

Fig. S1. A, Schematic showing the VSX2 predicted binding sites, min2 enhancer, and its deletion regions within the Vsx2-EN1. The highlighted sequence represents nucleotides that have been deleted. B, Luciferase assays of mouse min2 WT and min2 Mutant in the presence of VSX2. Data were shown as mean \pm S.E.M, $n = 4$, $P < 0.001$ (***) ; $P < 0.01$ (**); $P < 0.05$ (*). C, AP staining in the cross-section of the retinas electroporated with empty or Vsx2-EN1 enhancer cloned in Stagia3 reporter construct. D- O, Whole-mount images of zebrafish embryos at the indicated stages after injection of mouse (D-I) and human (J-O) Vsx2-EN1 enhancer. P- Q, Cross sections of the developing zebrafish retina 72 hpf after injection with the constructs. Panels H, N and P are shown in the main figures.

Supplementary Figure 2

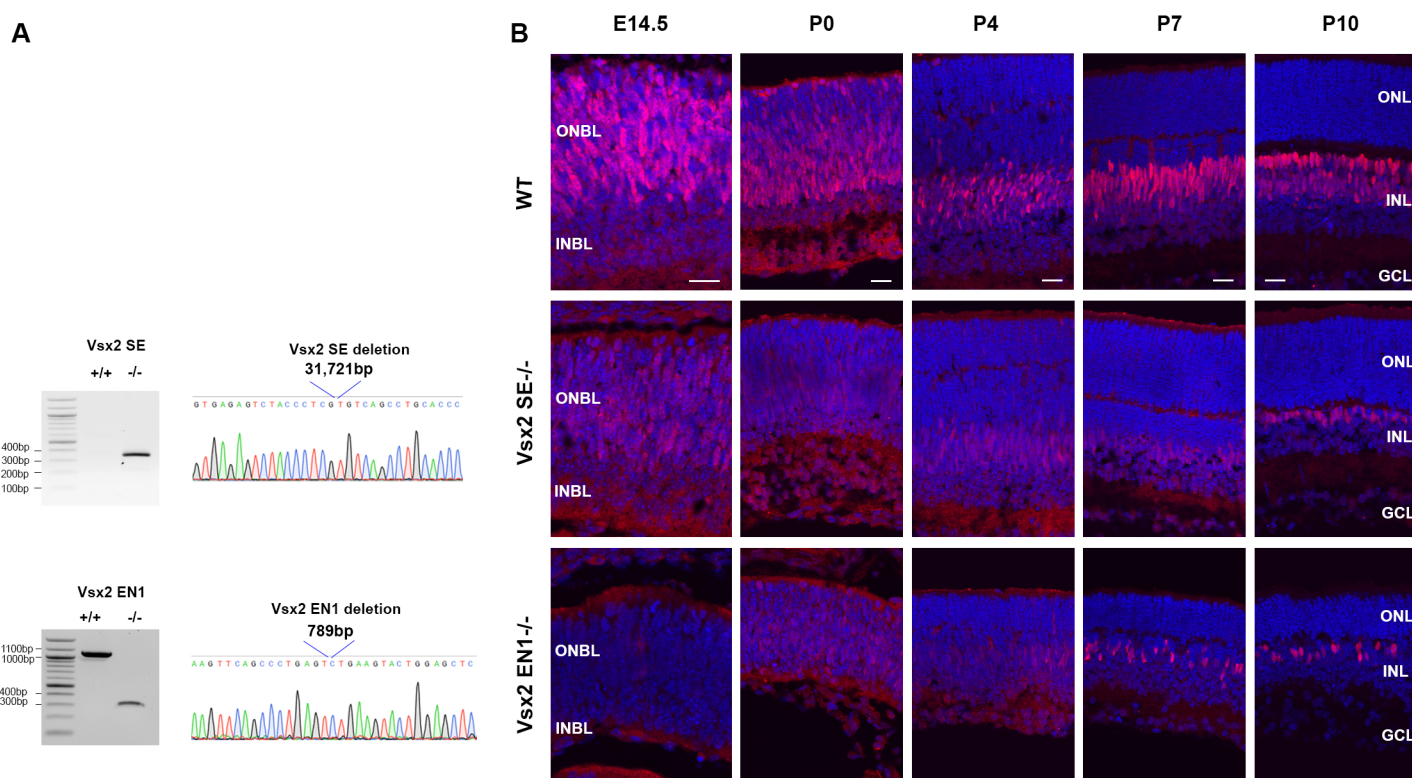


Fig. S2. A, Representative genotyping data (gel images of PCR products and sanger sequencing tracks) from *Vsx2*-EN1 and *Vsx2*-SE mouse lines. The deletion boundaries and sizes were indicated above each sequence track. B, Retinal cryosections at different developmental stages (E14.5, P0, P4, P7, P10) from WT, *Vsx2*-SE, and *Vsx2*-EN1 deficient mice immunoassayed with VSX2 antibody.

