Supplementary Methods and
Supplementary Figures S1-S5
for
Temporal requirement of *Mab21l2* during eye development in chick reveals stage dependent functions for retinogenesis

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Supplementary Methods

Design of Mab21l2 constructs

The Mab21l2 loss-of-function construct was made by using a 680bp long double stranded (ds) RNA from Mab21l2 cDNA and cloned into a pCRII vector using the following primers:
Forward primer: ATGGGCGTCTTCAACTTCGT;
Reverse primer: GAGATGAGCTGCAGCAGGA.

To make dsRNA, sense and antisense strands of RNA were synthesized using Sp6 and T7 polymerases, respectively. After elimination of the cDNA template, strands of RNA were annealed for 5 minutes at 95°C and then purified [1].

The gain-of-function construct was made by cloning Mab21l2 cDNA from RNA isolated from E6 chick eyes into a pCAG-P2A-EGFP-m5 vector. Full length Mab21l2 coding sequence was amplified from cDNA devoid of the stop codon to be translated continuously with the P2A peptide and GFP protein using following primers:
Forward: 5’-ATGATCGCCGCCCAG-3’
Reverse: 5’-TAGTTTTGTCGAGGCTTTTGGGATTG-3’
The full length Mab21l2 was cloned in front of P2A-EGFP sequences using Smal and Nhe1, and the sequence confirmed through sequencing.

In situ hybridization

Fragments of chick Mab21l1 (563bp) and Mab21l2 (680bp) were amplified from genomic DNA. Fragments of chick Atoh7/Ath5 (600bp) and NeuroD4/Ath3 (600bp) were amplified from cDNA derived from E6 eye tissue. The following primers were used:
Mab21l1 Forward: 5’-ACGAGATGGACAACCGCTAC-3’
Reverse: 5’-GCCCATCTGCAGTCTGTTCT-3’
Mab21l2  
Forward: 5’-ATGGGCGTCTTCAACTTCGT-3’
Reverse: 5’-GAGATGAGCTGCAGCAGGA-3’

Atoh7/Ath5  
Forward: 5’-TCCAGTCATTGGATTCAGGA-3’
Reverse: 5’-TCGCTGTGCATAAGGATCAC-3’

NeuroD4/Ath3  
Forward: 5’-TACATCTGGGCTCTGTCCGA-3’
Reverse: 5’-CTGCGTTTTGGAAGTGGGTG-3’

The PCR products were cloned into pCRII (Invitrogen) and the sequence confirmed through Sanger sequencing.

References

FIGURE S1. GFP over-expression does not affect retina morphology or differentiation. (A-D) Embryos electroporated at HH8-10 with only the GFP-expressing vector and cultured to approximately E2 (A,B; N=3) and E3.5 (C,D; N=4). GFP expression in the optic vesicle (A) does not affect Mab21l2 expression (B) (N=3/3), and GFP expression in retinal cells (C) does not affect MAP expression (D) (N=4/4). Scale bar 100µm for (A-B, C-D).
FIGURE S2. Electroporation of dsMab21l2 in the prospective optic vesicle does not affect midbrain development, but results in smaller lenses.

(A,B) Transversal sections of embryos electroporated at HH8-10 with the Mab21l2-expressing vector and cultured to approximately E6 (A; N=4) and E2 (C,D; N=4), showing both control and dsMab21l2 sides. (A) H&E staining indicates a rudimentary retina in the dsMab21l2 side (arrowhead) associated to a smaller lens, but with an apparent normal midbrain (N=4/4). The length of the lens equator (dotted line) in the electroporated side was significantly shorter (617±27 µm) compared to the control side (972±8 µm) (N=4; ***t test; P<0.0001). (B). In situ hybridization indicates that dsMab21l2-electroporation results in specific down-regulation of Mab21l2 in the electroporated side, compared to the control side, and without affecting the morphology and the patterning of the forebrain (N=4/4). Scale bars 1mm for (A) and 100µm for (B).
FIGURE S3. dsMab21l2 RNA inhibitory effect maintain suppression of Mab21l2 and Vsx2 expression in the retina at E6, but does not affect lens patterning.

(A-F) dsMab21l2-electroporated embryos at HH11-12 and cultured to E6, and analysed by Mab21l2 and Vsx2 in situ hybridization and Prox1 immunohistochemistry. (A,D) Mab21l2 expression is still decreased in the GCL in the dsMab21l2-electroporated side (D) compared to the control side (A) (N=4/4). (B,E) Vsx2 expression is reduced in the electroporated side (E) compared to the control side (B) (N=4/4). (C,F) Prox1 expression in the lens of the control side (C) and dsMab21l2-electroporated side (F), reveals reduced size, but no apparent changes in patterning in the dsMab21l2-electroporated side (N=4/4). Scale bar 100µm for (A-F).
FIGURE S4. Down-regulation of *Mab21l2* at HH11-12 results in disrupted maturation of post-mitotic neurons.

(A-C) Immunohistochemistry analysis using RA4 antibody against chicken MAP on transversal sections of ds*Mab21l2*-electroporated embryos at HH11/12 and cultured to E6 (N=4/4). (A, B-left) The control retina show a GCL-restricted MAP expression (N=4/4). (B-right, C) The ds*Mab21l2*-electroporated eye exhibit a radial MAP expression pattern throughout the retina width, and a malformed optic head with an excavated shape (N=4/4). *Scale bars* 1mm for (A,C) and 5mm for (B).
FIGURE S5. No change in the retinal progenitor pool after *Mab21l2* over-expression.

(A-H) Electroporation with a *Mab21l2*-over-expressing vector at HH8-10 and cultured to E2.5. GFP in (E) and *Mab21l2* in (G) indicates the electroporated area of the eye compared to the non-electroporated control side (A,C). No change in pHH3$^+$ proliferative cells (B,F; N=5; $P=0.14$), or the expression of *Vsx2*, indicative of RPCs (D,H), were detected between control and electroporated side (N=5/5). *Scale bar* 100µm for (A-H).