SUPPLEMENTARY MATERIAL

Zebrafish Lbh-like Is Required for *Otx2*-mediated Photoreceptor Differentiation

Wen-Hua Li[1,2], Li Zhou[1]*, Zhi Li[1], Yang Wang[1], Jian-Tao Shi[1], Yan-Jing Yang[1,2] and Jian-Fang Gui[1]*

[1]State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences,

[2] Graduate University of the Chinese Academy of Sciences, Wuhan 430072, China

* Corresponding Authors: Jian-Fang Gui and Li Zhou.

Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, Hubei, China;

Tel.: +86-27-68780707; Fax: +86-27-68780123.

E-mail: jfgui@ihb.ac.cn and zhouli@ihb.ac.cn

Supplementary table

Table S1. Primers used for all of the studies

Name	Sequence (5‘ to 3’)	Usage
Lbh-like F:	AGCCTACAGAAAGTGTCAGTTTGTC	RT-PCR
Lbh-like R:	AGGATAAAGACGGTAAGGCAAGG	
Lbh F:	TGTGTAGCCGTGATGACTGA	
Lbh R:	CTCTCCGCTCTCCACCTCT	
Gs F:	TTTTCTGTTGGTGTCAAGGTG	
Gs R:	TCTCCGGTTCCATCTATCCA	
Cahz F:	GGAACACAGCGAAAACCATT	
Cahz R:	GCTTCAGAGAAGGGTCATGC	
Vsx1 F:	TCATGCAGAATCTGGAGCAG	
Vsx1 R:	CATGTCGTGTCGCTGTCTTT	
Ath5 F:	CCAGAGACCCGGAGAAGTTT	
Ath5 R:	ATCCGATTGAGGGCCATGAT	
Insm1a F:	GAGAACTTCCCGAGCAGGAT	
Insm1a R:	TGGGAAGCACTGGTTTAAGG	
Alcama F:	GTCCAGCACCTCTTCCAGAC	
Alcama R:	AGACATCCTTGTCCGTCACC	
Ptf1a F:	CGCCTAGCAATTGGCTACAT	
Ptf1a R:	CAACCCGTAGTCTGGGTCAT	
Th F:	CAGCTCCACATCTTCCACAA	
Th R:	TTCCATCGCTCTCCTCAAAC	
Opn1sw2(*blue*) F:	CCTTGCCATTTCCAACTTGT	
Opn1sw2(*blue*) R:	CAGTCAGGTCCACAAGAGCA	
Opn1mw1 (*green*) F:	ACAGCCCAGCACAAGAAACT	
Opn1mw1 (*green*) R:	TGTTAAGCATGCAGCTACGG	
Opn1lw1(*red*) F:	CGTCACCCTCTCAACTGGAT	
Opn1lw1(*red*) R:	CTTCCTTCTCGGCCTTCTGT	
Opn1sw1(*UV*) F:	CCTAGCAGGCTTCATTTTCG	
Opn1sw1(*UV*) R:	GGTCTTGCTGGAAACCTCTG	
Rho F:	AGCCATGAACGGTACAGAGG	
Rho R:	CTTCTTGTGCTCGATGGTGA	
Otx2 F:	CAACCACCTTACACGGTCAA	
Otx2 R:	TATCCGGGTAGCGTGTTTTC	
Crx F:	AGCCCCATTATGCTGTGAAC	
Crx R:	TCGGGAAGGTTGATTTTCAG	
Nr2e3 F:	AGCCAACACTCCAACAGTCC	
Nr2e3 R:	CTGCCGTACATCGGAGAACT	
NeuroD F:	CAGCAAGTGCTTCCTTTTCC	
NeuroD R:	TAAGGGGTCCGTCAAATGAG	
Notch1a F:	GGAATATGCGAGTACAAGCCC	

