# 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'-TAGTTTGTCGAGGCTTTTGGGATTG-3'

The full length *Mab21l2* was cloned in front of P2A-*EGFP* sequences using SmaI 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'-TCCAGTCATTTGGATTCAGGA-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***

1. Pekarik, V., et al., *Screening for gene function in chicken embryo using RNAi and electroporation.* Nat Biotech, 2003. **21**(1): p. 93-96.

***Supplementary Figures***

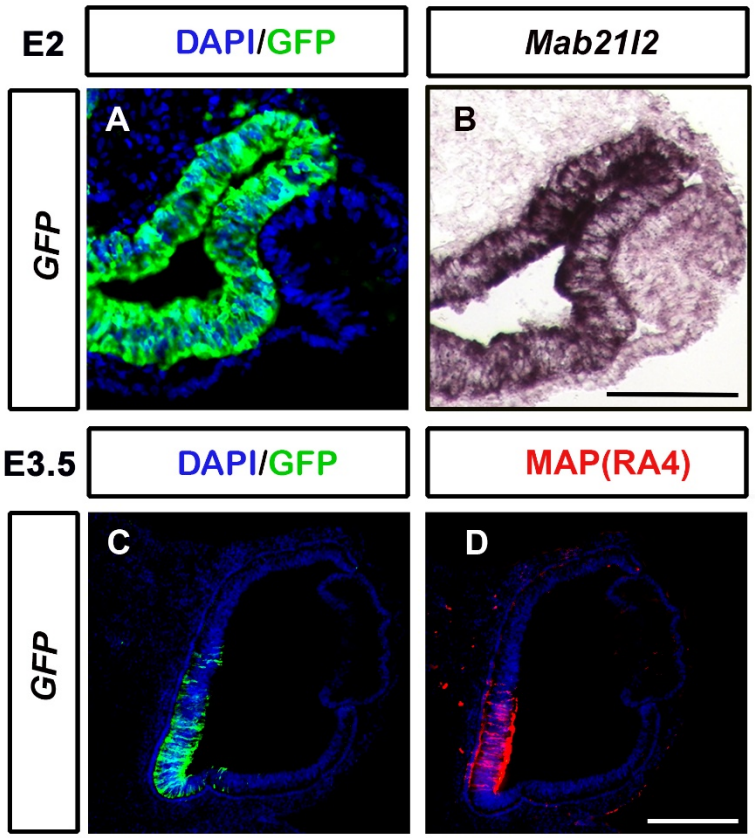


**FIGURE S1.** GFP over-expression does not affect retina morphology or differentiation. (**A-D**) Embryos electroporated at HH8-10 with only the *GFP*-expressing vector and cultured to approximately E2 (A,B; N=3) and E3.5 (C,D; N=4). GFP expression in the optic vesicle (**A**) does not affect *Mab21l2* 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**).

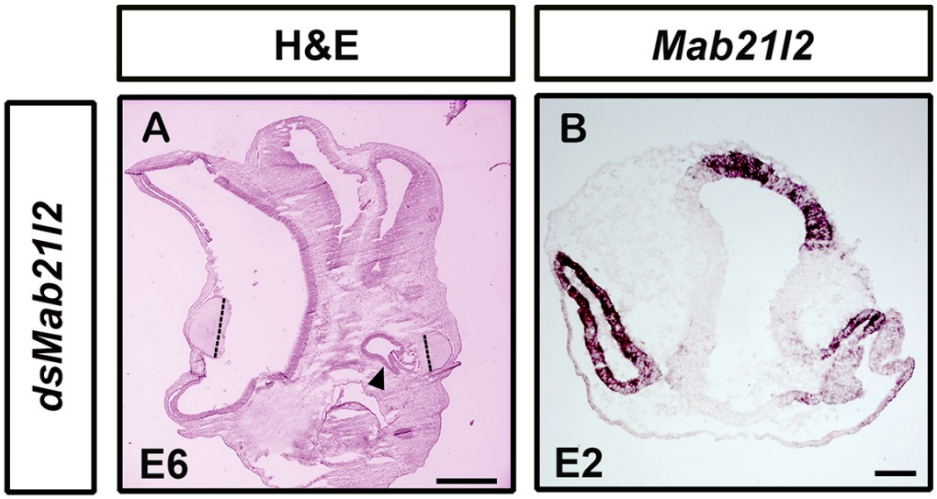


**FIGURE S2.** Electroporation of ds*Mab21l2* in the prospective optic vesicle does not affect midbrain development, but results in smaller lenses.

