

**Table 1. Retinoblastoma cell lines and associated patient characteristics**

Cell line	Laterality	Male / Female	Age at diag (months)	RB1 mutations <sup>1</sup>	MYCN Amplification
BCH-RB31	bilateral	F	19	c.1039dupT (blood)	Y
BCH-RB32	unilateral	F	22	46,XX,del(13)(q12q14) (blood)	N
BCH-RB33	bilateral	M	20	46,XY,del(13)(q14.1q14.2) (blood)	N
BCH-RB34	unilateral	M	26	None found in tumour	N
BCH-RB35	unilateral	F	26	None found in tumour <sup>2</sup>	N
BCH-RB36	unilateral	M	31	c.751C>T p.(Arg251Ter), c.1363C>t p.(Arg455Ter)	N
BCH-RB37	unilateral	M	25	c.184C>T p.(Gln62Ter), LOH	N
BCH-RB38	unilateral	M	15	c.1961-1G>T, RB1 whole gene deletion	N
BCH-RB39	unilateral	M	32	c.1060-1061delCA, LOH	N
BCH-RB40	unilateral	M	32	Hypermethylation of RB1 promoter	N
BCH-RB41	unilateral	F	21	n.d.	N
BCH-RB42	unilateral	M	14	Whole gene deletion, c.1383C>T p.(Arg455Ter)	Y
BCH-RB43	unilateral	M	52	Tumour whole gene deletion (homozygous)	N
BCH-RB44	unilateral	M	10	Whole gene deletion	N
BCH-RB45	bilateral	F	3	c.958 C>T p.(Arg320Ter) (blood)	N
BCH-RB46	bilateral	F	26	Deletion exon 24 to 27 (blood)	N
BCH-RB47	bilateral	F	23	Whole gene deletion	N
BCH-RB49	unilateral	F	28	c.1399C>T p.(Arg467Ter) blood), LOH	N
BCH-RB50	unilateral	M	18	Whole gene deletion	N
BCH-RB51	unilateral	F	15	c.2520+1G>C (mosaic), c.2053C>T p.(Gln685Ter)	N
BCH-RB52	unilateral	F	5	c.380+1G>A, c.2359C>T p.(Arg787Ter)	N
BCH-RB53	bilateral	F	34	c.763C>T p.(Arg255Ter)	N
BCH-RB54	unilateral	F	45	c.1251_1252delAA, LOH	N
BCH-RB55	unilateral	F	27	c.1363C>T p.(Arg455Ter), LOH	N
BCH-RB56	Unilateral	M	29	c.1454_1457 del CTTT p.(Ser458Tyrfs*9), LOH	N
BCH-RB57	unilateral	M	n.r.	c.1363C>T p.(Arg455Ter) mosaic in blood	N
BCH-RB58	unilateral	F	n.r.	n.d.	N
BCH-RB59	bilateral	M	n.r.	13q-	N

<sup>1</sup>Mutations taken from clinical reports. <sup>2</sup>Clinical report: no mutation found; this study (RNA-seq) found a chr13 rearrangement RB1-RCBTB2. n.r. : not recorded.