Notch1a R:	AACACACAGTCGCACTTCAC	
Hes5 F:	TGGCTCCTGTGTATATGACTGA	
Hes5 R:	TTGTTGATGCGATCTCYGCG	
Anti *lbh-like* F:	ATGTGCAGTGACATTTGGAGTATCG	WISH
Anti *lbh-like* R:	TAATACGACTCACTATAGGGAGATCAGTTCAGAGCTGAAGCACTTTCTG	
Anti *lbh* F:	ACACAACACCAAGCATTAGAGAC	
Anti *lbh* R:	TAATACGACTCACTATAGGGAGAACATCTACACAACAAGAGAAAC	
Gs F:	CTAATTCGCACATGTTTGTTAGATG	
Gs R:	TAATACGACTCACTATAGGGAGACTTTTAGCATCTTCAGGTTTA	
Cahz F:	AAGCATCTCAAATTGAAGTATGACC	
Cahz R:	TAATACGACTCACTATAGGGAGATTTATGACAAACGCAGACAGT	
Vsx1 F:	GGAACTCTCAAAAGAGGAAAAAGAG	
Vsx1 R:	TAATACGACTCACTATAGGGAGAGCAACAACATAAAAACCCTT	
Ath5 F:	CGGAATTACATCCCAAGAACAT	
Ath5 R:	TAATACGACTCACTATAGGGAGACTACTCTGGCTACGGTACAA	
Insm1a F:	ATCTGAAGGTGGTACAACAGGTTAG	
Insm1a R:	TAATACGACTCACTATAGGGAGAAGACGTATCTTGGTACAGAA	
Alcama F:	TATCCCACTGAGAAGGTGAGTCTAC	
Alcama R:	TAATACGACTCACTATAGGGAGACTCCTCCAGTTTCTTACTCT	
Ptf1a F:	CATTCACAGGCTTAGACTCTTTCTC	
Ptf1a R:	TAATACGACTCACTATAGGGAGAAAAAGGCTGAAACACAGATAG	
Th F:	TCTTCACTCTCAGGTGCTCTAAAAG	
Th R:	TAATACGACTCACTATAGGGAGACAGTGAACCAGTACATTGTC	
Opn1sw2(*blue*) F:	TTCTTACCATAGTTTGCACAATTCA	
Opn1sw2(*blue*) R:	TAATACGACTCACTATAGGGAGAAAGCAAAAATTCCTATTGGG	
Opn1mw1(*green*) F:	AGTTGAACTGCTCATTCATATTTCC	
Opn1mw1(*green*) R:	TAATACGACTCACTATAGGGAGAAAGTCTTAGAGAAGAAGGCT	
Opn1lw1(*red*) F:	TATCCTAGGACATCCCATGTGTATT	
Opn1lw1(*red*) R:	TAATACGACTCACTATAGGGAGAAAACTGTAACATCATTCCTG	
Opn1sw1(*UV*) F:	CGACACGTTCTCTGTAAGTCAAGTA	
Opn1sw1(*UV*) R:	TAATACGACTCACTATAGGGAGAAGGCGTAGATTAGGGGATTA	
Rho F:	GGCTAAAGTCGCTTGTAGTACTGG	
Rho R:	TAATACGACTCACTATAGGGAGACTCTCAAAACTGTCTTTTGGT	
Gnat1 F:	GTTCATTGTCATCATCTACAGCAAC	
Gnat1 R:	TAATACGACTCACTATAGGGAGAGAGATTCTCCTTGATGATGA	
Gnat2 F:	CTGCTGGATACTACTTGAACGAAAT	
Gnat2 R:	TAATACGACTCACTATAGGGAGACCACCTGTGGATGTTCTTTT	
Irbp F:	GGCAAAAATATTCATGGACAACTAC	
Irbp R:	TAATACGACTCACTATAGGGAGAAGCCCTTTTAAGGTTTTTAA	
Otx2 F:	GTATAAACATAGGCCATTTGACCAC	
Otx2 R:	TAATACGACTCACTATAGGGAGATAGCAGTTATGACCAATGAA	
Crx F:	TGATTCATGTGATCTTAGAGGTGAA	
Crx R:	TAATACGACTCACTATAGGGAGAAAACAAAGCCACAATTAATG	

Nr2e3 F:	ATGAGAACATTGATGTGACCAGTAA	
Nr2e3 R:	TAATACGACTCACTATAGGGAGATGTTTATCGTAGCATTCACA	
NeuroD F:	CCACGAAGGGCATGAAACTATCATAACAAGC	
NeuroD R:	TAATACGACTCACTATAGGGAGAGCCATAACAGAATACCGTGT	
Rag1 F:	CTGACGAACTGTCTCATCCTAAGTT	
Rag1 R:	TAATACGACTCACTATAGGGAGAGTGTGATATTCTTTACCCAC	
Notch1a F:	ATGAACCGTTTCTTGGTGAAATTAAC	
Notch1a R:	TAATACGACTCACTATAGGGAGATCGTCGCCAGTCCAACCGTTCA	
Hes5 F:	TATGACTGAATACTCCAAGCTTTCC	
Hes5 R:	TAATACGACTCACTATAGGGAGACTCCTGCTTGATGTGTGTGTG	
Lbh-like F:	GAATTCTACCAAGCTTGGGCTGCCTGGAGATCTGCACCAC	Rescue experiment
Lbh-like R:	CTCGAGGAGGTTTACTGTGCTCAAATTGC	
Lbh F:	CGGAATTCCGATGACTGACGTGATGATCAGCGCA	
Lbh R:	CCCTCGAGGGATGCTTGGTGTTGTGTGTGTTCTGC	
Otx2 F:	CGGAATTCCGATGTCGTATCTCAAGCAACCA	
Otx2 R:	CCCTCGAGGGTCACAACACTTGGAATTTCCAGGAGGAA	
NeuroD F:	CGGGATCCCGTCCGACATGACGAAGTCATACAG	
NeuroD R:	GCTCTAGAGCTCACGAGTCGTGAAATATCGCGT	
Otx2 0.6kb FM F:	GCTGGAATTGCTCTGGTCTTTTTCA	Luciferase assay
Otx2 0.6 kb FM R:	CCAACTCTAAAATCTAACATCACGT	
Otx2 1.2kb F:	CTGTTTTAAGTGACAGATTGGGAGG	
Otx2 1.2kb R:	GCTAAAGATGGTTGTGGGGG	
GFP F:	CGGGATCCCGATGGTGAGCAAGGGCGAGGAGCT	
GFP R:	GCTCTAGAGCTTACTTGTACAGCTCGTCCATGC	

