

Rbpj direct regulation of *Atoh7* transcription in the embryonic mouse retina

Joel B. Miesfeld^{1#}, Myung-soon Moon^{1,2#}, Amy N. Riesenber² Ashley N. Contreras^{3,4}, Rhett A. Kovall³
and Nadean L. Brown^{1,2*}

¹Department of Cell Biology & Human Anatomy, University of California Davis School of Medicine,
One Shields Avenue, Davis, CA 95616

²Division of Developmental Biology, Cincinnati Children's Hospital Research Foundation, 3333 Burnet
Avenue, Cincinnati, OH 45229

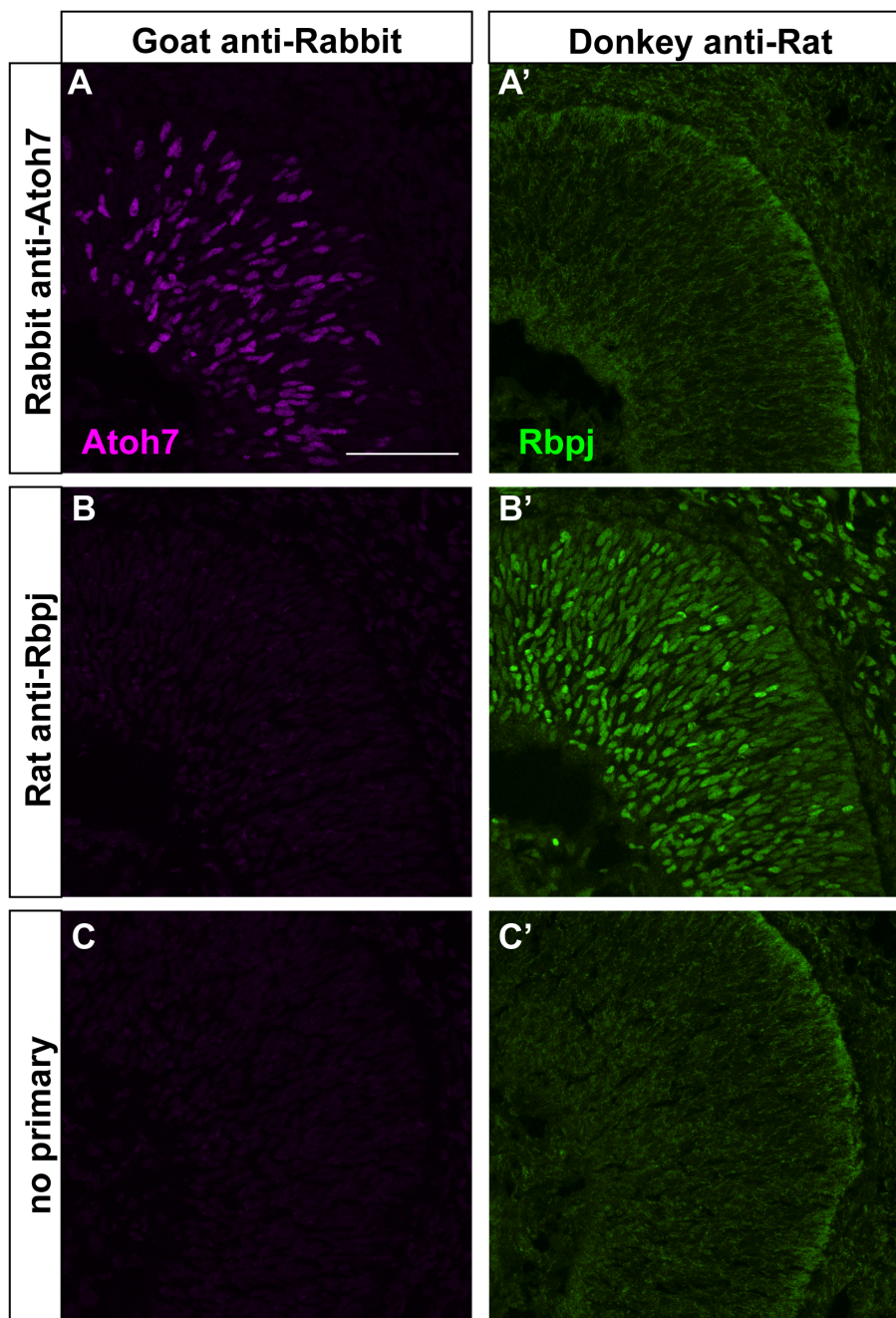
³Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati School of
Medicine, Cincinnati, OH 45267

