

Supplementary Materials

Involvement of a Basic Helix-Loop-Helix Gene *BHLHE40* in Specification of Chicken Retinal Pigment Epithelium

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Figure S1

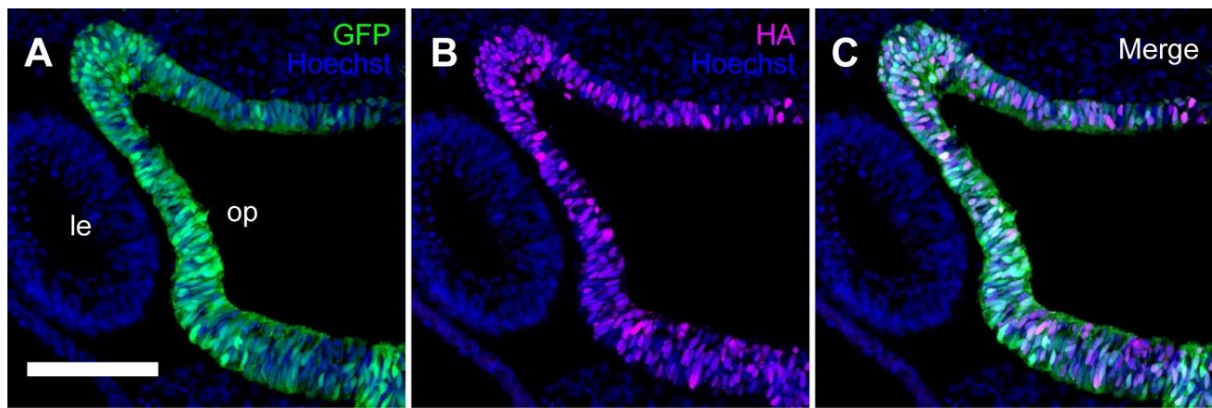


Figure S1 (data related to Figures 2 and 3): In the optic cup after *BHLHE40-HA* overexpression (from embryo #17, same as shown in Figure 3I-N), co-electroporated GFP fluorescence (**A**) is overlapped with BHLHE40-HA protein localization (**B**). Merged image of (**A**) and (**B**) is shown in (**C**). Cell nuclei were stained with Hoechst 33342. Shown images are maximum intensity projections of confocal z-series. le, lens vesicle; op, optic cup. Scale bar: 100 μ m in (**A**) for (**A-C**).