Supplementary figures

Figure S1

> *lbh-like* cDNA

ATGTGCAGTGACATTTGGAGTATCGGCTTGCAGTCTTTTCCGATTAACCCAGAGTCA
GGCATCGTCCTGGAAGAACAGTGCCCGCCGATGGGGCCTGCGGAGGGGCAGTGCT
GTGGGAGCTGCCCTTCTCTGCCCCCTATCATCATACCAGGCATGGGCAGCGTGGAGA
TCTGCACCACCCAAGAGGACGACTGCTTGCCCACCGATGGAGGGATGGGATACCCA
GAAGCCCAAGCCATGGATGAGTTTCCAGCCCAGCAGAAAGGGGACAGACTGCCCT
TTCAGATCTTCCCAGACCCGGTGGAAGTGGTTTTGAGCACTGAAGGCACTCTGACC
TGTTCTCCGCAGGGGAAGGAGCGCCTGCCGTCCATTGTGGTGGAGCCCACAGATGT
GAGCGAGGTGGAGAGCGGAGAGCTGCGCTGGCCGCCAGAAGACATGGACTTTGAT
GAAGATGAAGATCTATTCTTGGAGCAGTGCATCCCACCAGCAAACATCGCCGACTG
GGGGGAAGACGAGGAGGAGAGCTCTGTTATTGAAAACCACCAGCAGTCCGCTTCC
CTGTTAGATCTCAAATCGGACGACTTCCGGGACGACACCCCGACTCTACCTCCAGA
AAGTGCTTCAGCTCTGAACTGATGCCCTCTCAACTTCTCATCTTCGACCCGGCATTG
CCACATGTGACTGTGGTGTCTGACTATGGTCAAACAATATACCATGAAACTGAAACA
TTGCATATACTAGAAAAGAGTGCGAAGGAATTGACACACTGTAAAACTCAAAAAGT
TAAGGCAACTCAAACCGTTTGAGAAACCGATTGCAACAAACCATTCACATTGAAAA
ACTAATCCTGATGAGTACTGTGAACTTAATCCATTTGAGTAAACAAAGCAATTTGAG
CACAGTAAACCTCAATAAATGAAGAGAACTCAAGCCAACTGAGTACTGTGAAACCT
AAGTTAAGGCAACTCAAGCGATTTCAATAAACCATTTAAGTTCAAAAACTAACCTAT
ATAGGTTACTGTGACCATGCTCCTTTTAAATTTGAAGTAATAAGGGGTATTTGTTTAC
TCATTACCTTCAACACAGAGTTTAATACTCTTTTCAAATGAGTAGAATGAACTTTCAG
TAAATGTTGAGTTAACTACACTCATTTCATTTGATAAAGTTCTGTTTCACAGTGTATA
ATGTAATTTAGTACACTCAAAAAATAATAATTGTTCAAACTACTTAATTAATATGAGCC
AAAACAACACGATTCTTGAGATATTTTTGGGACAACTTAATTGTTTGATGTTTCATCC
ACTTAAATTTGTCAAAACCAATTAACTTATGTGACTTGTGTTGGGACGACATGGATG
AATACTGAGGAAGCCTGCATTTCTACAAAGTAGCTCAGTTTACTTTACTGCTGATCA
GTTTATCACAGTAGACATCTTTTCTTTACTTTTGATTTTCCAACAAGTCAGCATTTTCA
CACTTCCCTTTATCATGCATATCTGACCTATGCGGATAAATCAATAAAAAAAGGAAGC
AAGTTGGCGGAAACATGAAGTGGTGATGCTACACAAACATATGATCTTGAGACAGA
CGTTTATGTTATGGAGAAGTTGAAATGCATTACTCGAAATGAAAGTTTAATTTGTACT
ATTTTCGTCCCCTTATTTCACCCATGTCAGTTTCTGTGTTGTGCTTCACTCATGTCCAG
CTGCGTTTTAACCATTAAGTGCACCTCAGAGTAGAAAAACGTATTCAAACAAGCAAT
GTACTGTGTGTTTTATACCAGACATGTTACATTATGTCCAACATCAATCAGCCGTGTA
GCATTTCTCATTGCAGTATGTACATACAATGTTTTTCCTCGTATGATTGTGTACAAGTT
GAAAACTATTTAATAAACCGCGTCAGTACCAAAAAAAAAA

Fig. S1. The full length cDNA sequence of zebrafish *lbh-like*. The open reading frame of *lbh-like* is marked by red color.

