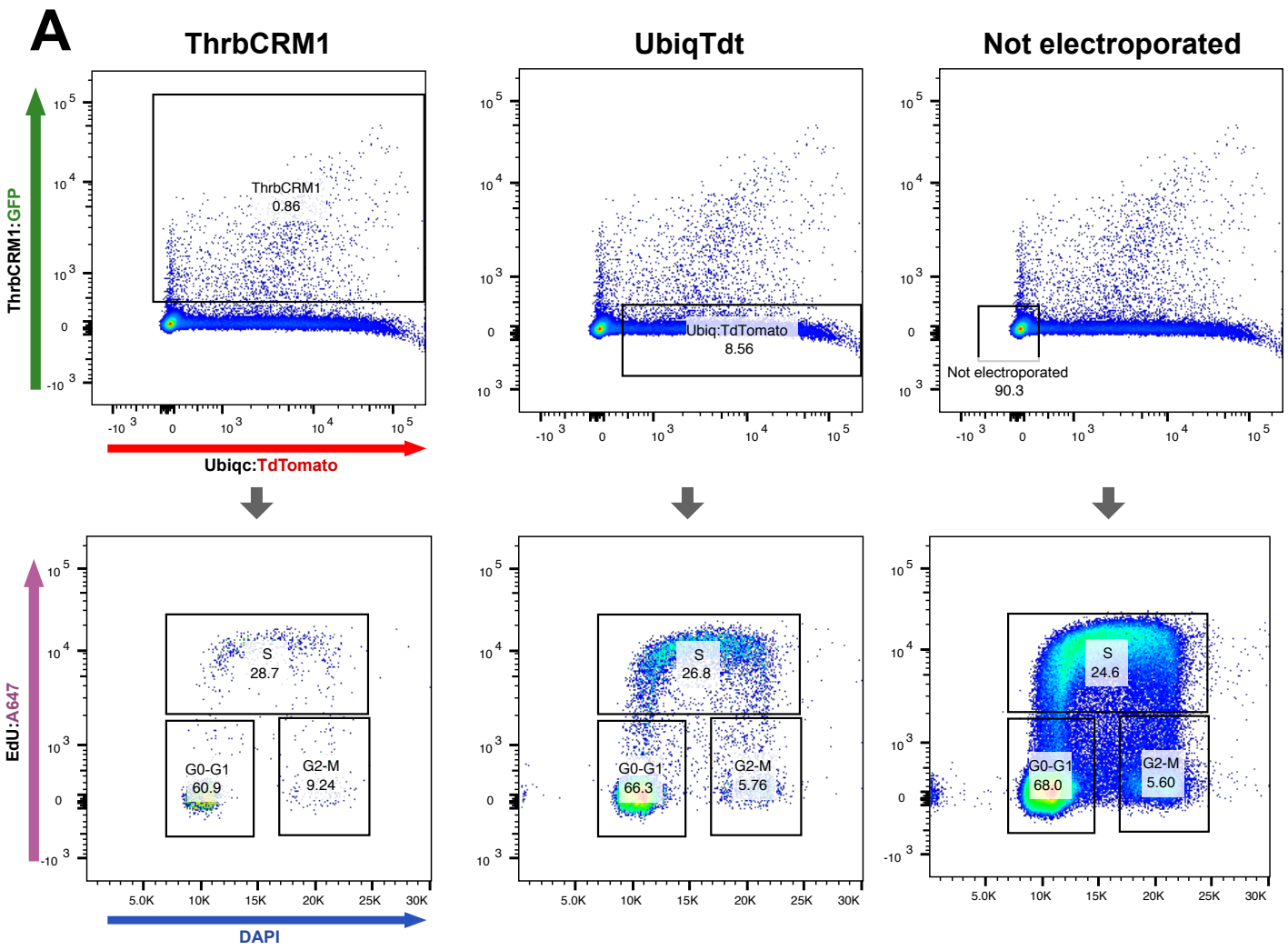
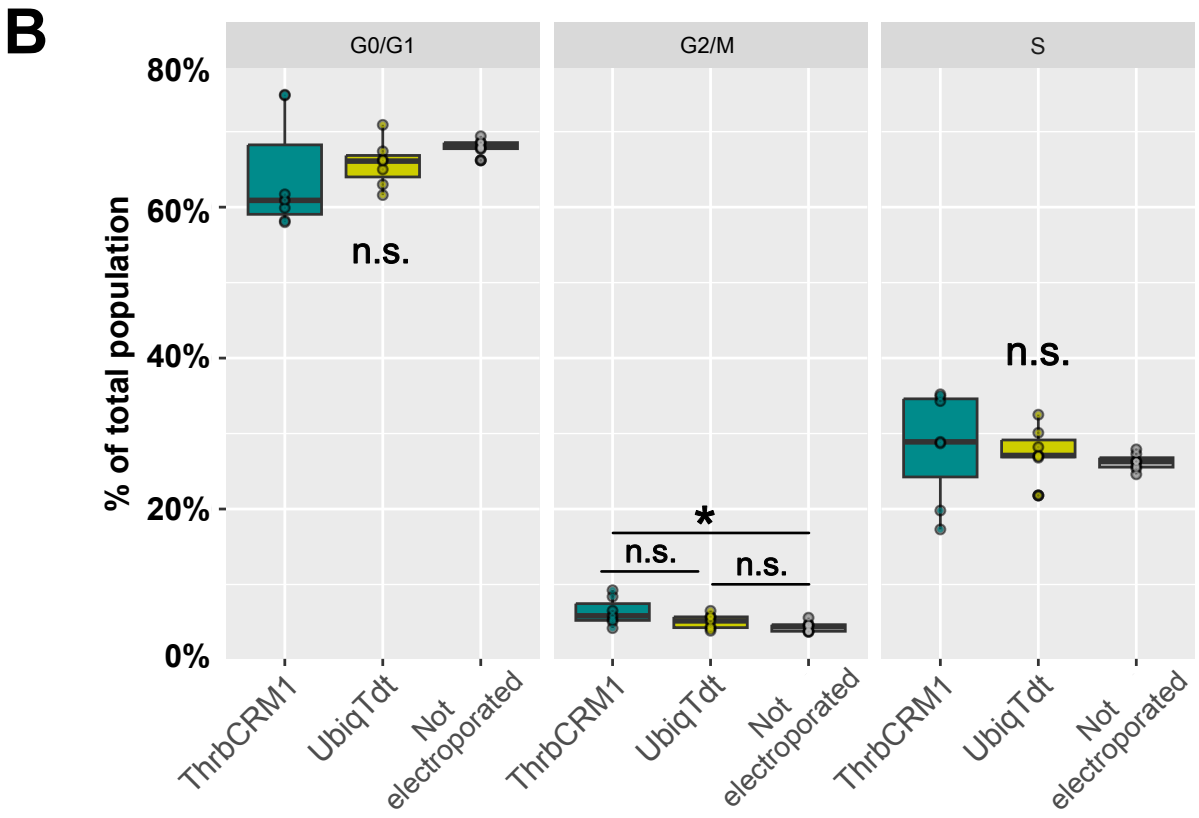
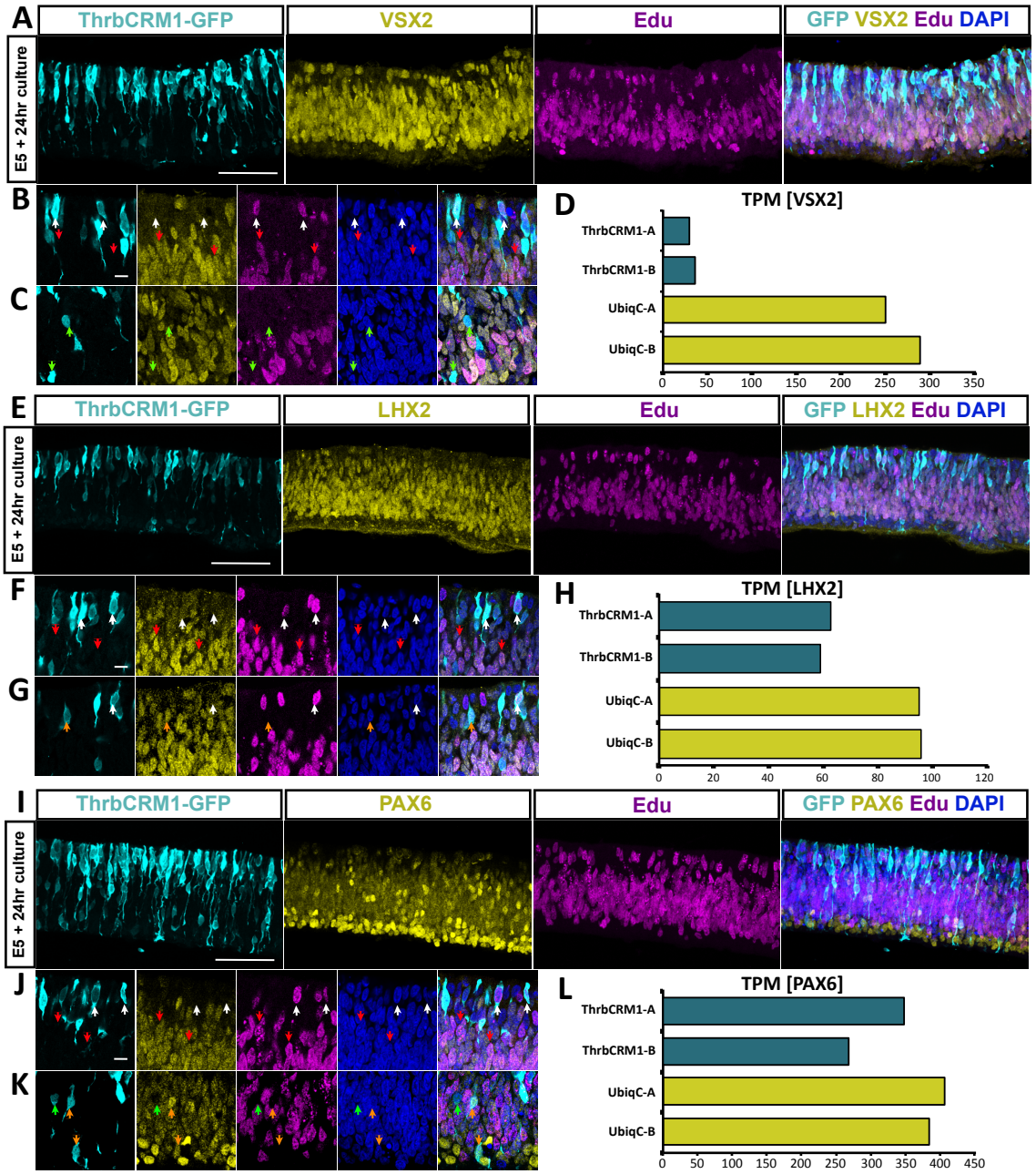




