal effect on eye size (n=28). (B) Co-injection of Ezh2 ATG MO with two different doses of p15 MO failed to rescue the eye size defects caused by injection of Ezh2 ATG MO alone. Injection of p15 MO alone also causes reduced eye size, potentially owing to expression in the neural tube and very early optic vesicle (not shown).
Fig. S5. Changes in optic vesicle gene expression after injection of control MO versus Ezh2 ATG MO by microarray analysis. Relative changes in gene expression shown by heatmap of log2-transformed microarray signals for the indicated genes, with the color scale shown at the bottom in log base 2 units. Data represent an average of four biological replicates.

Supplemental Figure S5

Fig. S6. Control MO has no effect on eye specification genes. (A-E) Frontal view of stage 20 embryos injected with control MO plus mRNA encoding β-galactosidase to mark injected side. X-gal stain is in light blue. The expression domain as well as expression levels of all tested genes appear normal in the injected side. Embryos with normal expression: 100%, n=13 for Rx; 91%, n=11 for Six3; 94%, n=16 for Fz5; 100%, n=15 for Sox2; 93%, n=15 for Cyclin D1.

Supplemental Figure S6
Fig. S7. Ezh2 UTR MO and Suz12 MO phenocopy the Ezh2 ATG MO effect. (A-F) Frontal view of embryos injected with Ezh2 UTR MO (A-C) or Suz12 MO (D-F) showing reduction in expression domain of the progenitor genes Rx, Vsx1 and Fz5 on the injected side. (G-N) Lateral view of embryos injected with Ezh2 UTR MO (G-J) or Suz12 MO (K-N) exhibiting reduced levels of the bHLH factors Xath5 and Xash1 on the injected side.
**Fig. S8. Ezh2 MO injection does not affect Wnt or Notch signaling.** Lateral view of stage 27 embryos injected with Ezh2 ATG MO. (A-D) There are relatively normal levels of expression of Fz5 and the downstream target Sox2 on the injected side, although the eye domain is smaller. (E-L) Likewise, there is normal expression of Notch signaling components Delta, Notch, Esr-1 and Nrarp.

**Fig. S9. Overexpression of p15 does not affect retinal progenitor specification or neural differentiation.** (A-F) Lateral view of stage 27 embryos after injection of p15 mRNA. The progenitor gene Rx and neural differentiation genes NeuroD and Xath5 show normal levels of expression.
Fig. S10. Blocking PRC2 in retinal progenitors alters the normal complement of retinal cell types. Analysis of GFP-labeled retinal cell types in stage 41 retinal sections after injection of GFP mRNA plus MO into a dorsal animal blastomere at the 32-cell stage. Injection of either the Ezh2 ATG MO or the Ezh2 UTR MO caused an increase in the representation of Müller glia when compared with GFP mRNA alone or control MO, and a corresponding decrease in several major retinal cell types (ganglion cells and bipolar cells). Amacrine cells are significantly increased whereas horizontal cells and photoreceptor cells are unchanged, although this analysis does not distinguish rods and cones. Injection of mRNA for dominant-negative ΔSET-Ezh2 had a similar effect, confirming that the effect is due to loss of PRC2 function. RG, ganglion cells; C, horizontal cells; AM, amacrine cells; BP, bipolar cells; PR, photoreceptor cells; MG, Müller glia. The percent representation of each cell type was calculated as a weighted average; error bars represent s.e.m; *P<0.001 (Student’s *-test).