Figure S2

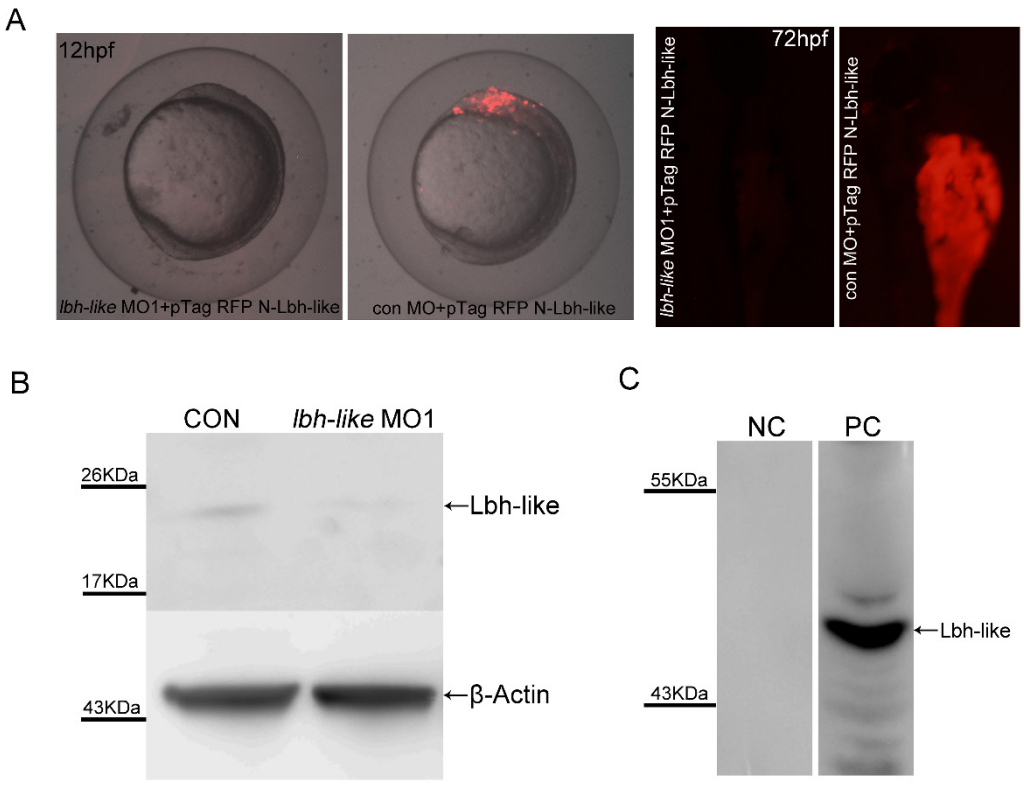
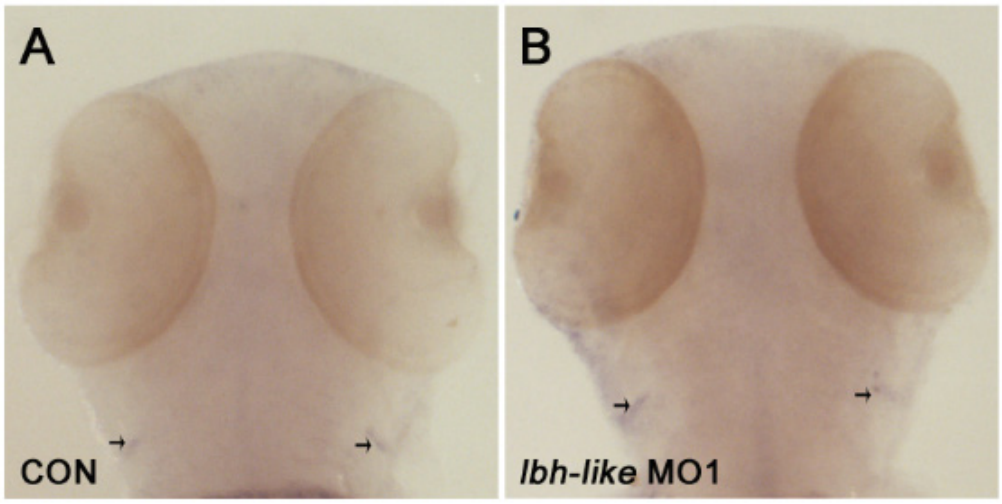


Fig. S2. Specificity confirmation of MO1 effectiveness. (A) Embryos were co-injected with either *lbh-like* MO1 or control MO and a pTag RFP N-Lbh-like plasmid containing the *lbh-like* MO1 binding site. From 12 hpf to 3 dpf, no any RFP expression is observed in the co-injected embryos with *lbh-like* MO1 and pTag RFP N-Lbh-like plasmid, whereas robust RFP expression is seen in the co-injected embryos with control MO and pTag RFP N-Lbh-like plasmid. (B) Western blot detection of the control (CON) and *lbh-like* MO1 embryo extracts at 48 hpf by anti-Lbh-like antiserum. The β-Actin was used as control. (C) Western blot detection of EPC cell extracts with Lbh-like-GST overexpression by pre-immune serum as negative control (NC) and anti-Lbh-like antiserum as positive control (PC).

41 Figure S3



42

43 **Fig. S3. Whole-mount *in situ* hybridization of *rag1* expression (arrows) in control**