Supplemental Figure 1. **Cell cycle analysis of ThrbCRM1 and UbiqTdt population.**
(A) Representative collection and cell cycle analysis plots for each population.
(B) Quantification of cell cycle analysis from E5 retinas electroporated and cultured approx. 20h. Constructs used were Ubiq-Tdt and ThrbCRM1-GFP. Cell populations used for Y-axis calculations are shown along the X-axis. Statistical significance was determined in n=7 biological replicates by Kruskal-Wallis with Dunn's posthoc test. (*: $p < 0.05$).



Supplemental Figure 2 - **Retinal Progenitor Gene expression at the single cell level and transcriptome level in ThrbCRM1(+) cells**

Retinas electroporated at E5 with ThrbCRM1-GFP, cultured for 24hand exposed to EdU for 1h. Large panels are maximum intensity projections Z-stacks (scale bar = 50um) and small panels are single planes of the same Z-stacks (scale bar = 5um).

(A-C) ThrbCRM1 cells include VSX2(-) RPCs located in the scleral side (B, white arrows). Majority of RPCs are VSX2(+) (B, red arrows). ThrbCRM1 activity seldom cross-labeled with VSX2 (C, green arrows)

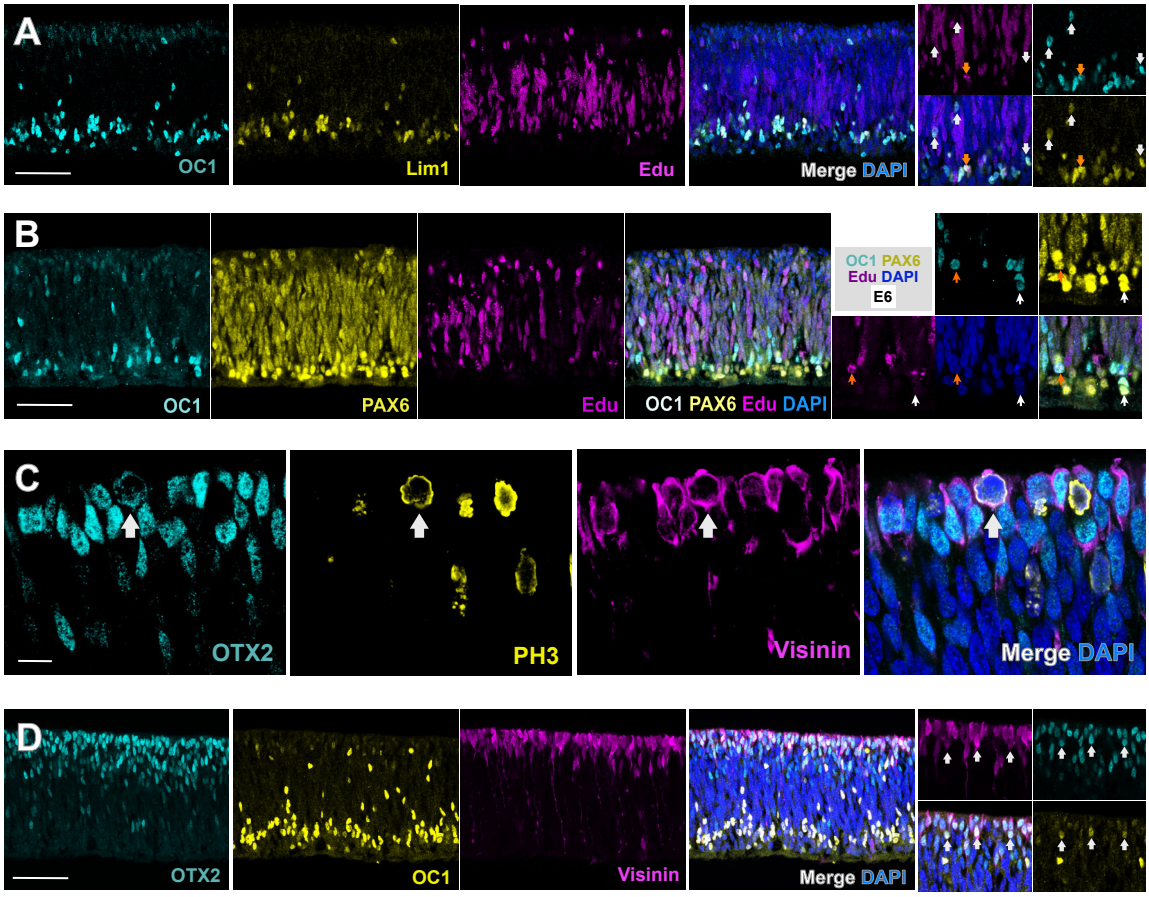
(D) Transcript expression of VSX2 in each replicate.