⁴Present address: Department of Biology, University of Cincinnati Blue Ash College, Cincinnati, OH
45236

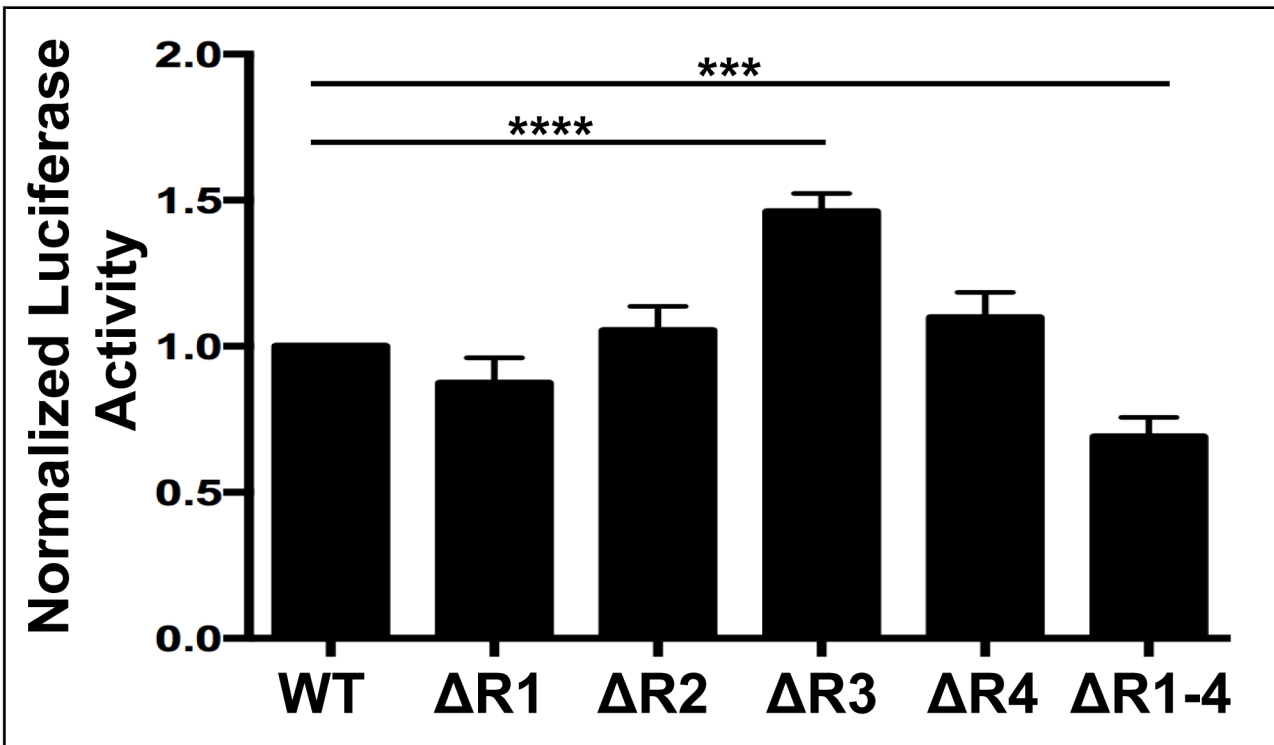
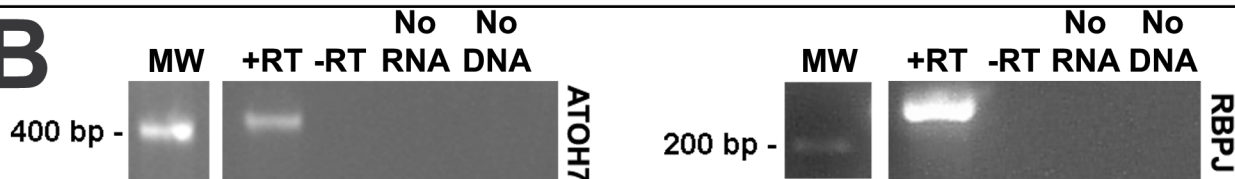
#Co-First Authors