(**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 ds*Mab21l2* sides. (**A**) H&E staining indicates a rudimentary retina in the ds*Mab21l2* 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 ds*Mab21l2*-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**).

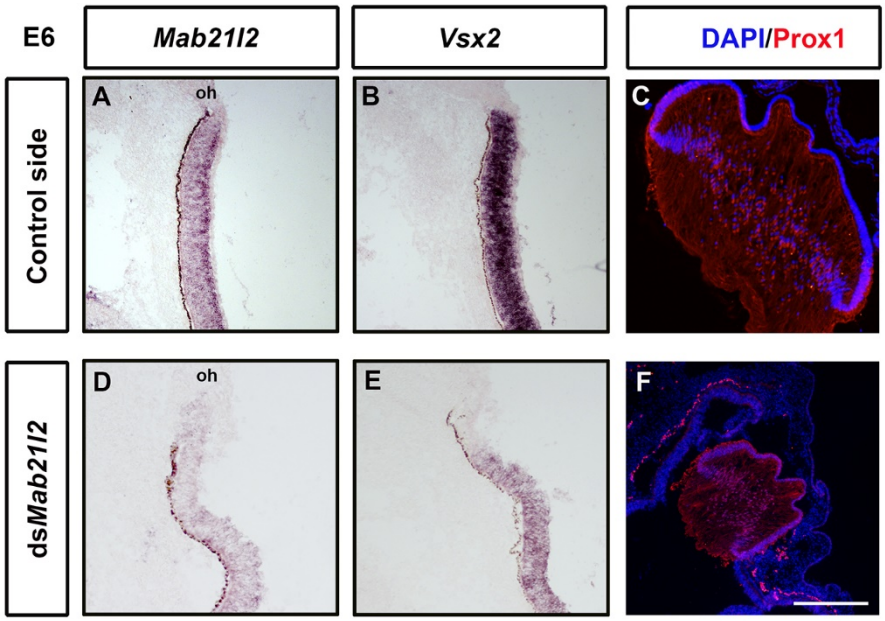


**FIGURE S3.** ds*Mab21l2* RNA inhibitory effect maintain suppression of *Mab21l2* and *Vsx2* expression in the retina at E6, but does not affect lens patterning.

(**A-F**) ds*Mab21l2*-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 ds*Mab21l2*-electroporated side (**D**) compared to the control side (**A**) (N=4/4). (**B,E**) *Vsx2* expression is reduced in the elctroporated side (**E**) compared to the control side (**B**) (N=4/4). (**C,F**) Prox1 expression in the lens of the control side (**C**) and ds*Mab21l2*-electroporated side (**F**), reveals reduced size, but no apparent changes in patterning in the ds*Mab21l2*-electroporated side (N=4/4). *Scale bar* 100µm for (**A-F**).