(E-G) ThrbCRM1 cells also include LHX2(-) RPCs located in the scleral side (F, white arrows). Majority of RPCs are LHX2(+) (F, red arrows). Some ThrbCRM1cells were also positive for LHX2 (G, orange arrows).

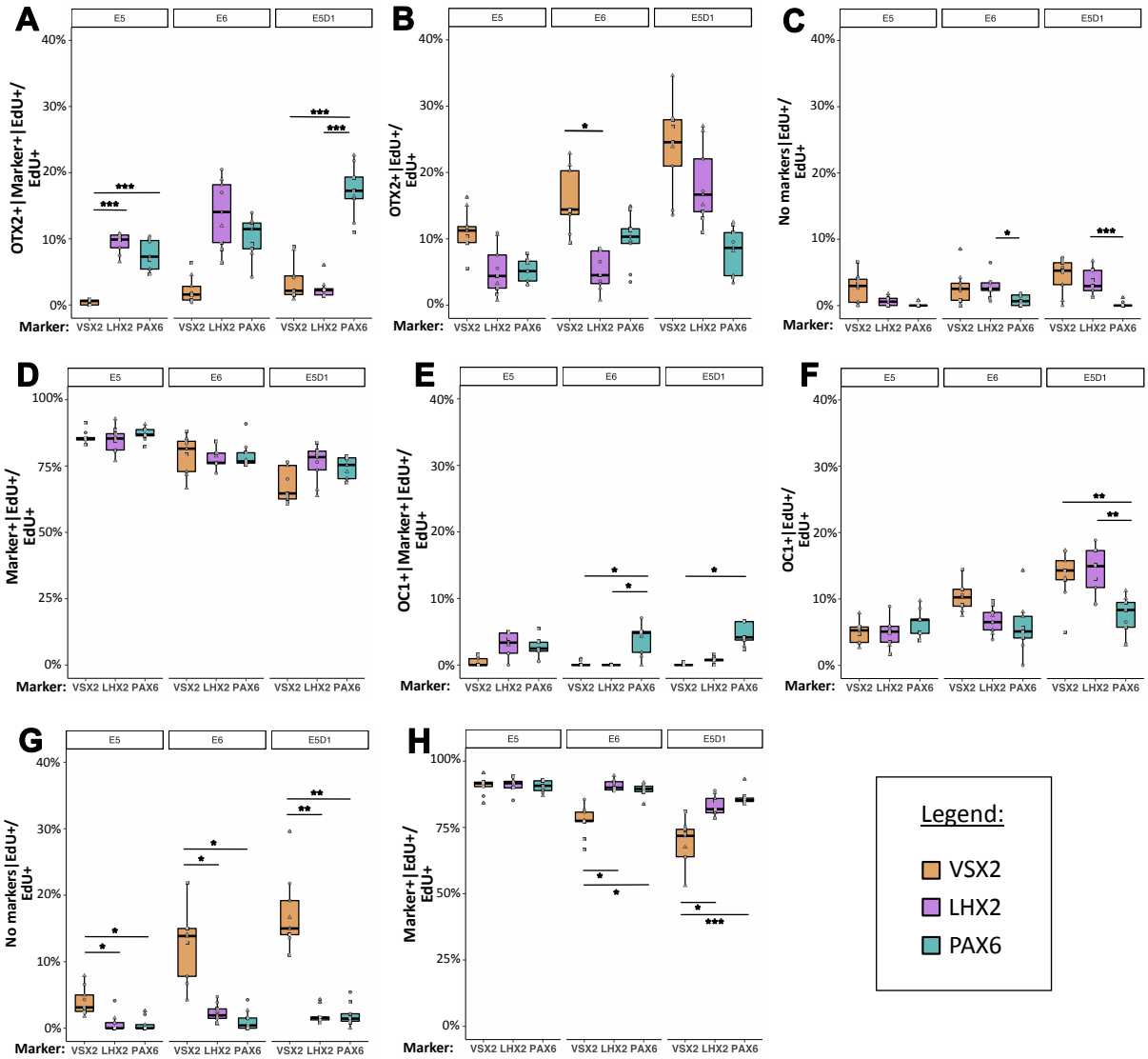
(H) Transcript expression of LHX2 in each replicate.

(I-K) ThrbCRM1 cells also include PAX6(-) RPCs located in the scleral side (J, white arrows). Majority of RPCs are PAX6(+) (J, red arrows). Several ThrbCRM1 cells were also positive for PAX6, sometimes strongly in the NBL and are usually located near the vitreal side (K, orange arrows). Many ThrbCRM1 cells in the NBL remained PAX6(-) (K, green arrows)