Figure S2

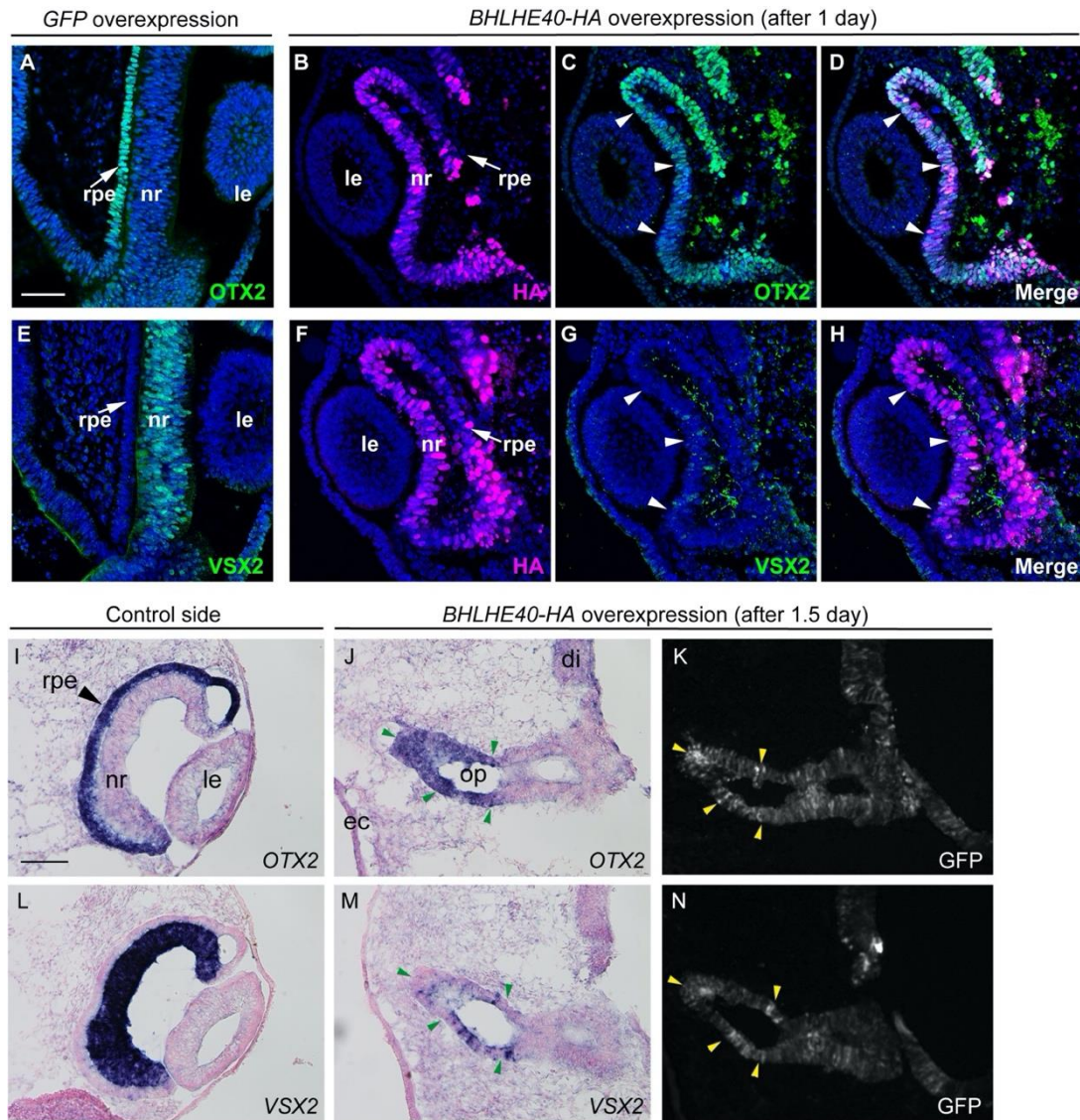


Figure S2 (data related to Figure 3): Similar data of Figure 3 from other embryos, except for those after GFP expression (A, E), which are identical images as shown in Figure 3A and E. Frontal sections of the optic cup from three embryos (*GFP*#2, *BHLHE40*#11, *BHLHE40*#22) are shown. Dorsal is to the up. (A-H) Optic cups at 1 day post electroporation (for *GFP* alone and HA-tagged *BHLHE40* overexpression). In embryo #11 after *BHLHE40* overexpression, OTX2 protein is localized to the inner layer of the OC (arrowheads in C, D), where VSX2 protein is not detected (arrowheads in G, H). Cell nuclei were stained with Hoechst 33342. Shown images are maximum intensity projections of confocal z-series. (I-N) Optic cups at one and half day post electroporation (HA-tagged *BHLHE40* overexpression). (I, L) Optic cups on the control side are shown. In (K, N), co-electroporated *GFP*, showing the domain of *BHLHE40* overexpression. (J, M) In embryo #22, optic cup development is disrupted and there is no lens formation after *BHLHE40* overexpression. OTX2 is expressed in the distal portion of the optic cup (J), where *BHLHE40* is ectopically expressed (arrowheads in J, K). In the same domain, VSX2 expression is downregulated but remains in some cells (arrowheads in M, N). Cell nuclei were stained with nuclear fast red. di, diencephalon; ec, surface ectoderm; le, lens vesicle; nr, neural retina; op, optic cup or deformed optic vesicle in (J-N); rpe, developing RPE. Scale bar: 50 μ m in (A) for (A-H), 100 μ m in (I) for (I-N).

Figure S3

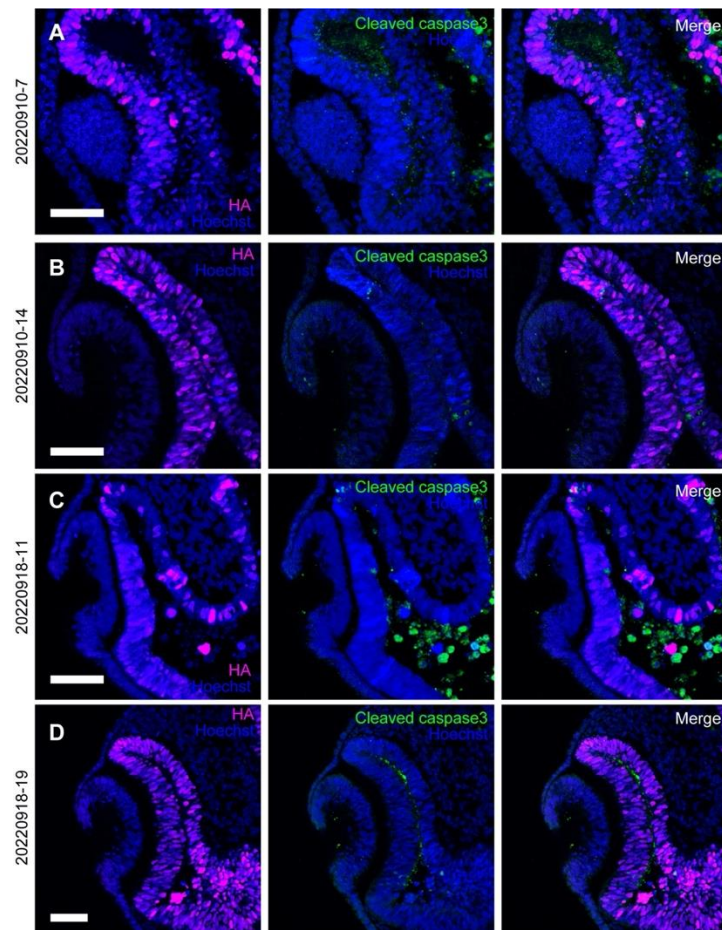


Figure S3: *BHLHE40*-HA expressing cells do not show cell death as revealed by immunofluorescence using anti-cleaved caspase 3. In four embryos examined (A-D) (embryo identification numbers are shown on the leftmost), none of the HA-immunoreactive cells are positive for cleaved caspase 3. Cell nuclei were stained with Hoechst 33342. Shown images are maximum intensity projections of confocal z-series. Scale bar: 50 μ m in (A-D) for all images.