44 **embryos (A) and the Lbh-like morphants (B) at 3 dpf.**

45

Figure S4

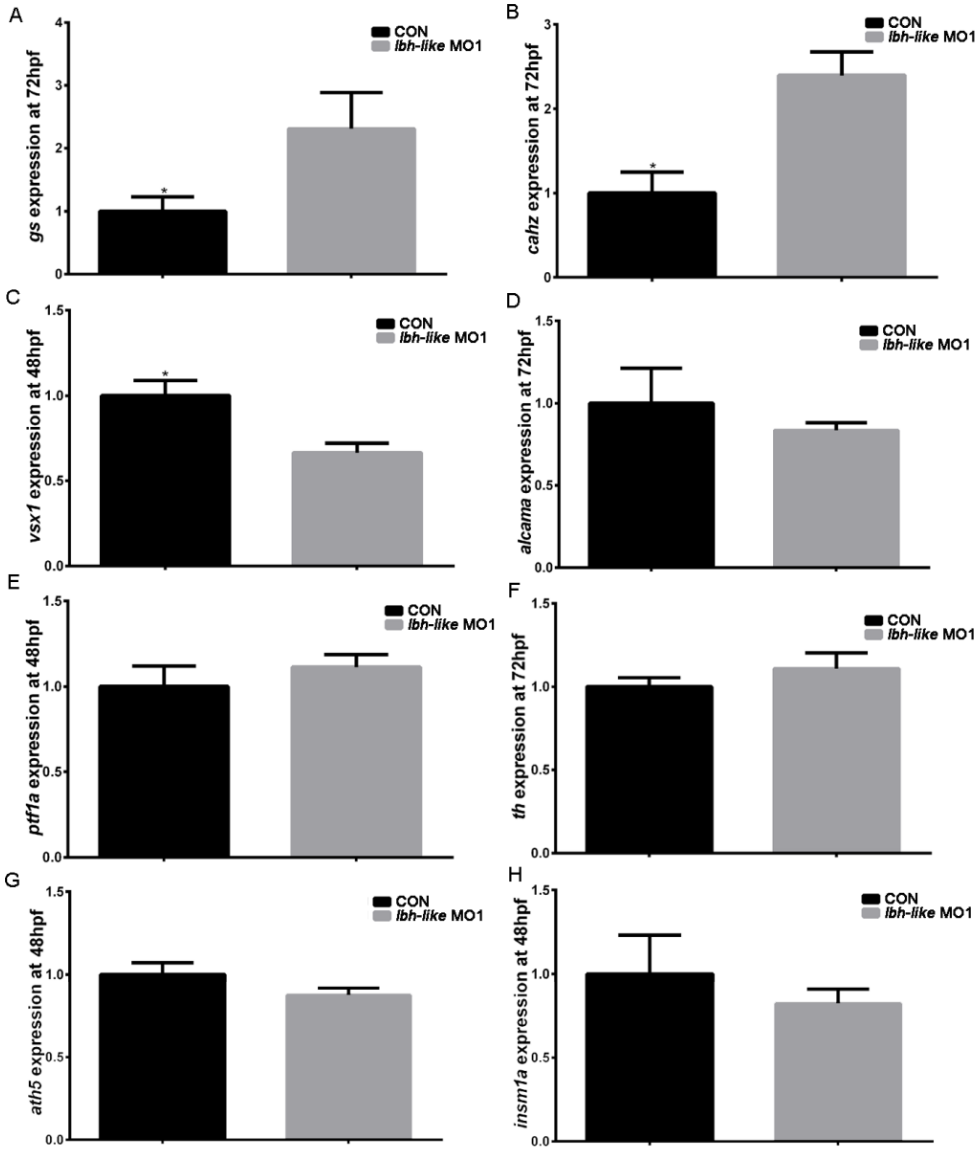


Fig. S4. Relative expression levels of several retina lamination markers at embryonic development stages checked by real-time PCR. The relative expression was normalized to the expression level of *β-actin*. Error bars represent SD (n=3, *p<0.05). Gene names and development stages are marked in the graph left.

Figure S5

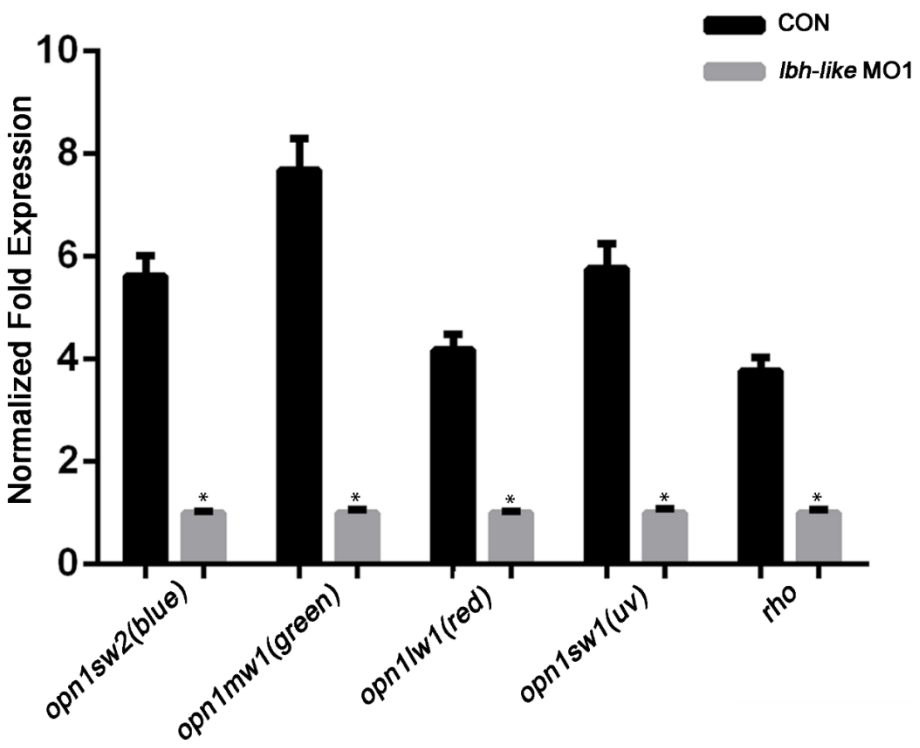


Fig. S5. Expression of four *opsins* and *rho* in embryos at 3 dpf was checked by real-time PCR. The relative expression was normalized to the expression of *β-actin*. Error bars represent SD (n=3, *p<0.05). Gene names are marked in the bottom of graph.