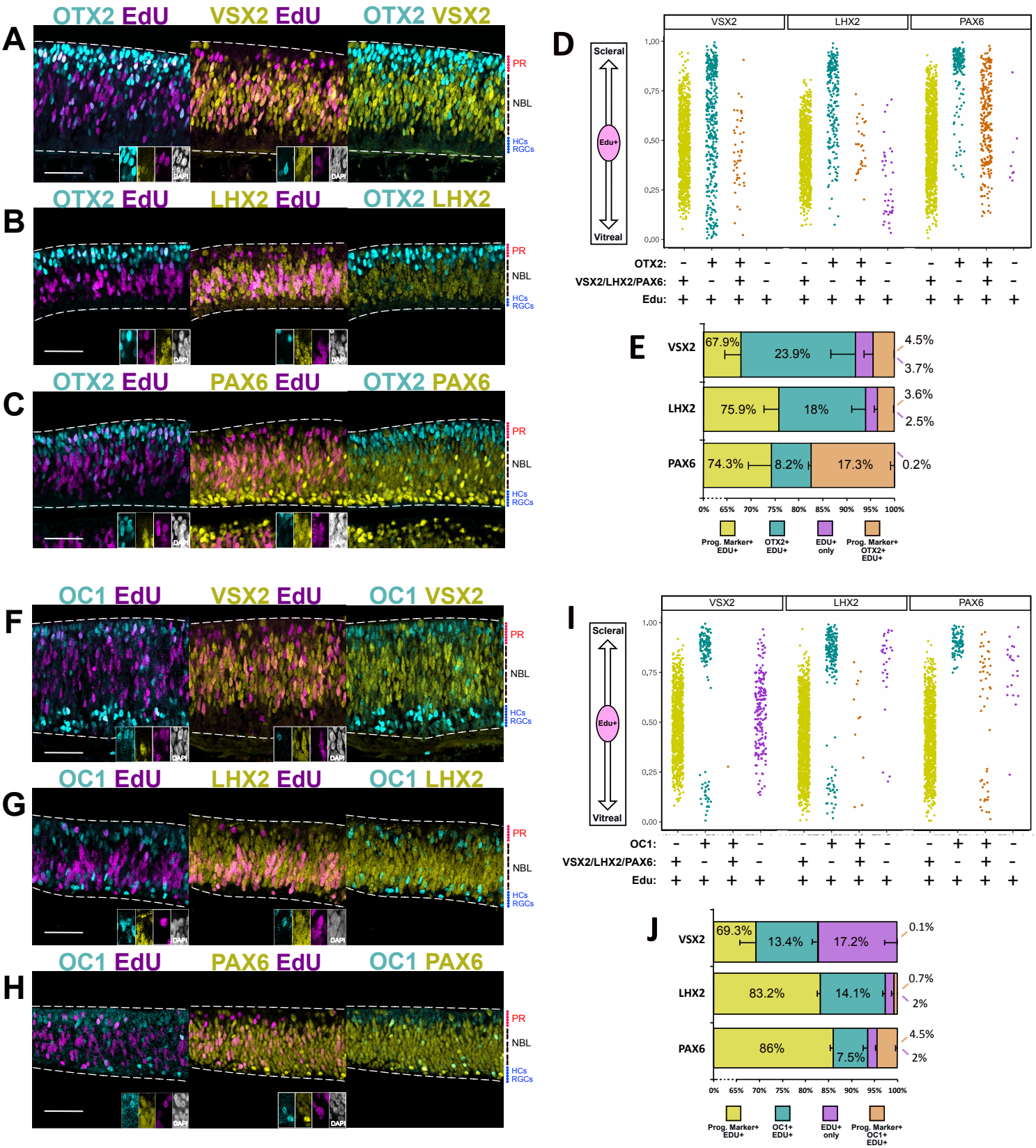
(L) Transcript expression of PAX6 in each replicate.



Supplemental Figure 3 - **One population of OTX2 and OC1 progenitors upregulate Visinin and another upregulates LHX1 and migrate to the vitreal side**
(A) OC1, LHX1, DAPI and EdU on E6 chicken retina. White arrows point to EdU negative cells that are OC1 and LHX1 positive in the NBL. Orange arrow points to an OC1, LHX1, EdU triple positive cell near the vitreal retina.
(B) E6 chicken imaged for PAX6, OC1 and developed for EdU.There are cells positive for OC1, PAX6 and either positive (orange arrow) or negative (white arrow) for EdU.
(C) OTX2, PH3, Visinin, and DAPI in the E6 chick retina Single Z-plane images of each channel as denoted. Arrow points to an OTX2, PH3, and Visinin triple positive cell.
(D) Maximum intensity projection of Z-stack of an E6 chick retina imaged for OTX2, OC1, Visinin, and DAPI. Small panels are single Z-planes of the same Z-stack. Arrows point to OTX2, OC1, and Visinin triple positive cells. Scale bar represents 50um for (A), (B) and (D), 5um for (C).



Supplemental Figure 4 - **Differences in proportion of OTX2 and OC1 progenitors that are positive or negative for progenitor markers.**
(A-D) Percentage of EdU+ progenitors (A) expressing OTX2 and progenitor markers in all EdU(+) cells, (B) OTX2(+) but not expressing progenitor markers, (C) no OTX2 or progenitor markers or (D) only progenitor markers.
(E-H) Percentage of EdU(+) progenitor cells that (E) express OC1 and progenitor markers, (F) express OC1 and do not express progenitor markers, (G) no OC1 or progenitor markers or (H) only progenitor markers. Shapes (triangle, circle and square) denote data points of 3 technical replicates per each biological replicate. Statistical significance was determined in n=3 biological replicates by ANOVA if normally distributed and Kruskal-Wallis with Dunn's posthoc test if not normally distributed. (*: $p < 0.05$, **: $p<0.01$, ***: $p < 0.001$).



Supplemental Figure 5 – **Analysis of the distribution of OC1 and OTX2 relative to progenitor genes after 24 hours of culture.** E5 chicken retinas were cultured for 24hrs, exposed to EdU for 1hr. Single z-plane images of each channel as denoted.

(A-C) Retinal sections imaged for OTX2, EdU, and (A) VSX2, (B) LHX2, (C) PAX6. Edu+ cells positive for OTX2 or respective progenitor markers are enlarged in insets, shown in all channels imaged

(D) Scatter plot of the location in the apical-basal axis of the retina of EdU(+) cells positive for OTX2. Progenitor markers used denoted on top and immunofluorescence signal on the bottom.

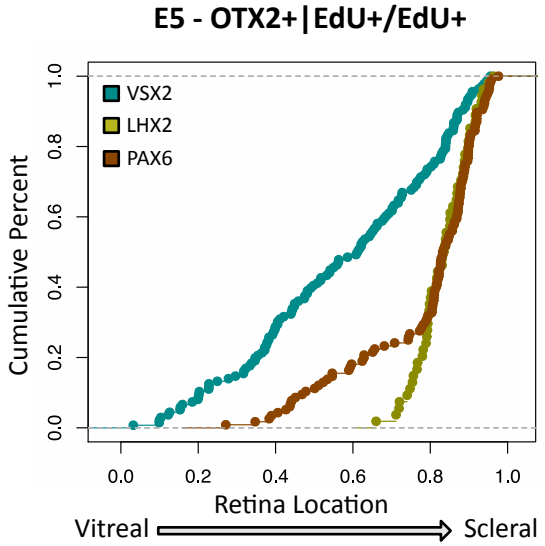
(E) Stacked bar chart of percentages of EdU(+) cells labeled by combinations OTX2 and progenitor markers.

(F-H) Retinal sections imaged for OC1, EdU, and (D) VSX2, (E) LHX2, (F) PAX6. Edu+ cells positive for OC1 or respective progenitor markers are enlarged in insets, shown in all channels imaged

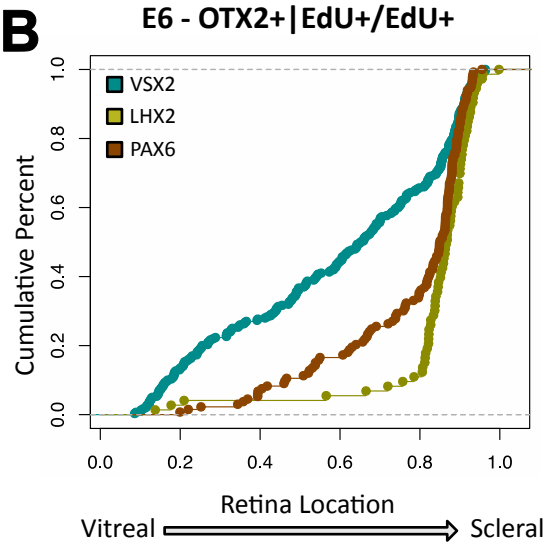
(I) Scatter plot of the location in the apical-basal axis of the retina of EdU(+) cells positive for OC1. Progenitor markers used denoted on top and immunofluorescence signal on the bottom.

(J) Stacked bar chart of percentages of EdU(+) cells labeled by combinations OC1 and progenitor markers. Error bars denote SEM. Scale bar represents 50um for (A-C) and (F-H). PR: Presumptive photoreceptor layer; NBL: Neuroblast layer; HCs: Horizontal Cells; RGCs: Retinal Ganglion Cells.