Figure S4

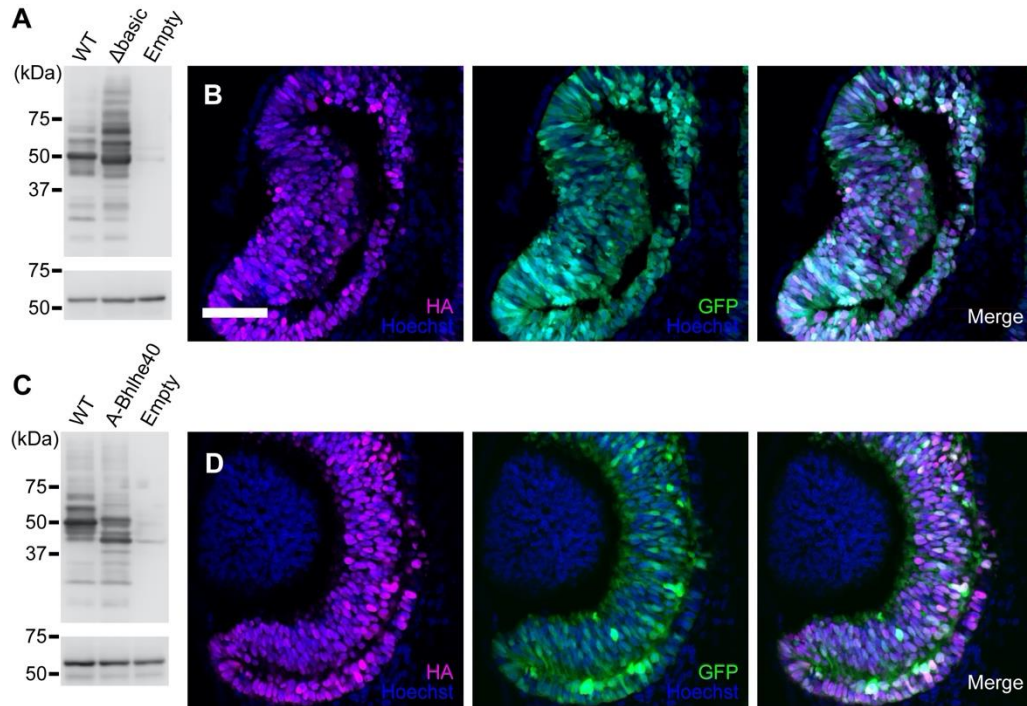


Figure S4: Validation of expression of HA-tagged presumed dominant negative forms of BHLHE40. (A, C) Western blot analysis of HEK293T cells transfected with chicken Δ basic *BHLHE40*-HA/pCAGGS (A) or Δ basic with acidic extension *BHLHE40*-HA (C) vectors. Alpha tubulin from the same samples was detected for loading reference. (B, D) Confocal microscopic images show that HA-tagged presumed dominant negative forms of BHLHE40 are detected all over the optic cup by immunofluorescence using the anti-HA antibody. Secondary antibody to anti-HA was Alexa Fluor 568-conjugated anti-rat IgG. Co-electroporated GFP and merged images are shown in the middle and right panels, respectively. Cell nuclei were stained with Hoechst 33342. Shown confocal images are maximum intensity projections of z-series. Scale bar: 50 μ m in (B) for all confocal images.

Figure S5

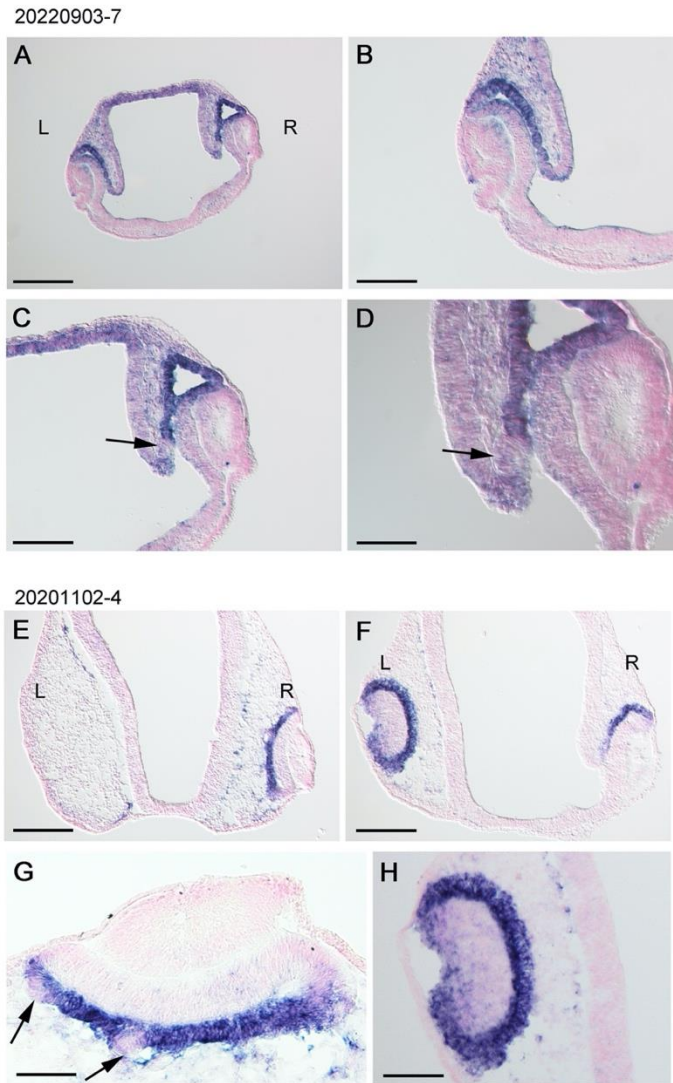


Figure S5 (data related to Figure 4): In situ hybridization (ISH) on the frontal section of the chicken embryonic head after 1 day post electroporation (embryo #7 for **A-D** and #4 for **E-H**). Enlarged view of the optic cup (OC) in (**A**) on the left (L) control side is shown in (**B**), and that of the right (R) *Lhx1*-overexpressed one is shown in (**C, D**). *BHLHE40* expression is downregulated in a portion of the outer layer of the OC (arrows in **C, D**). In embryo #4, the head sections were cut oblique and the left control OC is not seen in (**A**). Enlarged view of the right OC is shown in (**G**) and that of the left control OC is shown in (**H**). *BHLHE40* expression is downregulated in two portions of the outer layer of the OC (arrows in **G**). Cell nuclei were stained with nuclear fast red. Scale bars: 200 μm (**A, E, F**), 100 μm (**B, C, H**), 70 μm (**G**), 50 μm (**D**).

Figure S6

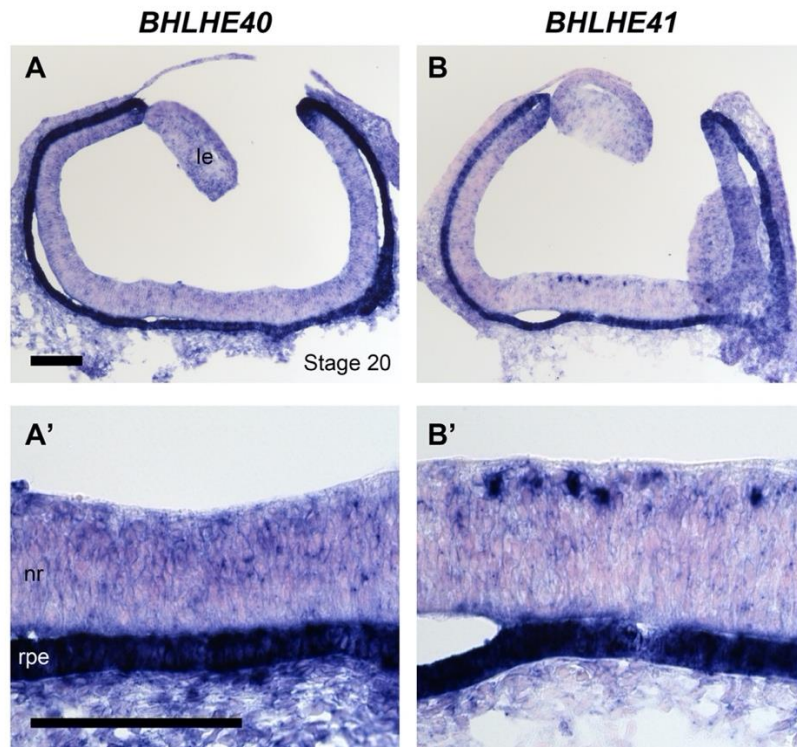


Figure S6: Expression patterns of *BHLHE40* (A,A') and *BHLHE41* (B,B') in the developing chicken eye at stage 20. *BHLHE41* is expressed by developing retinal ganglion cells at the central retina in addition to the developing RPE and ciliary marginal zone. le, lens vesicle; nr, neural retina; rpe, developing RPE. Scale bars: 100 μ m.