Figure S6

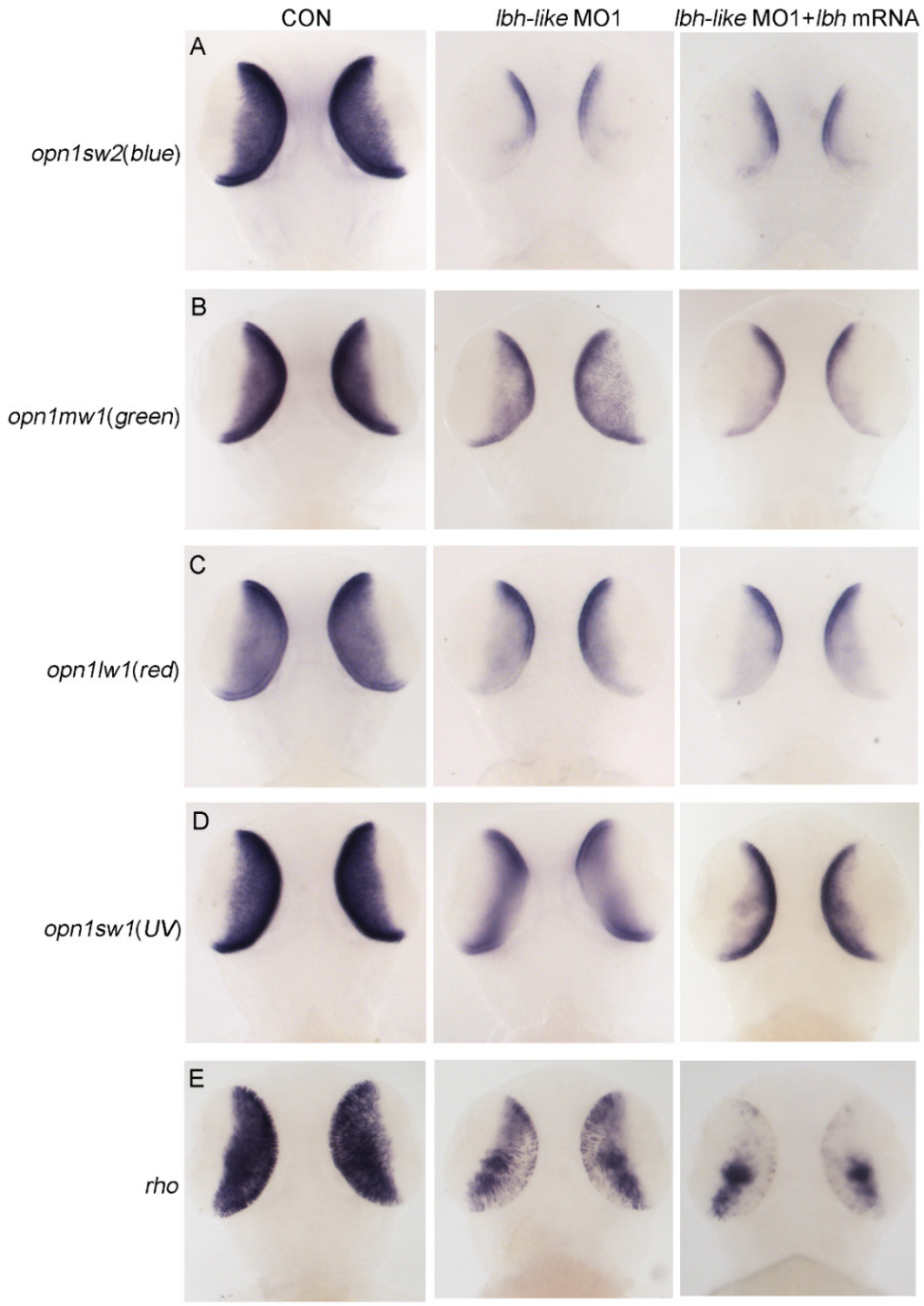


Fig. S6. The defects in Lbh-like morphants can't rescue by zebrafish *lbh* mRNA. Four *opsins* (A-D) and *rho* (E) were analyzed by WISH in the embryos at 3 dpf. In each panel, the left embryos were injected with 4 ng control morpholino, the middle embryos with 4 ng *lbh-like* MO1, and the right embryos with 4 ng *lbh-like* MO1 and 0.1 ng lbh mRNA. Dorsal views.