**Table 2. BCOR mutations in retinoblastoma cell lines**

Cell line	Male/female	Method <sup>1</sup>	% Mutation positive reads	Exon	cDNA NM_001123383.1	gDNA NG_008880.1	Protein (predicted) NP_001116855.1	LCL/Str <sup>2</sup>	Validation 2nd cell line <sup>3</sup>	Validation Tumour <sup>4</sup>
RB31	F	SS		13-14	c.5003_5237del	g.[127955_127957del;127961_128713del]	p.Asp1547Glyfs*15	wt	Y	Y
RB32	F	SS		4	c.1285C>T	g.107906C>T	p.Gln308*	wt	n.d.	Y <sup>A</sup>
RB33	M	RNA-seq	n.a.	-	None found	-	-	-	-	-
RB34	M	SS		1-15	c.(?_299)_(5536_?)del	g.(?_4909)_(130376_?)del	p.1_1721del	wt	Y	n.d.
RB35	F	RNA-seq		4-6	c.3601_3602ins[NC_00007.14:145323333;145323371;1010_3601]	g.(111358_1117730)ins[NC_00007.14:145323371;107631_(111358_1117730)]	p.Cys1080*	wt	n.d.	Y
RB36	M	SS		9-10	c.4109_4689del	g.119363_120216del	p.Leu1250Serfs*1	wt	Y	Y <sup>A</sup>
RB37	M	RNA-seq	93	8	c.3970dupG	g.118584dupG	p.Val1203Glyfs*8	wt	n.d.	Y <sup>A</sup>
RB38	M	RNA-seq	100	4	c.2687T>A	g.109308T>A	p.Leu775*	wt	Y	N
RB39	M	SS		7	c.3703G>T	g.117832G>T	p.Glu1114*	wt	Y	n.d.
RB40	M	RNA-seq	n.a.	-	None found	None found	-	-	-	-
RB41	F	SS		-	None found	None found	-	-	-	-
RB42	M	SS		-	None found	None found	-	-	-	-
RB43	M	RNA-seq		1-15	c.1_6390del	g.(?_4909)_(130376_?)del	No protein	wt	Y	n.d.
RB44	M	SS		2-5	no expression	g.(98055_98478)_(110641_10766)del	(p.1_1017del)	wt	No 2nd line	No tissue
RB45	F	RNA-seq	n.a.	-	None found	None found	-	-	-	-
RB46	F	RNA-seq	37	7	c.3744_3765del	g.117873_117894del	p.Ser1127Argfs*25	wt	No 2nd line	N
RB47	F	SS		2,3,partial 4	c.[324_2837del;324_2754del]ins[NG_008880.1:g62491_62968]]	g.[(5314_?)_(?_62491)del;62969_109375del]	p.?	wt	Y	No tissue
RB49	F	SS		-	None found <sup>5</sup>	None found <sup>5</sup>	-	-	-	-
RB50	M	SS		7	c.3703delG	g.117832delG	p.Glu1114Serfs*45	no cells	n.d.	Y

Variants are reported relative to the reference sequence LRG\_627(BCOR): genomic sequence source: NG\_008880.1; transcript LRG\_627t1 sequence source: NM\_001123383.1, initiation codon at position 364; protein sequence source: NP\_001116855.1. <sup>1</sup>SS: Sanger sequencing. <sup>2</sup>Genotype of lymphoblastoid cell line or ocular stromal cells: wt: wild type; <sup>3</sup>Mutation present in both cell lines established from the original tumour: Y: yes, n.d.: not done. <sup>4</sup>Cell line mutation present in tumour: Y: yes, N: no, <sup>A</sup>both mutant and wildtype sequence present; <sup>5</sup>RB49 showed significantly reduced BCOR expression (Figure 2), but no mutation was detected.

**Table 3.** GO categories associated with altered gene expression in BCOR-mutated retinoblastoma cell lines

**DOWN-REGULATED GENES**

Annotation Cluster	Enrichment Score	Fold Enrichment	P value
Category	Term		
<b>Annotation Cluster 1</b>	<b>Enrichment Score: 10.01</b>		
GOTERM_BP_FAT	GO:0050953~sensory perception of light stimulus	6.48	6.95E-14
GOTERM_BP_FAT	GO:0007601~visual perception	6.48	6.95E-14
GOTERM_BP_FAT	GO:0007600~sensory perception	2.05	1.92E-04
<b>Annotation Cluster 2</b>	<b>Enrichment Score: 3.54</b>		
GOTERM_CC_FAT	GO:0000267~cell fraction	1.91	1.93E-04
GOTERM_CC_FAT	GO:0005624~membrane fraction	2.07	2.52E-04
GOTERM_CC_FAT	GO:0005626~insoluble fraction	2	4.60E-04
<b>Annotation Cluster 3</b>	<b>Enrichment Score: 3.40</b>		
GOTERM_BP_FAT	GO:0009583~detection of light stimulus	10.63	8.12E-06
GOTERM_BP_FAT	GO:0007602~phototransduction	10.99	3.41E-05
GOTERM_BP_FAT	GO:0009581~detection of external stimulus	6.3	8.42E-05
GOTERM_BP_FAT	GO:0009582~detection of abiotic stimulus	6.38	2.40E-04
GOTERM_BP_FAT	GO:0009416~response to light stimulus	3	1.72E-02
GOTERM_BP_FAT	GO:0009314~response to radiation	2.33	3.96E-02
<b>Annotation Cluster 4</b>	<b>Enrichment Score: 2.38</b>		
GOTERM_BP_FAT	GO:0032496~response to lipopolysaccharide	5.39	6.83E-04
GOTERM_BP_FAT	GO:0002237~response to molecule of bacterial origin	4.82	1.32E-03
GOTERM_BP_FAT	GO:0009617~response to bacterium	2.15	7.92E-02
<b>Annotation Cluster 5</b>	<b>Enrichment Score: 2.10</b>		
GOTERM_MF_FAT	GO:0010853~cyclase activator activity	23.48	6.51E-03
GOTERM_MF_FAT	GO:0030250~guanylate cyclase activator activity	23.48	6.51E-03
GOTERM_MF_FAT	GO:0030249~guanylate cyclase regulator activity	20.54	8.57E-03
GOTERM_MF_FAT	GO:0010851~cyclase regulator activity	18.26	1.09E-02