* Corresponding Author: Nadean L. Brown (nlbrown@ucdavis.edu)

Address: Department of Cell Biology & Human Anatomy, Room 4407, Tupper Hall University
of California, Davis Davis, CA 95616



Supplemental Figure S1. Colocalization of Atoh7 and Rbpj proteins is not due to cross-species reactivity of secondary antibodies. A-C') E13.5 retinal cryosections were incubated sequentially with one primary antibody (indicated at left) and one secondary antibody (indicated at top), and processed as described in Methods. Panels in each column were imaged identically. Secondary antibodies were goat anti-rabbit IgG Alexa-647 and donkey anti-rat IgG Alexa-488, respectively. Section in C-C' received no primary antibody (blocking solution only). Bar = 50µm.

A**B**

Supplemental Figure S2. Luciferase activity in Ad12HER10 retinal cells. A) Single and quadruple Rbpj binding site mutant luciferase assay comparison using a retinoblast cell line. The same outcomes were observed as in HEK293T cells, only ΔR3 site mutant derepressed Atoh7-luciferase activity, whereas simultaneous removal of all four CSL sites R1-4 reduced Atoh7-luciferase activity over wild type. *** $p \leq 0.001$; **** $p < 0.0001$; $n = 9$ biological replicates (each in technical triplicate). B) RT-PCR analysis of AD12Her10 cell mRNA showing endogenous RBPJ and ATOH7 expression. Uncropped gels provided in Suppl Figure S3.