A



B



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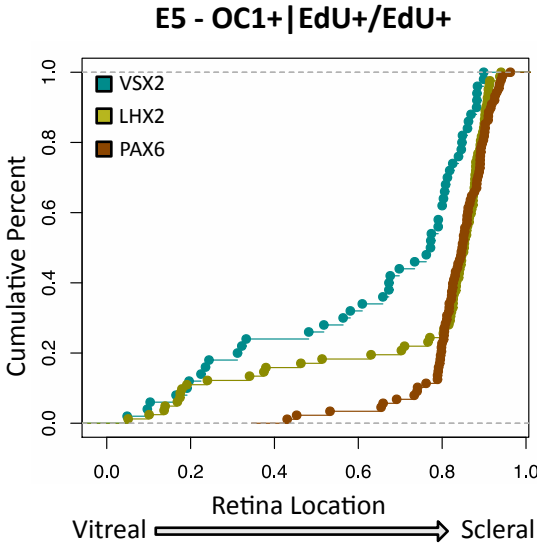

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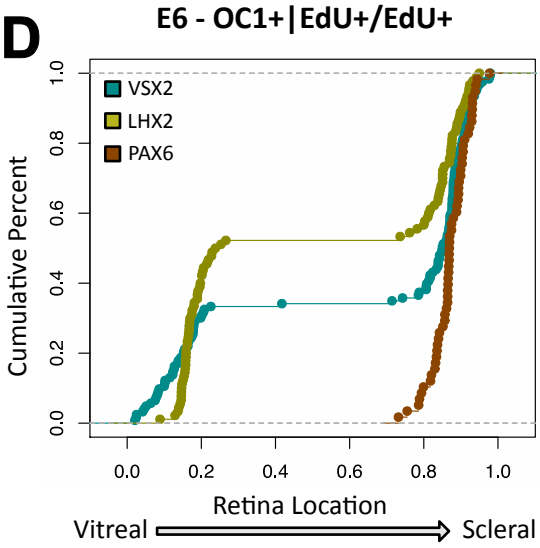


E

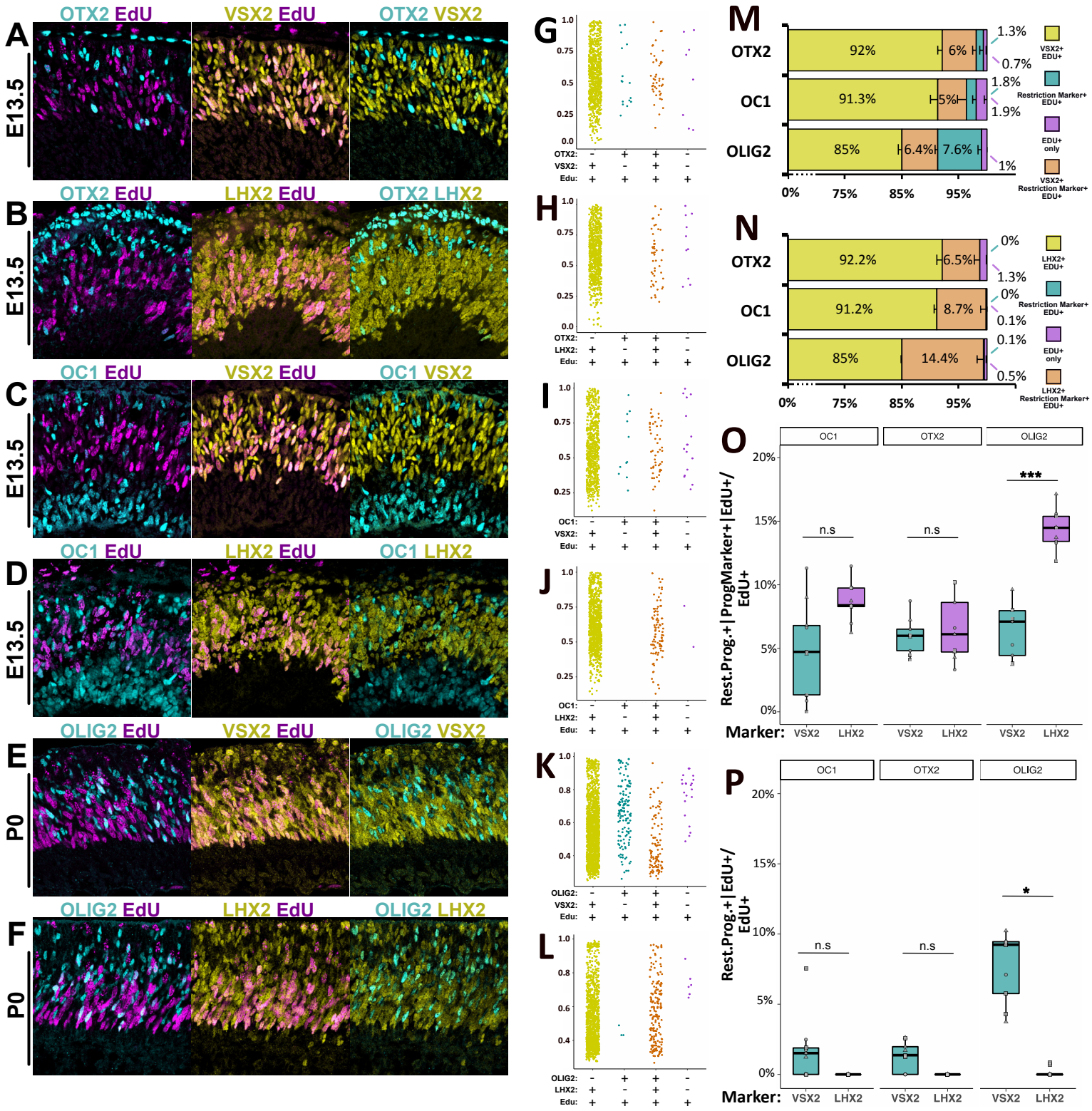
OTX2-only progenitors			
E5	VSX2	LHX2	PAX6
VSX2			
LHX2	2.887E-14		
PAX6	6.897E-11	0.04934	

OTX2-only progenitors			
E6	VSX2	LHX2	PAX6
VSX2			
LHX2	9.992E-16		
PAX6	5.686E-09	0.005194	

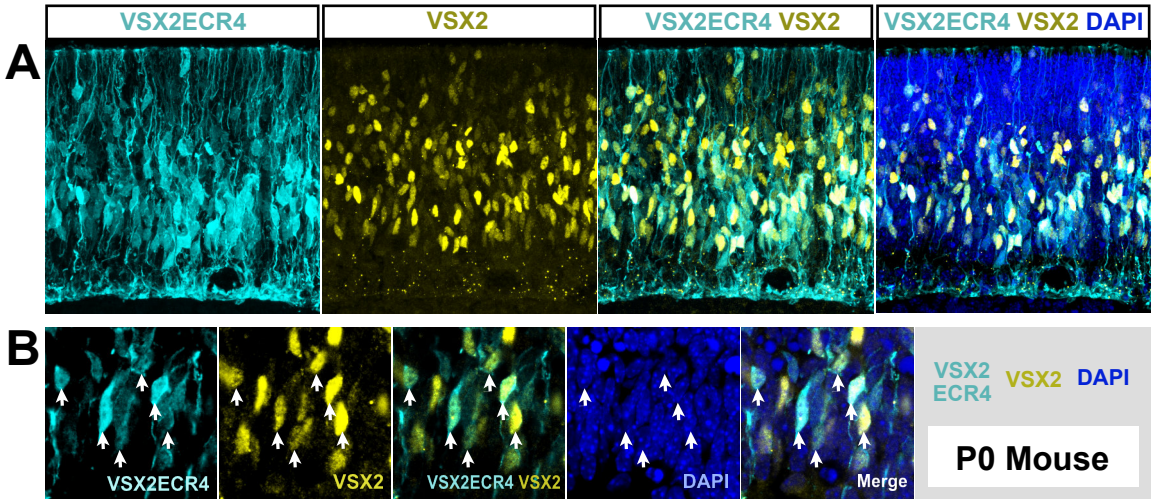
OC1-only progenitors			
E5	VSX2	LHX2	PAX6
VSX2			
LHX2	0.00001229		
PAX6	0.00001337	0.1921	