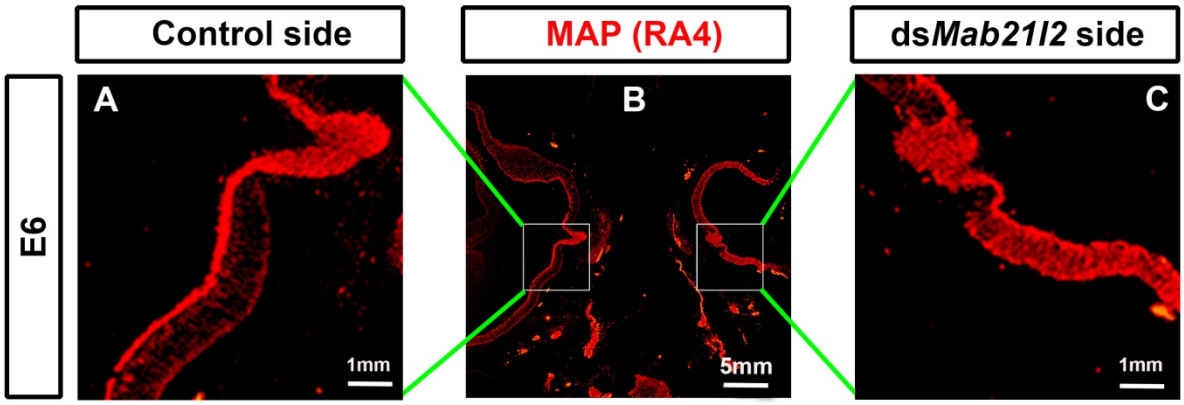


**FIGURE S4.** Down-regulation of *Mab21l2* at HH11-12 results in disrupted maturation of post-mitotic neurons.

(**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**).

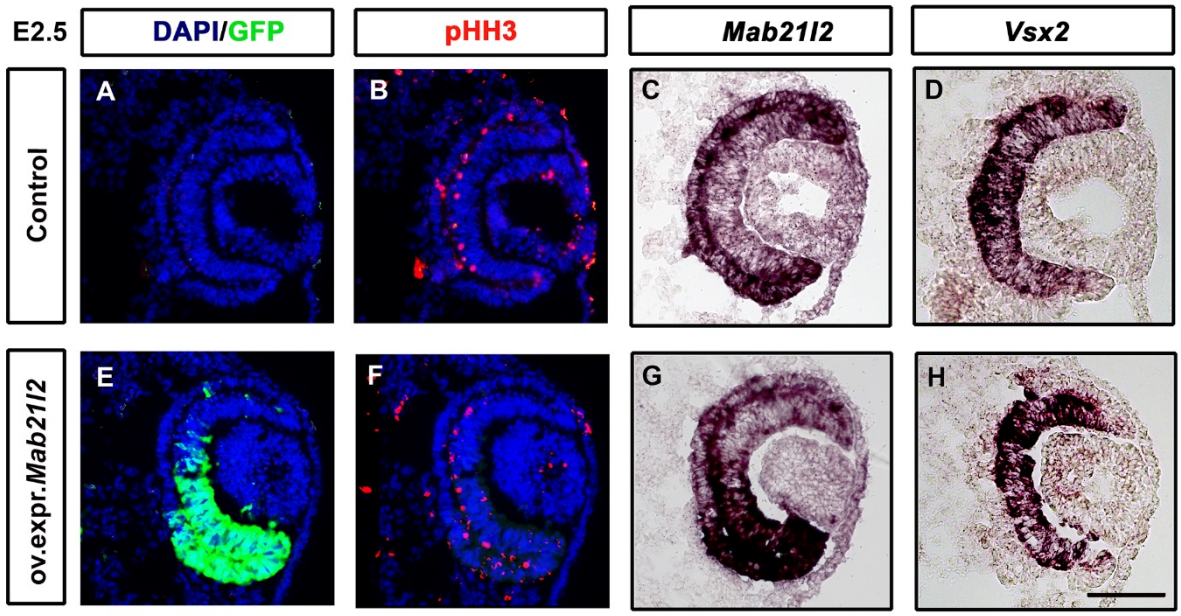


**FIGURE S5.** No change in the retinal progenitor pool after *Mab21l2* over-expression. (**A-H**) Electroporation with a *Mab21l2*-over-expressing vector at HH8-10 and cultured to E2.5. GFP in (**E**) and *Mab21l2* in (**G**) indicates the electroporated area of the eye compared to the non-electroporated 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**).