Supplementary Figure 3

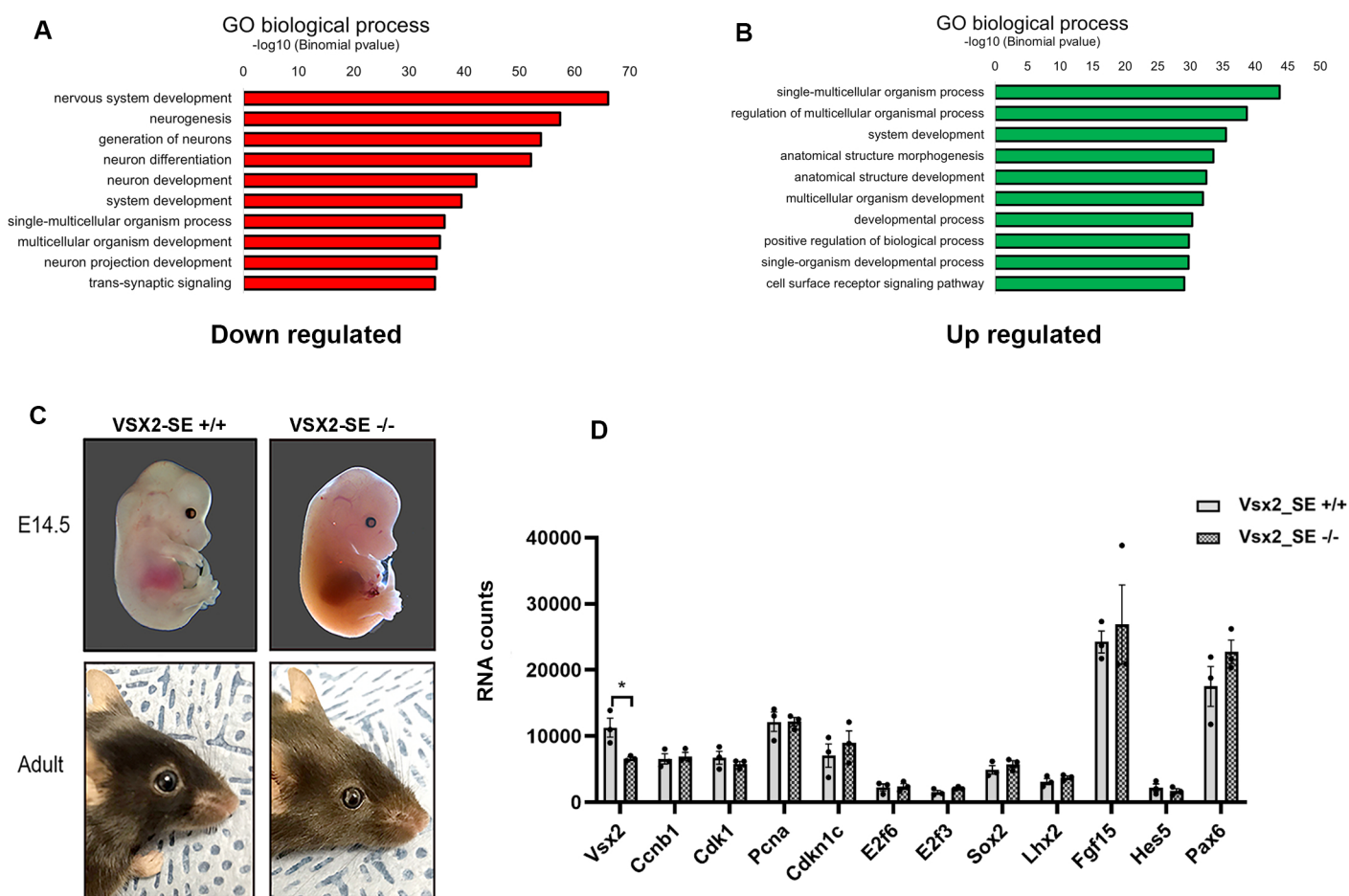


Fig. S3. A-B, Results of Gene Ontology (GO) enrichment analysis of downregulated (A) and up-regulated (B) genes in VSX2-EN1 KO mice at E14.5 retina. The lengths of the bars indicate the $-\log_{10}$ -transformed P-values. Data were generated by Database for Annotation, Visualization, and Integrated Discovery (DAVID) software. C, VSX2-SE KO mice exhibit normal eye size compared to WT mice. D, Bar plot of RNA levels of selected RPC genes as determined by RNA-Seq analysis performed on VSX2-SE $^{-/-}$ and WT retinæ at E14.5. Noticed that only *Vsx2* is significantly downregulated.