OC1-only progenitors			
E6	VSX2	LHX2	PAX6
VSX2			
LHX2	0.0124		
PAX6	0.00000238	8.836E-09	

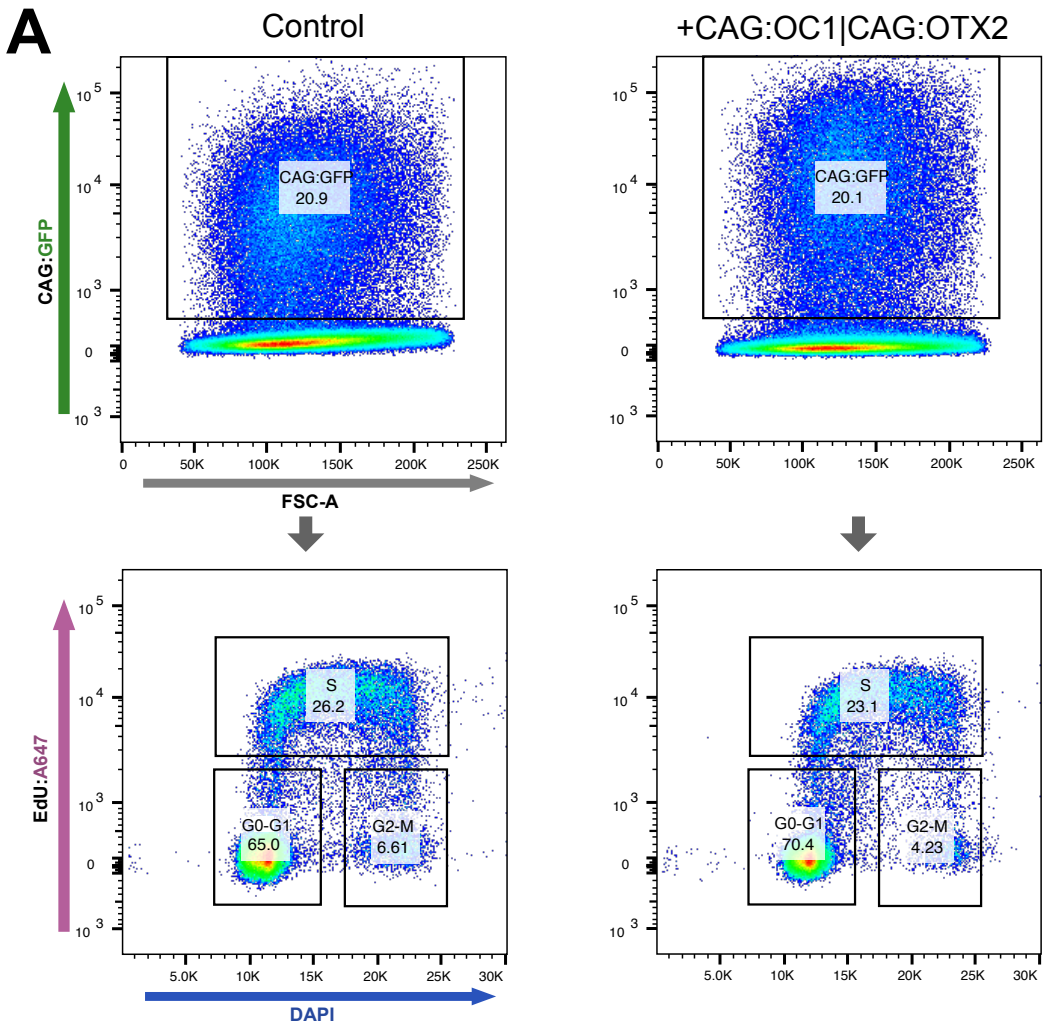
Supplemental Figure 6. **Differences in the spatial distribution of OTX2 and OC1 progenitors**
(A-B) Cumulative distribution graphs of OTX2(+)|EdU(+) cells in the chick retina at (A) E5 and (B) E6.
(C-D) Cumulative distribution graphs of OC1(+)|EdU(+) cells in the chicken retina at (C) E5 and (D) E6. N=3.
(E) Tables of p-values from Kolmogorov-Smirnov tests in each timepoint.