**UP-REGULATED GENES**

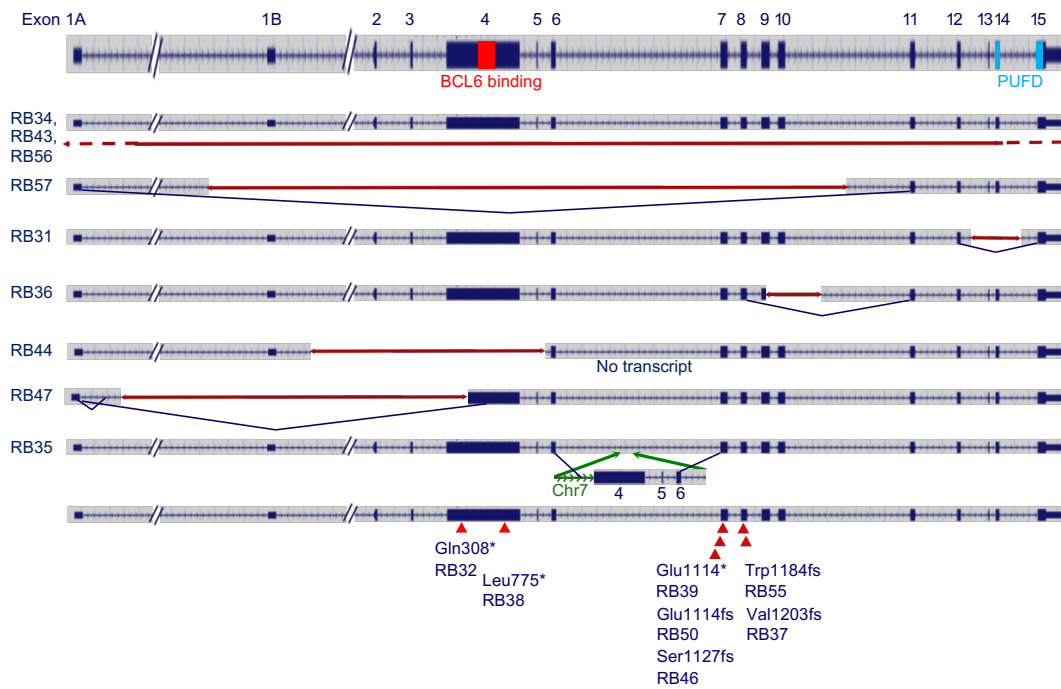
Annotation Cluster	Enrichment Score	Fold Enrichment	P value
Category	Term		
<b>Annotation Cluster 1</b>	<b>Enrichment Score: 6.76</b>		
GOTERM_BP_FAT	GO:0007409~axonogenesis	5.74	5.32E-09
GOTERM_BP_FAT	GO:0048812~neuron projection morphogenesis	5.2	2.51E-08
GOTERM_BP_FAT	GO:0048858~cell projection morphogenesis	4.76	4.06E-08
GOTERM_BP_FAT	GO:0032990~cell part morphogenesis	4.56	8.20E-08
GOTERM_BP_FAT	GO:0048666~neuron development	3.96	8.26E-08
GOTERM_BP_FAT	GO:0031175~neuron projection development	4.33	4.07E-07
GOTERM_BP_FAT	GO:0030030~cell projection organization	3.49	1.36E-06
GOTERM_BP_FAT	GO:0000902~cell morphogenesis	3.44	3.13E-06
GOTERM_BP_FAT	GO:0032989~cellular component morphogenesis	3.08	1.58E-05
<b>Annotation Cluster 2</b>	<b>Enrichment Score: 5.79</b>		
GOTERM_MF_FAT	GO:0022836~gated channel activity	4.19	6.31E-08
GOTERM_MF_FAT	GO:0005216~ion channel activity	3.52	6.12E-07
GOTERM_MF_FAT	GO:0022838~substrate specific channel activity	3.41	1.02E-06
GOTERM_MF_FAT	GO:0015267~channel activity	3.29	1.81E-06
GOTERM_MF_FAT	GO:0022803~passive transmembrane transporter activity	3.29	1.89E-06

**Table 4.** Genes associated with specific retinal lineages that are upregulated in BCOR mutated retinoblastoma cell lines.