Figure S7

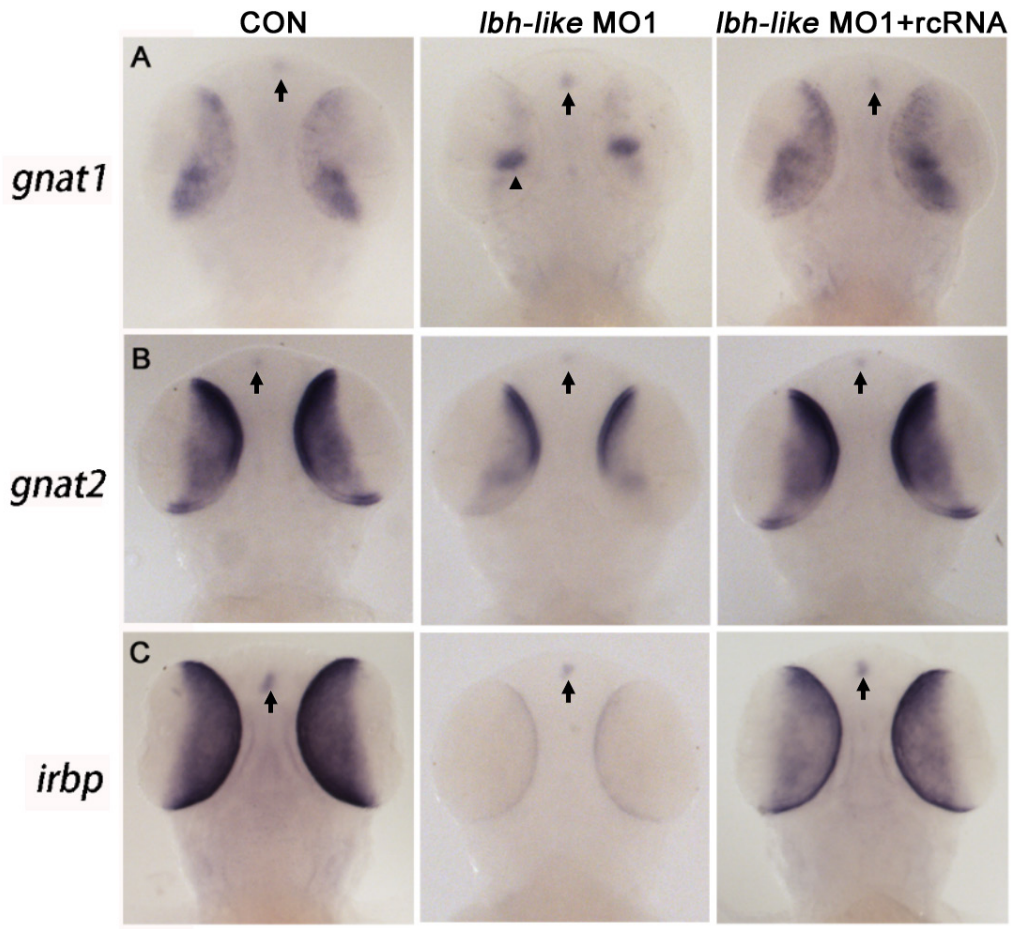
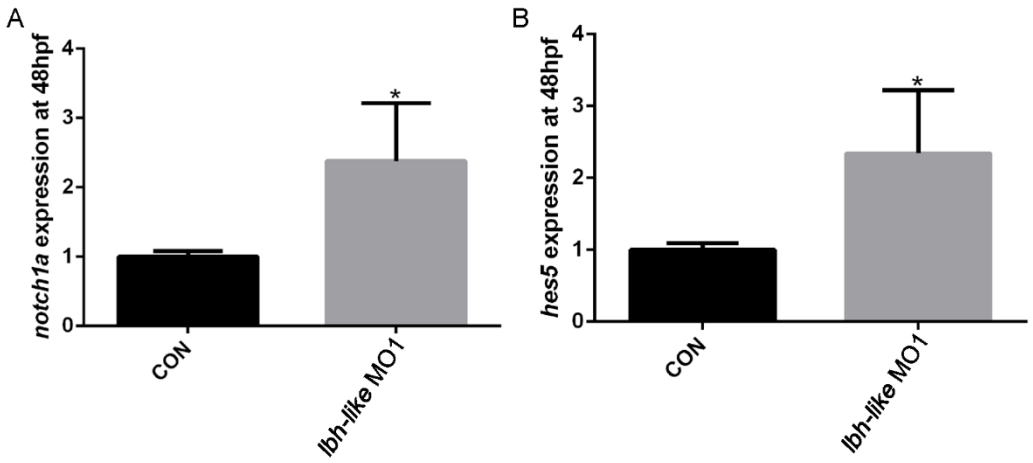


Fig. S7. Effects of Lbh-like knockdown on photoreceptor cell differentiation. Photoreceptor-specific gene *gnat1* (A)*, gnat2* (B), and *irbp* (C) were tested to evaluate photoreceptor cell differentiation in embryos at 3 dpf. In each panel, the left embryos were injected with 4 ng control morpholino, the middle embryos with 4 ng *lbh-like* MO1, and the right embryos with 4 ng *lbh-like* MO1 and 0.1 ng rcRNA. Dorsal views. Arrows: pineal gland. Arrowheads: ventral patch of differentiating retina.

77 Figure S8



78

79 **Fig. S8. Expression of Notch 1 (A) and its downstream target genes (B) was**

80 **checked by real-time PCR.** The relative expression was normalized to the expression

81 of *β-actin*. Error bars represent SD (n=3, *p<0.05). Gene names and development

82 stages are marked in the left of graph.