Supplementary Figure 4

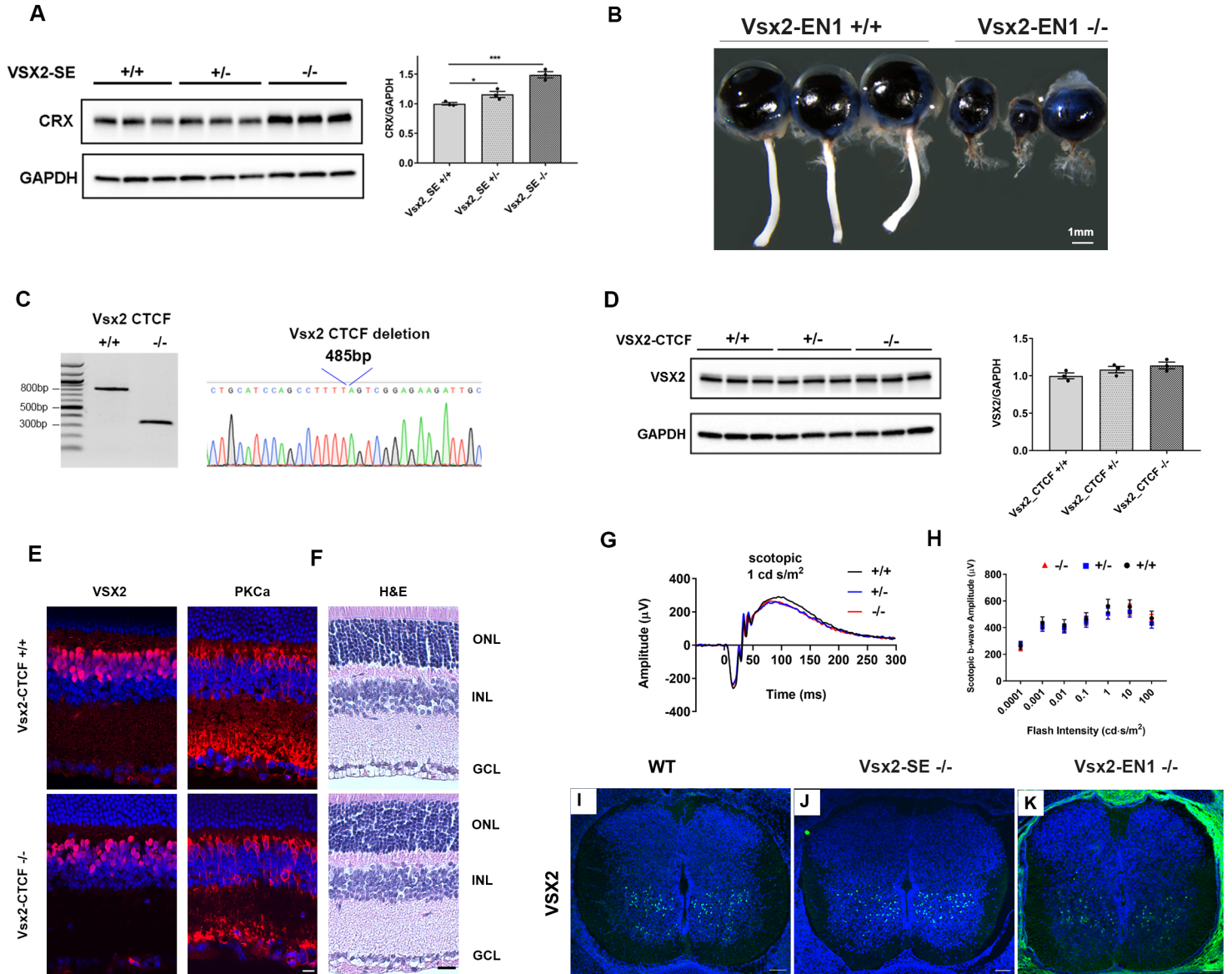


Fig. S4. A Western blot analyses of CRX performed on adult retinae from VSX2-SE^{-/-}, heterozygous, and WT littermates with quantification. B, Adult eyes from VSX2-EN1 deficient mice are microphthalmic and exhibit optic nerve aplasia. C, Representative gel image of PCR products and sanger sequencing tracks from Vsx2-CTCF mouse lines. D, Analysis of Vsx2 expression in adult Vsx2-CTCF^{-/-}, heterozygous and WT littermates by Western blot with quantification. E, Immunohistochemistry of VSX2 and PKCa of the adult retina from Vsx2-CTCF^{-/-} and littermate control. F, Hematoxylin and Eosin (H&E) staining of adult Vsx2-CTCF^{-/-} retina and control littermates. G-H, ERG was recorded from different groups of mice at 6 weeks of age. Scotopic ERG responses from different groups of mice stimulated by a flash of light at 1 cd·s/m² (G) and plots of scotopic b- wave amplitudes (H) did not show any change in different groups (data were shown as mean ± S.E.M, n = 3, *P* < 0.05 (*)). I-K, Immunostaining of VSX2 on cryosections from WT (I), VSX2-SE^{-/-} (J), and VSX2-EN1^{-/-} (K) of the developing spinal cord at E14.5. Note that VSX2 remains expressed in the ventral spinal cord in VSX2 enhancer knockouts (scale bar 80 μm).

Table S1. Genomic coordinates (mm10) of VSX2 ChIP-Seq peaks in E14.5 and adult mouse retina

[Click here to download Table S1](#)

Table S2. Genomic coordinates (mm10) of VSX2 ChIP-Seq peaks near photoreceptors and bipolar genes in adult retina

[Click here to download Table S2](#)

Table S3. RNA-Seq data from VSX2-EN1 knockout retina at E14.5

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Table S4. RNA-Seq data from VSX2-SE knockout retina at E14.5

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Table S5. Genomic coordinates (hg38) of VSX2 ChIP-Seq peaks in adult human retina, and lists of photoreceptor and bipolar genes with VSX2 peaks

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Table S6. Primer sequences for genotyping