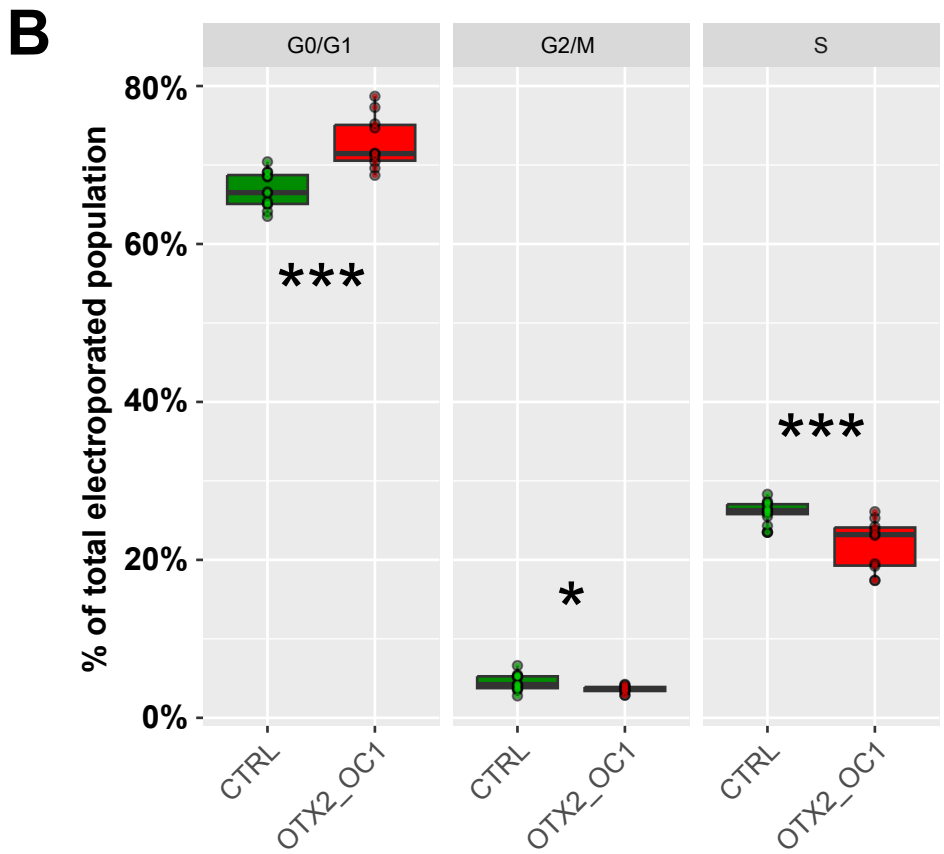


Supplemental Figure 7 - **Restricted progenitors in the mouse are not spatially segregated but still regulate VSX2 and LHX2 differently at P0**. Retinas were harvested at E13.5 or P0 and exposed to EdU for 2 hours.
(A-D) EdU-pulsed E13.5 mouse retinal sections imaged for (A) OTX2, VSX2 and EdU (B) OTX2, LHX2 and EdU, (C) OC1, VSX2 and EdU, (D) OC1, LHX2 and EdU.
(E-F) EdU-pulsed P0 mouse retina imaged for (E) OLIG2, VSX2 and EdU or (F) OLIG2, LHX2 and EdU.
(G-L) Scatterplot of EdU(+) cells location in the apical-basal axis of the retina imaged the same as the panels to the left of the scatterplot
(M-N) Stacked bar chart of percentages of EdU(+) cells labeled by combinations of (M) OTX2 or (N) OC1 and respective progenitor markers. Error bars denote SEM.
(O-P) Quantitation of OC1, OTX2, or OLIG2 expressing progenitors in all EdU(+) cells (O) that also express VSX2 or LHX2 and (P) that do not express VSX2 or LHX2. Statistical significance was determined by two-tailed t-test if normally distributed and using Mann-Whitney tests if not normally distributed (*: $p < 0.05$, ***: $p < 0.001$), n=3.

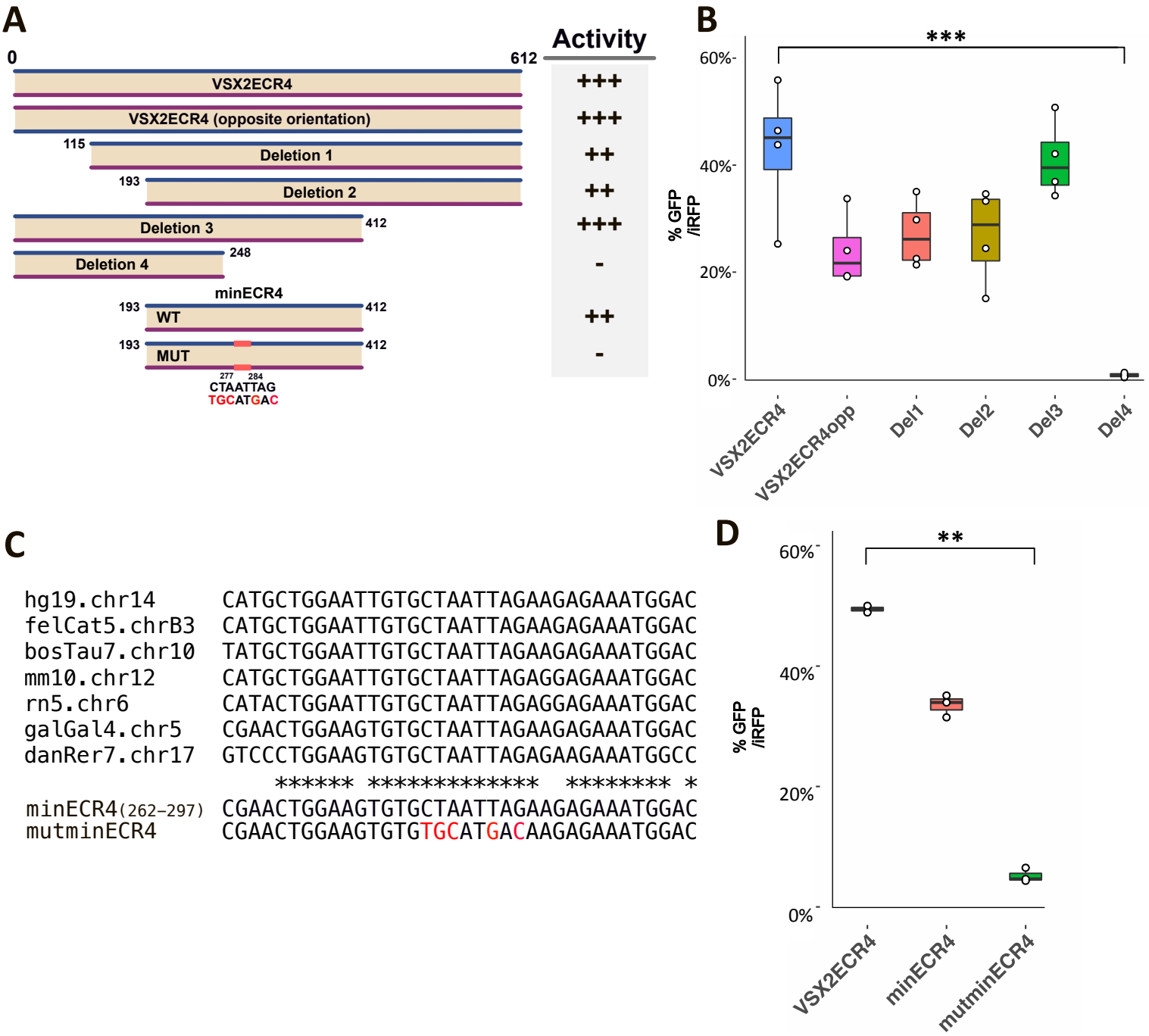


Supplemental Figure 8 - **VSX2ECR4 is active at P0 in the mouse retina**
(A) Maximum intensity projection of VSX2 stained Z-stack of a chicken retina electroporated at E5 with VSX2ECR4-GFP, cultured for 24hr and exposed to EdU for 1hr.
(B) Single Z-plane of the same confocal Z-stack. White arrows point to cells that are VSX2ECR4(+) and VSX2(+).





Supplemental Figure 9. **Cell cycle analysis of OTX2 and OC1 misexpression.**
(A) Representative collection and cell cycle analysis plots for GFP+ population, with and without CAG:OTX2 and CAG:OC1.
(B) Quantification of cell cycle analysis from E5 retinas electroporated and cultured approx. 20h. Constructs used were CAG:GFP, with and without CAG:OTX2 and CAG:OC1. Statistical significance determined by Mann-Whitney test, n>5, (*: $p < 0.05$, **: $p<0.01$, ***: $p < 0.001$).



Supplemental Figure 10 - **VSX2ECR4 activity is dependent on a highly conserved binding site.**
(A) Graphical summary of activity levels of VSX2ECR4 and subsequent deletions.
(B) Quantification of deletion constructs by FACS in E5 retinas electroporated and cultured for 24h. Constructs used were co-electroporated with CAG-iRFP. GFP Reporters used for Y-axis calculations are shown along the X-axis.
(C) Representative multi-species alignment of 36 bp portion of VSX2ECR4 that includes a highly conserved binding site. Asterisks denote conserved nucleotides across species. Red nucleotides indicate mutated bases in mutminECR4.
(D) Quantification of minimal and mutated constructs by FACS in E5 retinas electroporated and cultured for 24h. Constructs used were co-electroporated with CAG-iRFP. GFP Reporters used for Y-axis calculations are shown along the X-axis.
Statistical significance was determined in n=3 biological replicates by ANOVA if normally distributed and Kruskal-Wallis with Dunn's posthoc test if not normally distributed. (*: $p < 0.05$, **: $p<0.01$, ***: $p < 0.001$), n=4 for (B) and n=3 for (D), both experiments replicated additional 2 times.