Figure S7



Figure S7: Alignment of coding DNA sequences for chicken BHLHE40 and BHLHE41. The nucleotide identity is 63%. Identical nucleotides are highlighted in yellow.

Figure S8

		10	20	30	40	50	60	70	80	90	100
CHICK BHLHE40	M-ERIPSAQP	PPVCLGKLPA	LESADVPGLD	FAHMYQVYKP	RRGLKRSSEDS	KD TYKLPHRL	IEKKRRDRIN	ECIAQLKDLL	PEHLKLTTLG	H--LEKAVVI	
HUMAN BHLHE40	M-ERIPSAQP	PPACLCPKAPG	LEHGDLPGMY	PAHMYQVYKS	RRGFKRSSEDS	KET TYKLPHRL	IEKKRRDRIN	ECIAQLKDLL	PEHLKLTTLG	H--LEKAVVI	
MOUSE BHLHE40	M-ERIPSAQP	PPTCLCPKAPG	LEHGDLSGMD	FAHMYQVYKS	RRGFKRSSEDS	KET TYKLPHRL	IEKKRRDRIN	ECIAQLKDLL	PEHLKLTTLG	H--LEKAVVI	
DANRE BHLHE40	M-ERITSAQP	PP-CMSKHPS	LDISDMQMGD	FP-MY-VYKP	RRGMKRSSEDS	KD TYKLPHRL	IEKKRRDRIN	ECIAQLKDLL	PEHLKLTTLG	H--LEKAVVI	
CHICK BHLHE41	MDEGISRLPE	RQ-----L	LEHRDFLGL	YPALY-MCKP	KRGVKR-DES	KET TYKLPHRL	IEKKRRDRIN	ECIAQLKDLL	PEHLKLTTLG	H--LEKAVVI	
HUMAN BHLHE41	MDEGIPHLQE	RQ-----L	LEHRDFIGLD	YSSLY-MCKP	KRSMKR-DDT	KD TYKLPHRL	IEKKRRDRIN	ECIAQLKDLL	PEHLKLTTLG	H--LEKAVVI	
MOUSE BHLHE41	MDEGIPHLQE	RQ-----L	LEHRDFIGLD	YSSLY-MCKP	KRSLKR-DDT	KD TYKLPHRL	IEKKRRDRIN	ECIAQLKDLL	PEHLKLTTLG	H--LEKAVVI	
DANRE BHLHE41	MEERISRMQN	RQ-----F	LDHADFLGVE	YSSLY-MCKS	KRGMKR-EEG	KD AYKLPHRL	IEKKRRDRIN	ECIGQLKDLL	PEHLKLTTLG	H--LEKAVVI	
basic helix-loop-helix (bHLH) domain											
		110	120	130	140	150	160	170	180	190	200
CHICK BHLHE40	ELTLKHVKAL	TALIEQQQQK	ILALQSGLQA	GDLARSRLDS	SQEM FRSGGQ	LCAKELLQYV	AKHD--N-KE	LKAAQLVGH	HRVAEELLPG	G-----	
HUMAN BHLHE40	ELTLKHVKAL	TNLIDQQQQK	IIALQSGLQA	GELSGRNVET	GQEM FCSGGQ	TCAREVLQYL	AKHE--NTRD	LKSSQLVTHL	HRVVSSELLQG	G-----T	
MOUSE BHLHE40	ELTLKHVKAL	TNLIDQQQQK	IIALQSGLQA	GDLSGRNLEA	GQEM FCSGGQ	TCAREVLQYL	AKHE--NTRD	LKSSQLVTHL	HRVVSSELLQG	G-----A	
DANRE BHLHE40	ELTLKHVKAL	NNLLEQQQQK	IISLQNLQI	GEQGNPSEN	SEEM FRSGFH	LCAKEVLQFL	ANQE--TMRD	LTTAHIEHL	QKVASELIQS	P-----P	
CHICK BHLHE41	ELTLKHLKAL	TALTEQQHQK	IIVALQSGERS	VK---SPVQA	DLDA FHSGGQ	TCAKEVLQFL	SRSESWTPRE	QRCACLGLH	HAVSSQFLPG	PRLLSPPPPG	
HUMAN BHLHE41	ELTLKHLKAL	TALTEQQHQK	IIVALQNGERS	LK---SPIQS	DLDA FHSGGQ	TCAKEVLQYL	SRFESWTPRE	PRCQQLINHL	HAVATQFLPT	PQLLTQQV-P	
MOUSE BHLHE41	ELTLKHLKAL	TALTEQQHQK	IIVALQNGERS	LK---SPVQA	DLDA FHSGGQ	TCAKEVLQYL	ARFESWTPRE	PRCQQLVSHL	HAVATQLL-T	PQV-----P	
DANRE BHLHE41	ELTLKHLNAL	TAVTEQQHQK	IIVALQNGERS	LK---SSLQA	DLDA FHSGGQ	ACAKEVLQYL	NKVENNWTARE	QRCTRILNHL	HKVSAQFQPG	TGILQQ----	
Orange domain											
		210	220	230	240	250	260	270	280	290	300
CHICK BHLHE40	-----PK	DLKEKAVPG-	--GKAGEGRG	QNCVPIQRT	FAASSGQSGG	SDTDTDSGYG	GELEKSDSKP	EQP--YFPKD	TELKYAVQER	ISSIKQE--S	
HUMAN BHLHE40	SRKPSDPAKP	VMDKEKPPS	P-AKGSEGGP	KNCVPIQRT	FAHSSGQSGG	SDTDTDSGYG	GESEKGLDLS	EQP--CFKSD	HGRFFTMGER	IGAIKQE--S	
MOUSE BHLHE40	SRKPLDSAPK	AVDLKEKPSF	L-AKGSEGGP	KNCVPIQRT	FAPSGGEQSG	SDTDTDSGYG	GELEKGLDLS	EQP--YFKSD	HGRFFAVGER	VSTIKQE--S	
DANRE BHLHE40	SPRLDEPAK	AQESREKPSG	LQPKAAEGHA	KNCVPIQRT	YPHSS-EQSG	SDTDTDSGYG	GEYEKRDQKA	QRPDCYVKES	GALKYS----	--SIIKEE--Q	
CHICK BHLHE41	LAKG----PS	SSSSPPAP-L	RAPARKPEGQ	AHCVPVIQRT	H-AAE-PGAE	TDTDTSYG	GEAEARPERG	AGP-----	-----AGPLP	ALPVLQEPAG	
HUMAN BHLHE41	LSKGTG-APS	AAGSAAAPCL	ERAGQKLEPL	AYCVPVIQRT	QPSAE-LAAE	NDTDTDSYG	GEAEARPDRE	KGK-----	-----GAGAS	RVTIKQEPFG	