Figure 2C Western

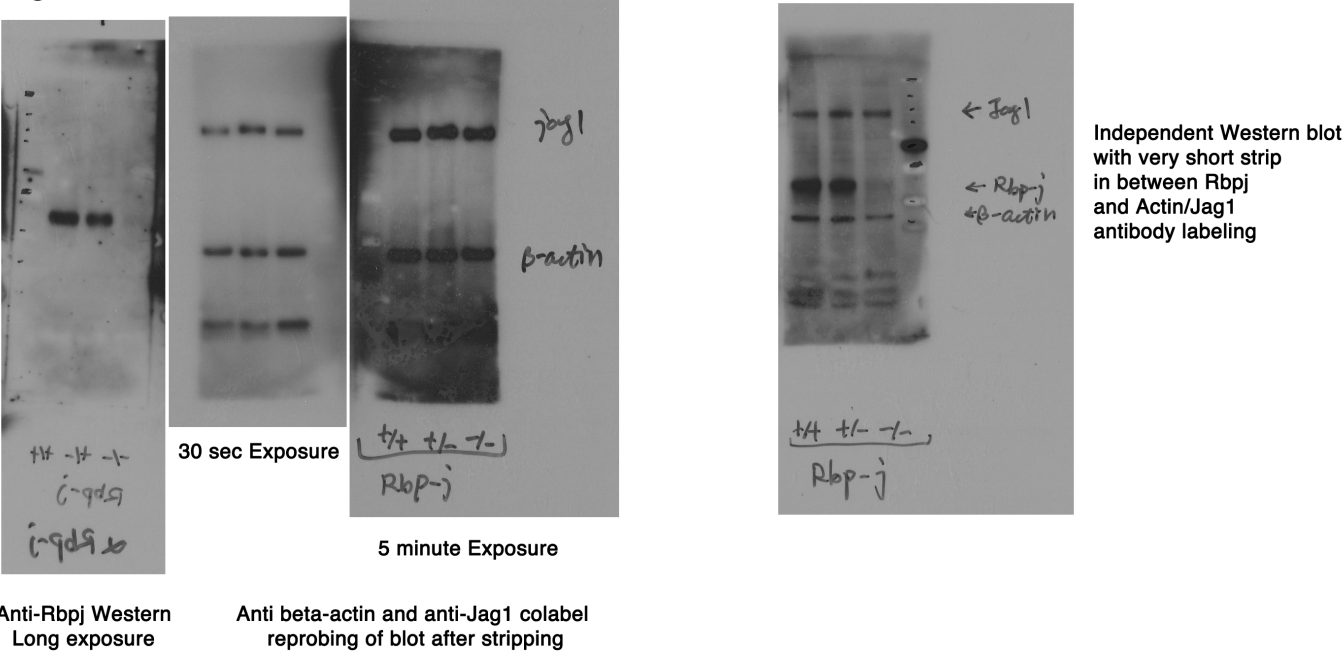


Figure 4A EMSA

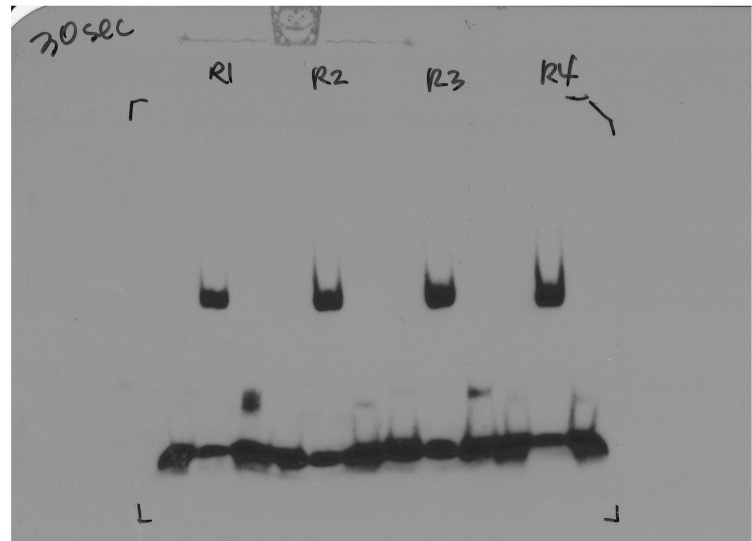
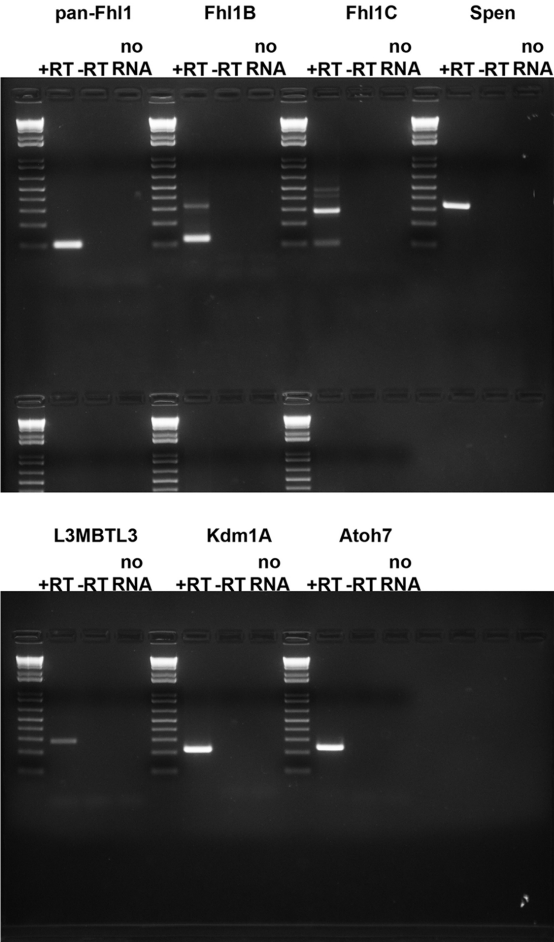
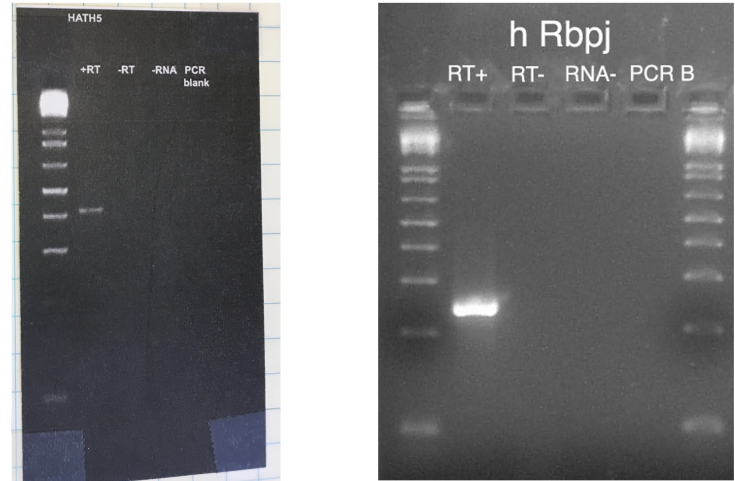