83

Figure S9

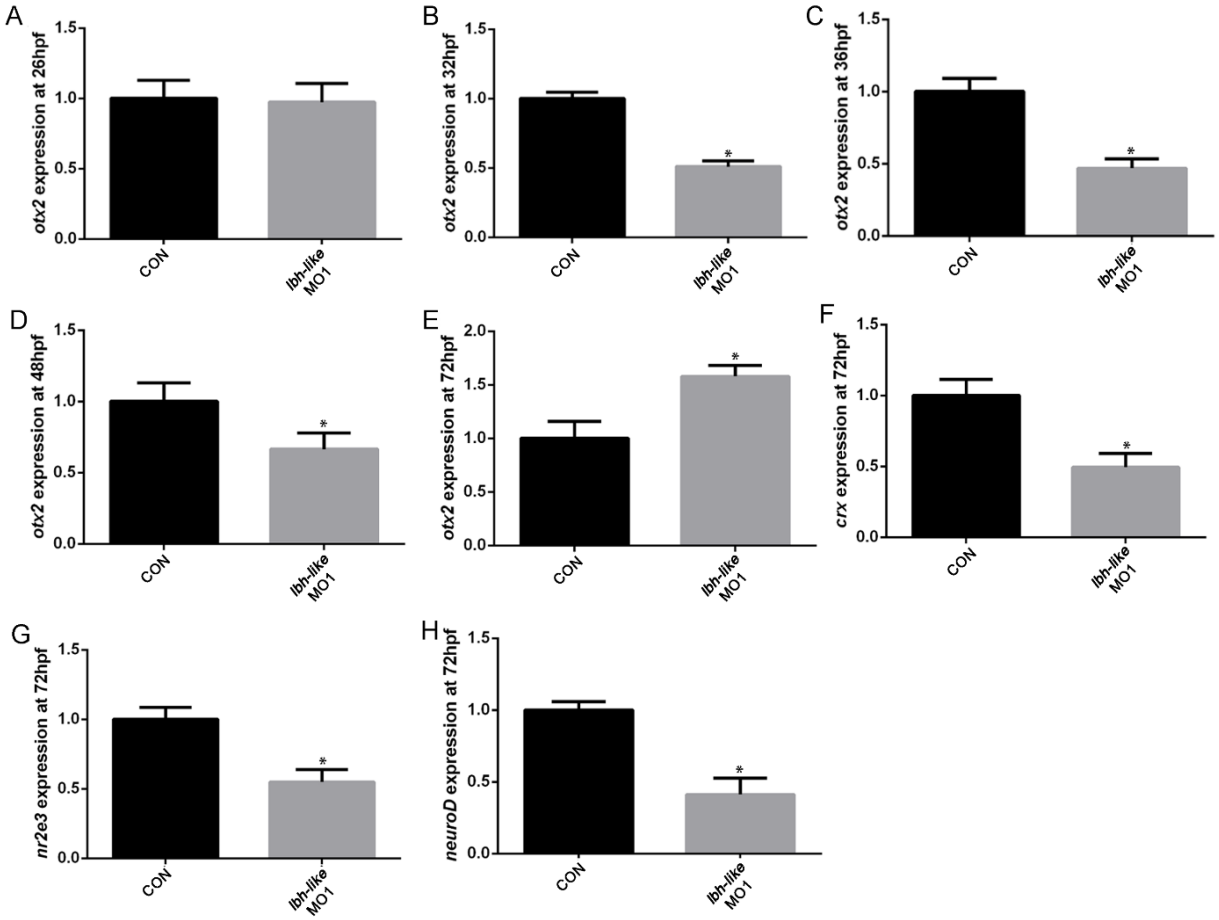


Fig. S9. Expression of early retinal marker *otx2* (A-E) and its downstream target genes (F-H) was checked by real-time PCR. The relative expression was normalized to the expression of *β-actin*. Error bars represent SD (n=3, *p<0.05). Gene names and development stages are marked in the left of graph.

Figure S10

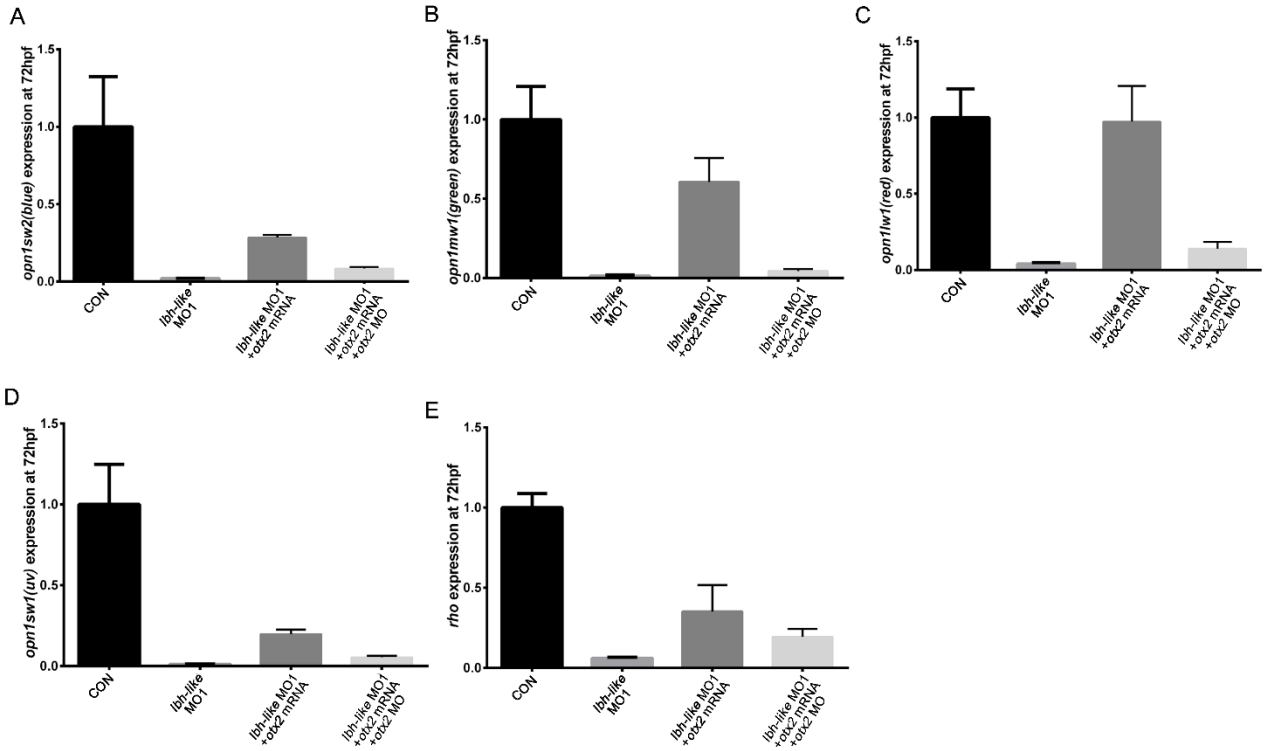


Fig. S10. *lbh-like* affects the expression of four *opsins* (A-D) and *rho* (E) by direct regulation of *otx2* checked by real-time PCR. The relative expression was normalized to the expression of *β-actin*. Error bars represent SD (n=3, *p<0.05). Gene names and development stages are marked in the left of graph.