MOUSE BHLHE41	SGRSGRAPC	SAGAAAA--	-----GPERV	ARCVPVIQRT	QPGTE---PE	HTDTSYG	GEAEQG----	-----	-----	RAAVKQEPFG	
DANRE BHLHE41	-----PV	AGDDAPER--	---DTQRDPQ	ANCVPIQRT	Q---NLELNE	NDTDTDSYG	GEAEKGDGKS	EKG-----	-----CDTAK	GVKIKQE-FG	
		310	320	330	340	350	360	370	380	390	400
CHICK BHLHE40	EDPPAKRSRL	EVPDDD----	-----	-----	-----	-----SPFG	GEVMGTAGGF	LPPHAHQPL	CLPFYLIPPS	AT-AYL-PM-	
HUMAN BHLHE40	EEPTTKNRM	QLSDDE----	-----	-----	-----	-----GHFTS	SDLIS--SPF	LGPHPHQPF	CLPFYLIPPS	AT-AYL-PM-	
MOUSE BHLHE40	EEPTTKSRM	QLSEEE----	-----	-----	-----	-----GHFAG	SDLMG--SPF	LGPHPHQPF	CLPFYLIPPS	AT-AYL-PM-	
DANRE BHLHE40	DEPPSKRPRS	DSSEDE----	-----	-----	-----	-----SLSG	HDVVGHSFY	VSFSPQP-PL	CMPFYLFPPG	AAAAYL-PM-	
CHICK BHLHE41	DEAPPAPKRP	RLERGG----	-----	-----SPPPG	AALPGAARGA	DAALLSSILMA	LGAGGGAAPF	GQP---AAPF	CLPFYFISPS	AAAAYVQPF	
HUMAN BHLHE41	EDSP-APKRM	KLDSRGGGSG	GGPGGAAAA	AAALLGPDPA	AA--AALLRP	DAALLSSSLVA	FGGGG--APF	PQPAAPAAAPF	CLPFCELSPS	AAAAYVQPF	
MOUSE BHLHE41	DSSP-APKRP	KLEARG----	-----	-----	-----ALLGP	EPALLGSLVA	LGGG---APF	AQP---AAPF	CLPFYLLSPS	AA-AVYQFWL	
DANRE BHLHE41	DERVTKAKM	NWSANG----	-----	-----GSDST	ST-----RP	DVALMNSLMG	MTGVGG----	-----QQTFF	CMPFYFINPS	AAAASYM-PLF	
		410	420	430	440	450	460	470	480	490	500
CHICK BHLHE40	----LEKCYW	PAS-----	VPVLYPGLP	-----AP	AAALTSFVGP	DKLSPPLLLP	QRLSPGPA-	-----	---RSPIDSS	ALLQALKQIP	
HUMAN BHLHE40	----LEKCYW	PTS-----	VPVLYPGLN	-----AS	AAALSSFMNP	DKISAPLLMP	QRLSPPLPA-	-----	---HPSVDSS	VLLQALKQIP	
MOUSE BHLHE40	----LEKCYW	PTS-----	VPVLYPGLN	-----TS	AAALSSFMNP	DKIPTPLLLP	QRLSPPLA-	-----	---HSSLDSS	ALLQALKQIP	
DANRE BHLHE40	----LEKCYW	PGA-----	MPVLYPGLG	-----SS	PASLS---P	EKLPSMVMMS	SRVGSVPST-	-----	---PTSMDSP	ALLQALKQVP	
CHICK BHLHE41	DKGGLEKLYL	PAA-----	PIPLLYPGIP	--AQAAAAA	AAAAAAAF	CLSSVLGPA-	EKAAGLSAP	HLP-----	---PFAAAA	AAVPAEPGE	
HUMAN BHLHE41	DKSGLEKLYL	PAAAAA----	PFPLLYPGIP	APAAAAAA	AAAAAAAF	CLSSVLSPPP	EKAGA-AAAT	LLPHEVAPLG	APHQPHPHGR	THLPFAGPGE	
MOUSE BHLHE41	DKSGLDKLYL	PAAAAA----	PFPLLYPGIP	-----AA	AAAAAAAF	CLSSVLSPPP	EKAGATAGAP	FLAHEVAPPG	PLRPQHAHSR	THLPRA----	
DANRE BHLHE41	DKSHLEKLYL	PAAAAAALT	PFPLLYPGIP	-----THAS	AAAAAAAF	NAST-----	DKTSGFDAAS	SKDDE----	---PESPD	LSNEADLAS	
alanine/glycine-rich region											
		510	520								
CHICK BHLHE40	PLNLETKD--	-----	---								
HUMAN BHLHE40	PLNLETKD--	-----	---								
MOUSE BHLHE40	PLNLETKD--	-----	---								
DANRE BHLHE40	PLNLETKD--	-----	---								
CHICK BHLHE41	EAEPAAAE--	---EPGA--	EGP								
HUMAN BHLHE41	PGNPSSAQE	DPSQPGK--	EAP								
MOUSE BHLHE41	-VNPESSQ-E	DATQPAK--	DAP								
DANRE BHLHE41	ASEDHGSEID	TSHQQRNDN	DGT								

Figure S8: Alignment of BHLHE40 and BHLHE41 proteins of chicken, human, mouse and zebrafish. bHLH and orange domains, and alanine and glycine-rich region in the C-terminus are shown. Gene IDs for referenced amino acid sequences are as follows: CHICK BHLHE40 (416108), HUMAN BHLHE40 (8553), MOUSE BHLHE40 (20893), DANRE BHLHE40 (324413), CHICK BHLHE41 (101750689), HUMAN BHLHE41 (79365), MOUSE BHLHE41 (79362), DANRE BHLHE41 (563771).