Figure 6C Full size gel images



Supplemental Fig S2 Full size gel images



Supplemental Table S4 PCR primers used for ChIP or RT-PCR**ChIP qPCR primer sets for mouse *Atoh7* locus**

Primer	Forward	Reverse	Amplicon	Start position
R1	TCCGTCATGAGTGTACAGG	ACTAAAAGGGTGTGAGGGGG	137	-2280
R2	AGCCCAAAGAAAGCAGAC	CCATCACTCCTCGGTCCTAA	132	-1863
R3	CCAACATCTGTCGCTCTGAA	GCCCCGTCTTCCCATAAATA	126	-1700
R4	GACGAAGGCAATGATGTCAG	TCAAGGAGGGAACAGGTGGT	129	-539
NegControl	GGGTCTTAGGGGAGAAAACCTCAATC	TACAAAACACAAGAGAAGTGGAGATA	114	+466
Neg-Pax6	ATCCTTCCCCTGACTGTGCCAC	CAAGATGCTTCCGCTTTTCTGG	208	+3026

RT-Primers Mouse

Primer	Forward	Reverse	Amplicon	NCBI gene ID
Pan <i>Fhl1</i> *	GACTGCCGCAAGCCCATAA	CCAAGGGGTGAAGGCACTT	118	14199
<i>Fhl1B</i> *	CTGCGTGGATTGCTACAAGA	CACACTGGGGACTTCCTAGC	126	14199
<i>Fhl1C</i> *	TGCCATGAGACCAAGTTCG	GTAGTCGTGCCAGGATTGTC	101	14199
<i>Spen</i>	CCACGAACCTTCCGAAACATC	TCGATCTCCGCTGTAGAGAAC	340	56381
<i>L3mbtl3</i>	AGGGGTTCTCAGAGAGCCAG	TCCAAACAAATACAAGGCGGAG	282	237339
<i>Kdm1A</i>	GTGGTGTTATGCTTTGACCGT	GCTGCCAAAAATCCCTTTGAGA	228	99982
<i>Atoh7</i>	ACAAGAAGCTGTCCAAGTAC	AATGGCCCCGAGGCTTAGCTG	244	53404

*Primer sequences for these splice variants based on Figures 1 and S1 from Domenighetti et al., 2014

RT-Primers Human

Primer	Forward	Reverse	Amplicon	NCBI gene ID
<i>ATOH7</i>	TGGGTCTCCACTGTGAGCACTT	GCCGAAGTGCTCACAGTGGAG	439	220202
<i>RBPJ</i>	AACAAATGGAACGCGATGGTT	GAGATGACTTTTATCCGCTTGCT	241	3516

Supplemental Table S5: Calorimetric data for RBPJ binding to *Atoh7* DNA sites.

<i>Atoh7</i> DNA	<i>Syringe</i>	<i>Cell</i>	$K (M^{-1})$	$K_d (\mu M)$	ΔG° (kcal/mol)	ΔH° (kcal/mol)	$-T\Delta S^\circ$ (kcal/mol)
	Hes1 Consensus	RBPJ	$1.1 \pm 0.6 \times 10^6$	1.05	-7.6 ± 0.2	8.8 ± 1.2	16.4 ± 0.9
	Site 1	RBPJ	$8.3 \pm 1.7 \times 10^5$	1.24	-7.5 ± 0.1	4.7 ± 0.2	12.2 ± 0.1
	Site 2	RBPJ	$3.4 \pm 1.7 \times 10^5$	3.64	-6.9 ± 0.3	3.7 ± 0.7	10.7 ± 0.4
	Site 3	RBPJ	n/a	>10	n/a	n/a	n/a
	Site 4	RBPJ	$1.3 \pm 0.4 \times 10^6$	0.81	-8.1 ± 0.4	5.1 ± 0.4	13.2 ± 0.4

All experiments were performed at 5°C, except for Site 3 (10°C). Values are the mean of at least three independent experiments and errors represent the standard deviations of multiple experiments. Weak binding was observed for Site 3, which only allowed for estimation of K_d . The Hes1 positive control DNA corresponds to the site – GTTACTGTGGGAAAGAAAG–.