Primer Name	Forward Primer 5'-3'	Reverse Prime 5'-3'	PCR size (bp)	
			no deletion	with deletion
VSX2-SE-homo	GCTCTGACCTTCCTGGAAGCC CGC	CTCAGGAGGTTACAAGGAG GTGTAG	none	300
VSX2-SE-Het	CATAACTGGCTGTATTCTGTG TGACTC	CTTACATCCTTTGACCCTGG CTATG	149	none
VSX2-EN1	GGCCAGGTAGGCCAAAGTAG	GTGTTCGTACCTCAGTCACCG	1091	302
VSX2-CTCF	TGTGGCTCTCTGGAAGATGC	GGCGCGGAAATGAATGC	817	330

Table S7. Primer sequences for cloning

Name	Forward Primer	Reverse Primer
mVsx2	GCCGGATCCGCCACCATGACGGGGAAAGC GGGGGAAG	GCCTCTAGA CTAAGCCATGTCCTCCAGCTG
mPAX6	GCCGGATCCGCCACC ATGCAGAACAGTCACAGCGG	GCCTCTAGA TACTGTAATCGAGGCCAGTAC
hVsx2	GCCGGATCCGCCACCATGACGGGGAAAGC AGGGGAAG	GCCTCTAGA CTAAGCCATGTCCTCCAGCTG
hPax6	GCCGGATCCGCCACC ATGCAGAACAGTCACAGCGG	GCCTCTAGA TACTGTAATCTTGGCCAGT
mVsx2_En1	GCCGATATCCCTTCCCACCTTTTGTCTGGT A	GCCAAGCTTTGTGCTCGTCGAGG GTAGACT
mVsx2_En2	GCCGATATCCCCGGGCTTAGAGAGCATT	GCCAAGCTTCAGGCACTTCCCAA GGAGAG
mVsx2_En1_mi n2_WT	GCCGATATCTTCTGCTTTGTCCCCACCAG T	GCCAAGCTTAGGGAATGGCTCTC TTTGAT
mVsx2_En1_mi n2_mutation	GTCCATTTCTCGCACAATTCC	GGAATTGTGCGAGAAATGGAC
hVsx2_En1	GCCGATATCTTGTCTATGCCTAAAGAGGC CA	GCCAAGCTTTTCTAGGGCAGAATT CACTCACG
hVsx2_En2	GCCGATATCGAGAAGCCTGAGGTTTAGCG ATT	GCCAAGCTTCTCCACAAGCATGA TCCTACTGA
mOTX2	GCCGGATCCGCCACCATGATGTCTTATCT AAAGCAACC	GCCTCTAGATCACAAAACCTGGA ATTTCC
mPrdm1_En	GCCCTCGAGAGCCCTGACAAAGAGAGTGG GAG	GCCGATATCGTCGACACAAAAGC TGAGCTTTGAG

Table S8. Antibodies used in western blots and immunoprecipitation

Antibody	Company	Catalog number	Dilution
CRX	Santa Cruz	Sc-377207	1:500
GAPDH	ThermoFisher	MA5-15738	1:1000
PRDM1	Santa Cruz	SC47732X	1:500
PAX6	Active Motif	61611	1:1000 (3 μ l in IP)
PAX6	Santa Cruz	Sc-81649	1:200
VSX2	Exalpha	X1180P	1:1000 (3 μ l in IP)
VSX2	Santa Cruz	SC-365519X	1:1000
OTX2	R & D	AF 1979	1:1000 (3 μ l in IP)

Table S9. Antibodies used in immunostaining

Antibody	Company	Catalog number	Dilution
PKC α	Santa Cruz	SC8393	1:100
SOX9	Millipore	AB5535	1:100
VSX2	Exalpha	X1180P	1:300
VSX2	Santa Cruz	SC-365519 X	1:500
Brn3a	Millipore	MAB1585	1/100
Calbindin	Sigma Aldrich	C9848	1/100
Cone Arrestin	Millipore	AB15282	1/300
Pax6	BioLegend	901302	1/300
Pax6	Active Motif	61611	1/300
Ki-67	Invitrogen	MA5-14520	1/100
OTX2	R & D	AF 1979	1:300