Figure S9

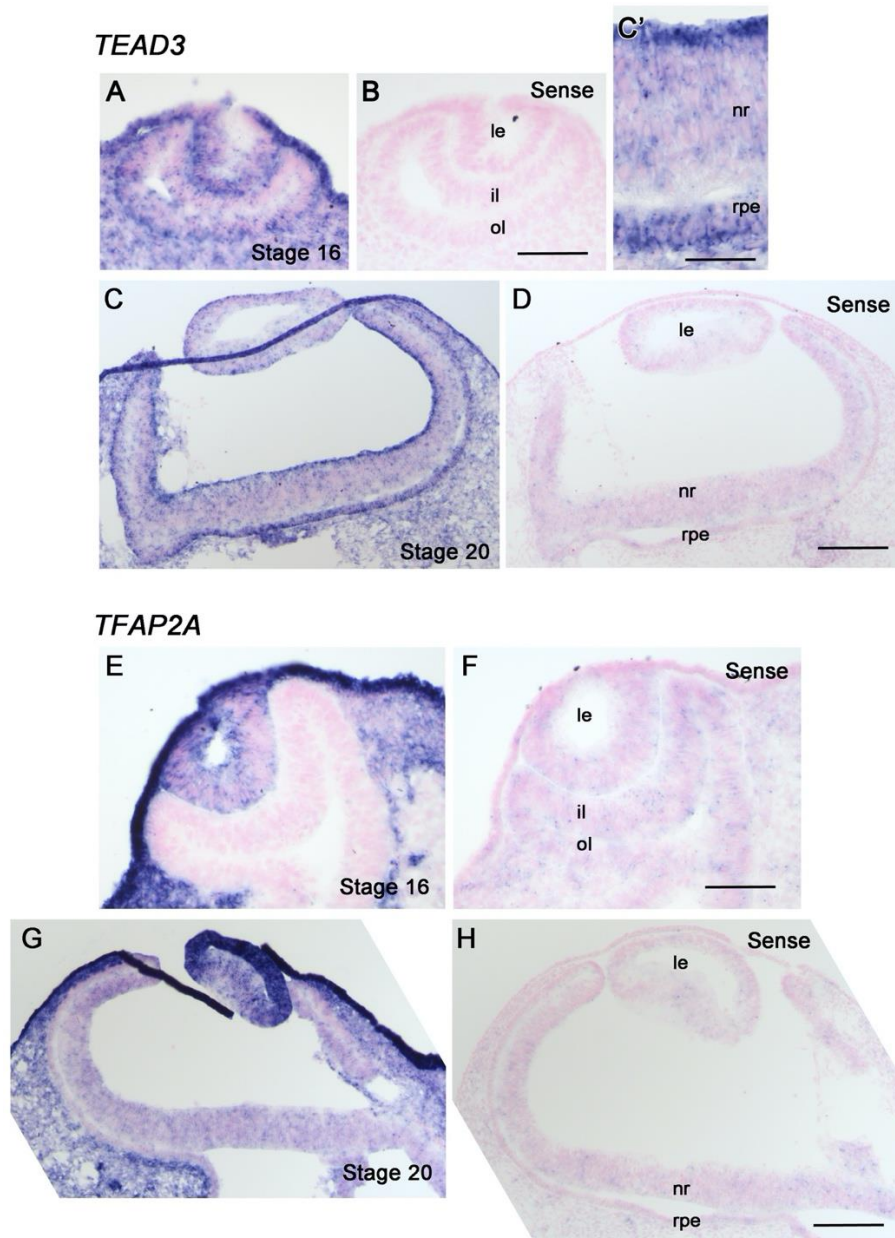


Figure S9: Expression patterns of *TEAD3* and *TFAP2A* in the developing chicken eye. (A-D) *TEAD3* is expressed in the surface ectoderm (SE) and developing eye at stages 16 (A,B) and 20 (C,C',D). (C') Enlarged view of the developing retina is shown. *TEAD3* mRNA signals are observed in the RPE as well as the basal vitread surface of the neural retina. (E-H) *TFAP2A* is expressed in the SE and lens vesicle. Results using sense control probes are shown in (B,D,F,H). il, inner layer of the optic cup; le, lens vesicle; nr, neural retina; ol, outer layer of the optic cup; rpe, developing RPE. Scale bars: 50 μ m (A,B,E,F), 100 μ m (C,D,G,H), 25 μ m (C').