Species	Restricted Progenitor Marker	Pan-Progenitor Marker	Timepoint	Pan-prog.+|EdU+/EdU+ Mean(±SEM)	Rest.Prog.+|EdU+/EdU+ Mean(±SEM)	No markers|EdU+/EdU+ Mean(±SEM)	Rest.Prog.+|Pan-prog.+|EdU+/EdU+ Mean(±SEM)
Chicken	OTX2	VSX2	E5	85.71(±0.65)%	11.20(±1.33)%	2.69(±0.47)%	0.40(±0.21)%
			E6	79.27(±5.28)%	15.68(±3.47)%	2.79(±1.01)%	2.25(±0.80)%
			E5D1	67.89(±3.35)%	23.89(±5.16)%	3.71(±1.91)%	4.51(±0.10)%
		LHX2	E5	84.77(±1.26)%	5.25(±1.50)%	0.59(±0.14)%	9.39(±0.30)%
			E6	78.16(±2.39)%	5.16(±0.97)%	2.79(±0.44)%	13.88(±2.91)%
			E5D1	75.86(±3.20)%	18.06(±2.93)%	2.51(±0.66)%	3.57(±0.26)%
		PAX6	E5	86.92(±0.97)%	5.29(±0.94)%	0.17(±0.17)%	7.62(±1.20)%
			E6	79.00(±2.16)%	9.96(±2.09)%	0.81(±0.29)%	10.24(±0.36)%
			E5D1	74.26(±0.71)%	8.21(±0.56)%	0.19(±0.11)%	17.34(±0.78)%
	OC1	VSX2	E5	90.29(±0.47)%	4.93(±0.22)%	4.42(±0.69)%	0.35(±0.22)%
			E6	77.07(±3.22)%	10.06(±1.04)%	12.68(±3.14)%	0.19(±0.10)%
			E5D1	69.26(±3.50)%	13.44(±1.40)%	17.21(±2.18)%	0.09(±0.09)%
		LHX2	E5	91.11(±0.44)%	4.9(±0.97)%	0.81(±0.47)%	3.18(±1.04)%
			E6	90.90(±0.91)%	6.81(±1.06)%	2.29(±0.61)%	0.00(±0.00)%
			E5D1	83.22(±0.66)%	14.08(±0.56)%	1.96(±0.60)%	0.74(±0.06)%
		PAX6	E5	90.54(±0.74)%	6.23(±1.06)%	0.58(±0.31)%	2.64(±0.60)%
			E6	89.17(±0.55)%	5.85(±2.13)%	1.15(±0.38)%	3.83(±1.38)%
			E5D1	86.04(±0.77)%	7.46(±0.53)%	1.97(±0.58)%	4.53(±0.66)%
Mouse	OTX2	VSX2	E13.5	92.03(±0.77)%	1.28(±0.65)%	0.71(±0.23)%	5.97(±0.56)%
		LHX2	E13.5	92.15(±0.99)%	0.00(±0.00)%	1.31(±0.03)%	6.53(±0.99)%
	OC1	VSX2	E13.5	91.26(±1.19)%	1.82(±0.67)%	1.90(±0.48)%	5.02(±1.51)%
		LHX2	E13.5	91.22(±0.51)%	0.00(±0.00)%	0.13(±0.07)%	8.65(±0.45)%
	OLIG2	VSX2	P0	84.96(±0.63)%	7.63(±0.64)%	1.04(±0.09)%	6.36(±0.43)%
		LHX2	P0	84.99(±0.06)%	0.17(±0.17)%	0.46(±0.25)%	14.38(±0.38)%

Supplementary Table 1 **- Results of restricted and multipotent RPC quantification in chick and mouse.**

Antibody name	Host Organism	Vendor	Catalog number	Dilution	RRID*
PH3	Rabbit	EMD Millipore	06-570	1 to 500	AB_310177
OLIG2	Rabbit	Millipore	AB9610	1 to 500	AB_570666
OTX2	Rabbit	EMD Millipore	AB9566	1 to 500	AB_2157186
OTX2	Goat	Novus biologicals	AF1979	1 to 500	AB_2157172
OC1	Rabbit	Santa Cruz	SC-13050	1 to 500	AB_2251852
VSX2	Sheep	Ex alpha	X1180P	1 to 200 (chick) 1 to 500 (mouse)	AB_2314191
LHX2	Goat	Santa Cruz	sc-19344	1 to 50 (chick) 1 to 500 (mouse)	AB_2618817
PAX6	Mouse IgG1	DSHB	Pax6-s	1 to 10	AB_2315070
LIM1+2	Mouse IgG1	DSHB	4F2-C	1 to 10	AB_2314743
VISININ	Mouse IgG1	DSHB	7G4-s	1 to 250	AB_528510

RRID: Repository Resource ID, http://antibodyregistry.org/

Supplementary Table 2 - **Antibody list**

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
CERS	AGATGTCTGTACTCCTCTGG	TCACATTTTGCTTTCAGTTTACC
CYP1B1	ACTTCACAAGTCGTAATTCAGT	AGCTAAAGGCAAGGTTTTACAT
FGF19	GCTAATGGGAATTCAGCATGTG	GCGAGCCTTTGGAAATGAGTG
GJA1	ACGTGATGAGGAAAGAAGAGA	GAGACATGGGAGACAAGGG
GNGT2	GCATCTGAAGTAGCAGCAGA	TGGTTGGAGTTACGAACGAG
LHX1	CTGGGTCTAAGTAAGTGATGAT	TGCTCAGCAGGCTGCAATG
NEUROD1	AGCGGGGCCACCAGCAAT	GTGCTAAGGCAACAGAACTTC
NR2E1	ACCCCAGTAGATATGATTCTG	TGTATAAAGCTCTTCTGTCATG
RBP4	GACTGAAGCAGAGTAGTCTC	CCACTACTGTACTGCACG
TFAP2A	CCCTCGCAGCCCATCAGT	AAGTTCACAAACTCAAGACAGAAC
TFAP2C	GCAGATCTGTAAGGAATTCACA	GTACCCAAATTGCTACGTTCC
THRB	TTGTGATGCTCAGGTCCTGC	TAATCCTCAAACACCTCCAGG

VSX2ECRs	Forward primer (5'-3')	Reverse primer (5'-3')
cVSX2ECR4	GCGCTGACTGCCGCTCG	CCTTCTGGATGGCTGATGG
cVSX2ECR2	CGCTAATGCTGCTAATCCG	TGGCGTTTCCTCAGAGCC
cVSX2ECR5	GTGCTGTTCTTAATGCATTGC	CCACAGTACGTAATTTGATCC
cVSX2ECR7	AGGATGTTATTTCCTGGCCG	CCTGGCACCATCAACAGC
cVSX2ECR8	GGGAGCAATCAGACTGGGC	GACAGAATGGTAGTGAAAACC
cVSX2ECR10	TGTCACTGTTATGTGTTTCCC	GGTCTAAGACTGAATGCAAGG

Supplementary Table 3 - **Primers used for the generation of mRNA probes for ISH and amplification of VSX2 ECRs**