Figure S10

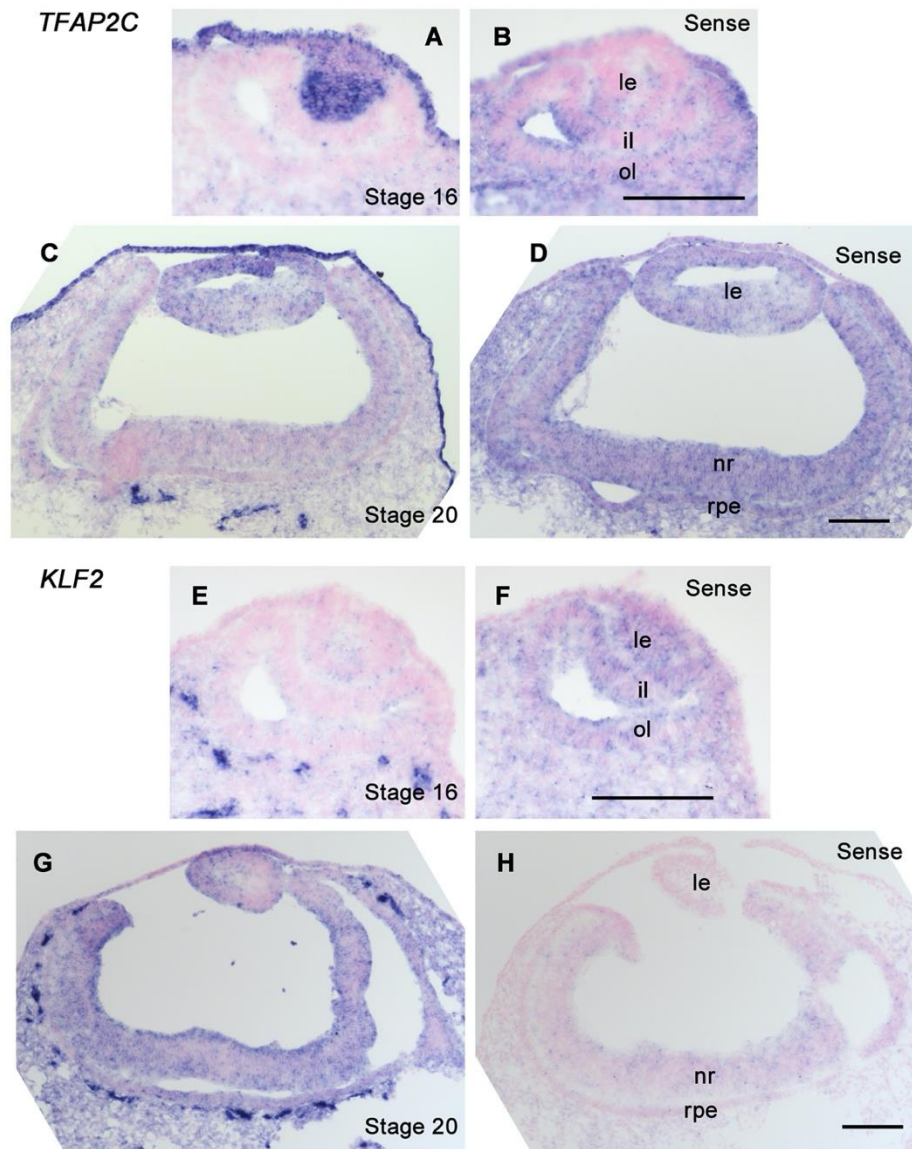


Figure S10: Expression patterns of *TFAP2C* and *KLF2* in the developing chicken eye. (A-D) *TFAP2C* is expressed in the surface ectoderm (SE), lens vesicle, and by a subset of cells in the periocular mesenchyme (C). (E-H) *KLF2* is expressed by a subset of cells in the periocular mesenchyme just beneath the RPE (G). il, inner layer of the optic cup; le, lens vesicle; nr, neural retina; ol, outer layer of the optic cup; rpe, developing RPE. Scale bars: 100 μ m.

Figure S11

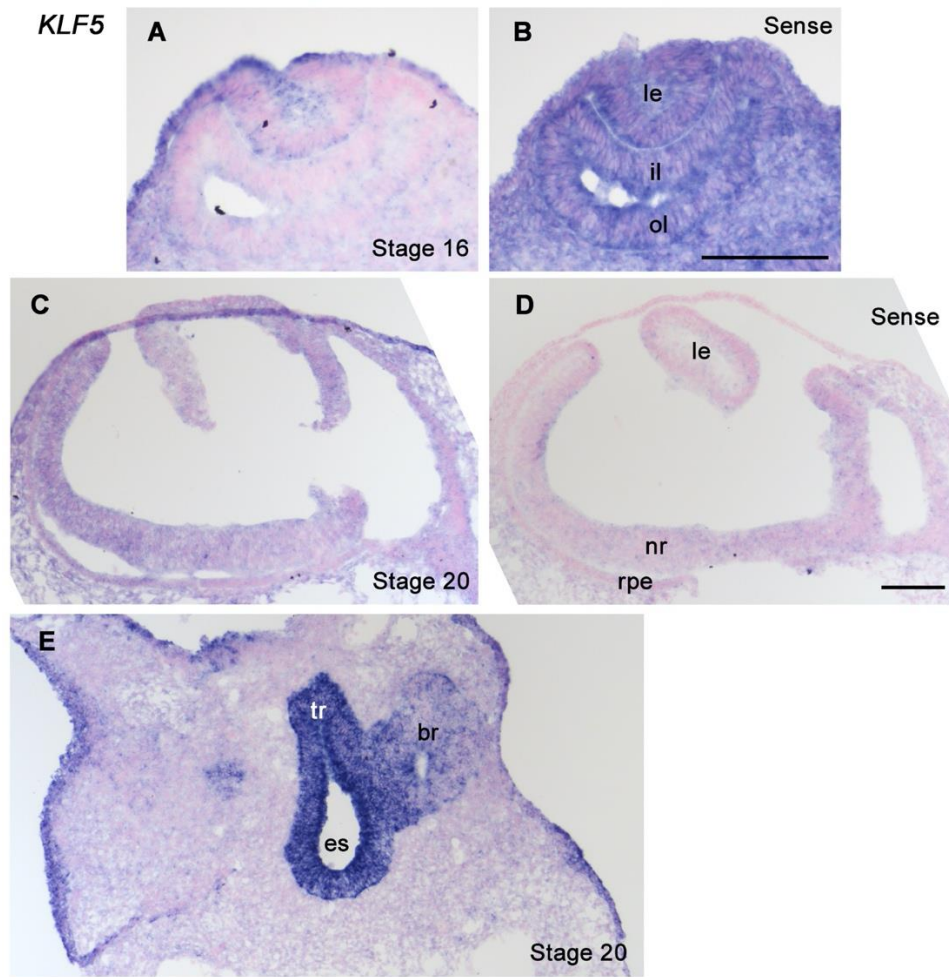


Figure S11: Expression pattern of *KLF5* in the developing chicken eye. *KLF5* is expressed in the surface ectoderm of the developing eye (A,C) and outside the eye (E) as well as the endoderm of developing trachea, bronchus and esophagus. br, bronchus; es, esophagus; il, inner layer of the optic cup; le, lens vesicle; nr, neural retina; ol, outer layer of the optic cup; rpe, developing RPE; tr, trachea. Scale bars: 100 μ m.

Table S1: PCR primer sequences used in this study.

Gene name	Amplicon size (bp)	Primer name	Primer/DNA sequences (5'→3')	Purpose
<i>chicken</i> <i>BHLHE40</i> (<i>cBHLHE40</i>)	1193	cBhlhe40 forward	CTGACCCCTGAAGCAGCTCAA	donating a template cDNA to synthesize riboprobes
		cBhlhe40 reverse	GACACATGGCAGCTACTGA	
<i>cBHLHE40</i>	1206	cBhlhe40 full forward cBhlhe40 full reverse	ATGGAGCGCATCCCGAG TTAGTCTTTGTTTTCCAAAGTTCAC	donating a full-coding sequence for <i>cBHLHE40</i>
<i>cBHLHE40</i>	1252	Bhlhe40_Fw Bhlhe40_Rv	TAGCGTTTAAACTTAACTCTGCACATGCGAGCGATCCCGACG ACGGCGCCCTCAGCTACGCTAGTCTTGTGTTTCCAAAGTTCAC	insertion of <i>cBHLHE40</i> into pCAGGS vector
<i>cBHLHE40</i>	1279	Bhlhe40_pCAG_Fw Bhlhe40_HA_pCAG_Rv	CGGGCTTAAACTTAAGCTTGGCCACATAGGACGGCATC CGGCGCCCTCTAGACTCTAGCAGCTTACGCATAATCCGGCACATACAGCATAGTCTTTGTTTCCAAAGTT	insertion of <i>cBHLHE40</i> into pCAGGS vector with HA-tag
<i>delta_basic</i> <i>cBHLHE40</i>	194	ggaBhlhe40_delbasic_Fw ggaBhlhe40_delbasic_Rv	AGCAGCGAGCAGCAGCAAGATAGGATCAACAGAGTGC GCACCTGTGTATCTATCTCTGCTCTGCTCGCTCT	amplification of N terminal portion of HA-tagged cBHLHE40 before basic region
<i>delta_basic</i> <i>cBHLHE40</i>	1076	pCAG_fw_HindIII pCAG_Rv_XhoI	ACGCTTTAAACTTAAGCTTC CGGCGCCCTCTAGACTCGAGC	amplification of C terminal portion of HA-tagged cBHLHE40 after basic region
<i>acidic</i> <i>extension</i> <i>cBHLHE40</i>	106	a-Bhlhe40_ins_Fw_pCAG a-Bhlhe40_ins_Rv	AGCCTTTAAACTTAAGCTTGGCCACATGGCAATCAATGACTGGA AACTCTTCAGACTCTTCTCATCTCTCTCATCGGGGTCTCTCCCATCTGCTGCTGACCTCCAGTCATGTGCGCAT	acidic extension sequence for replacement of N terminal portion of cBHLHE40 including basic region
<i>acidic</i> <i>extension</i> <i>cBHLHE40</i>	1068	a-Bhlhe40_Fw pCAG_Rv_XhoI	AGAACAGCTGGAAGAGTTGGAGATGCATGCCACGCTC CGGCGCCCTCTAGACTCGAGC	amplification of C terminal portion of HA-tagged cBHLHE40 after basic region
<i>chicken</i> <i>BHLHE41</i>	1776	gga_bhlhe41_Fw gga_bhlhe41_Rv	CCCGCGGATCCCGGAAGC CGACTCGGCGCGCCCATAA	
<i>chicken</i> <i>TEAD3</i>	1299	gga_lead3_Fw gga_lead3_Rv	ATGGACAAGAGCCCTGGACAATGACGGCGAG CTAGCTCTTCACAACTTGTAGACATCATG	
<i>chicken</i> <i>TFAP2A</i>	1640	gga_flap2a_Fw gga_flap2a_Rv	GGGATCGGAGCCCTCTCCCGC CTGGCTCCGTCGCCCTGTTT	
<i>chicken</i> <i>TFAP2C</i>	1347	gga_flap2c_Fw gga_flap2c_Rv	ATGTTGTGAAACTGGCACAACGTCAAG TTACTCTCTGCTGTGTCCATTATTATGAT	donating template cDNAs to synthesize riboprobes
<i>chicken</i> <i>KLF2</i>	1536	gga_klf2_Fw gga_klf2_Rv	CCATGGCGCTGACGGATACCA AACACCGAGCTACAGCAACGCTC	
<i>chicken</i> <i>KLF5</i>	1504	gga_klf5_Fw gga_klf5_Rv	GAGCAATGACAGCCCGGA GCCTCGGCAACAACTCTGGCT	

Table S2: Antibodies used in this study.

Antibody		Vender	Catalog#	Dilution	Final concentration	Diluted in	Antigen retrieval	Antibody concentration
Anti-OTX2	Rabbit polyclonal	abcam	ab21990	1000	1 ug/mL	IMMUNO SHOT immunostaining, Fine	None	1 mg/mL
Anti-VSX2	Sheep polyclonal	Ex Alpha	X1180p	400	0.25 ug/mL	IMMUNO SHOT immunostaining, Mild	in TE (pH 8.0) at 98°C, 45 min	1 mg/mL
Anti-Cleaved caspase 3	Rabbit monoclonal	R & D systems	MAB835	1000	0.5 ug/mL	IMMUNO SHOT immunostaining, Fine	None	0.5 mg/mL
Anti-HA	Rat monoclonal	Roche	ROAHAHA	200 or 400	0.5 or 0.25 ug/mL	IMMUNO SHOT immunostaining, Fine or Mild	None	0.1 mg/mL
Anti-OTX2 + Anti-HA				1000(OTX2), 200 (HA)		IMMUNO SHOT immunostaining, Fine	None	
Anti-VSX2 + Anti-HA				400(VSX2), 400 (HA)		IMMUNO SHOT immunostaining, Mild	in TE (pH 8.0) at 98°C, 45 min	
Anti-sheep IgG Alexa Fluor 488	Donkey polyclonal	Thermo Fisher Scientific	A-11015	1000	2 ug/mL	1% BSA, 0.1% Triton-X100 in PBS	Not applicable	2 mg/mL
Anti-rat IgG Alexa Fluor 488	Goat polyclonal	Thermo Fisher Scientific	A-11006	1000	2 ug/mL	1% BSA, 0.1% Triton-X100 in PBS		2 mg/mL
Anti-rat IgG Alexa Fluor 568	Donkey polyclonal	abcam	ab175708	1000	2 ug/mL	1% BSA, 0.1% Triton-X100 in PBS	Not applicable	2 mg/mL
Anti-rabbit IgG Alexa Fluor 647	Goat polyclonal	abcam	ab150087	1000	2 ug/mL	1% BSA, 0.1% Triton-X100 in PBS	Not applicable	2 mg/mL