Gene	Fold Change	BaseMean <sup>1</sup>	Cell Types <sup>2</sup>
BARHL2	24.3	288	Amacrine, Ganglion
BHLHE22	31.3	1740	Amacrine, Bipolar
EBF3	6.6	14258	Ganglion, Amacrine, Bipolar
NEUROD2	92.5	624	Ganglion, Amacrine
NOTCH1	8.9	3616	Retinal progenitor cells
ONECUT1	9.6	2260	Horizontal, cone
POU4F2	4.6	1707	Ganglion
SALL3	11.2	966	Horizontal, Cone,
SATB1	12.6	2997	Ganglion
SATB2	8.9	1349	Amacrine, Ganglion

<sup>1</sup>BaseMean is the the average of the normalized read counts taken over all samples (log normalized scale) and provides an indication of the overall level of expression of each gene (GAPDH BaseMean: 232817 Fold change: 1.03).

<sup>2</sup>Retinal cell type markers are as reported<sup>70-74</sup>



**Figure 1.** BCOR functional domains and BCOR mutations in RB cell lines. Red lines: deletions; Green lines: inserted sequence. Red triangles: small sequence alterations. Dark blue lines: aberrant splicing. Two transcripts were identified for RB47: in one, exon 1A was spliced to an internal position (c.2838) in exon 4; in the second, exon 1A was spliced to a segment of intron 1A which was joined to an internal position (c.2755) in exon 4 as a genomic rearrangement. The RB35 cell line showed a genomic rearrangement between an intergenic segment of chromosome 7q35 and BCOR exon 4.

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RB46 - Deletion (22bp) Exon 7 (direct & inverted repeats)
AGACCAGTGGCCTCGGACATGCTCACAGCCCAACCCCTCCGGTGGACAGGAACGCAAGTCTCAGGTGACAGCAGCCAC NG_008880 Exon 7
|||||
AGACCAGTGGCCTCGGACATGCTCACAG.....AAACGCAAGTCTCAGGTGACAGCAGCCAC RB46

RB55 - Deletion (19bp) Exon 8 (direct and inverted repeats)
AAACAACGCCACTTGCCTGACCTTAGAGAACGATGGGACGACGAGTGTGCGCAGCAGATGGCAAACCTGGCCGGCAAAG NG_008880 Exon 8
|||||
AAACAACGCCACTTGTCTGACCTTAGAGAAA.....GGCAGCAGATGGCAAACCTGGCCGGCAAAG RB55

RB31 - Deletion (759bp) exons 13 & 14 (slippage and inverted repeat; mirror repeat)
TGCCACGTGGCTCTGCCATCATTTGCTCTCAGCAGTAGATTTTATCTGTGTTTTTATTTTTTATT NG_008880 Intron 12
|||||
TGCCACGTGGCTCTGCCATCATTTGTTCT...CAGTAGTGAACCTTATGGAAGGTCAGCACCTTGG RB31
CTTAAGTCACTCCCCAGAGAAGTGGGTGACTGGAACCTTATGGAAGGTCAGCACCTTGG NG_008880 Intron 14

RB35 - Chr7-BCOR Rearrangement (4 base homology, direct repeat, mirror repeat)
ATGAAAATACAAGAGAATGATTAATTTTTTAAACATTACATTTAAACAAGTCAAGAATGTG Chr7
|||||
ATGAAAATACAAGAGAATGATTAATTTTTT...ACATGTCAAGGCCTTGCTACCTCAGCAGT RB35
TTTCTGACTCGCCAAATAAGTATTCTACTGACATGTACAGGCCTTGCTACCTCAGCAGT NG_008880 Exon 4

RB44 - Deletion Exons 2 - 5
The deletion boundaries were mapped to within 444bp and 125bp of 5' and 3' breakpoints respectively
but it was not possible to amplify a junction fragment. This may be a complex insertion/deletion event

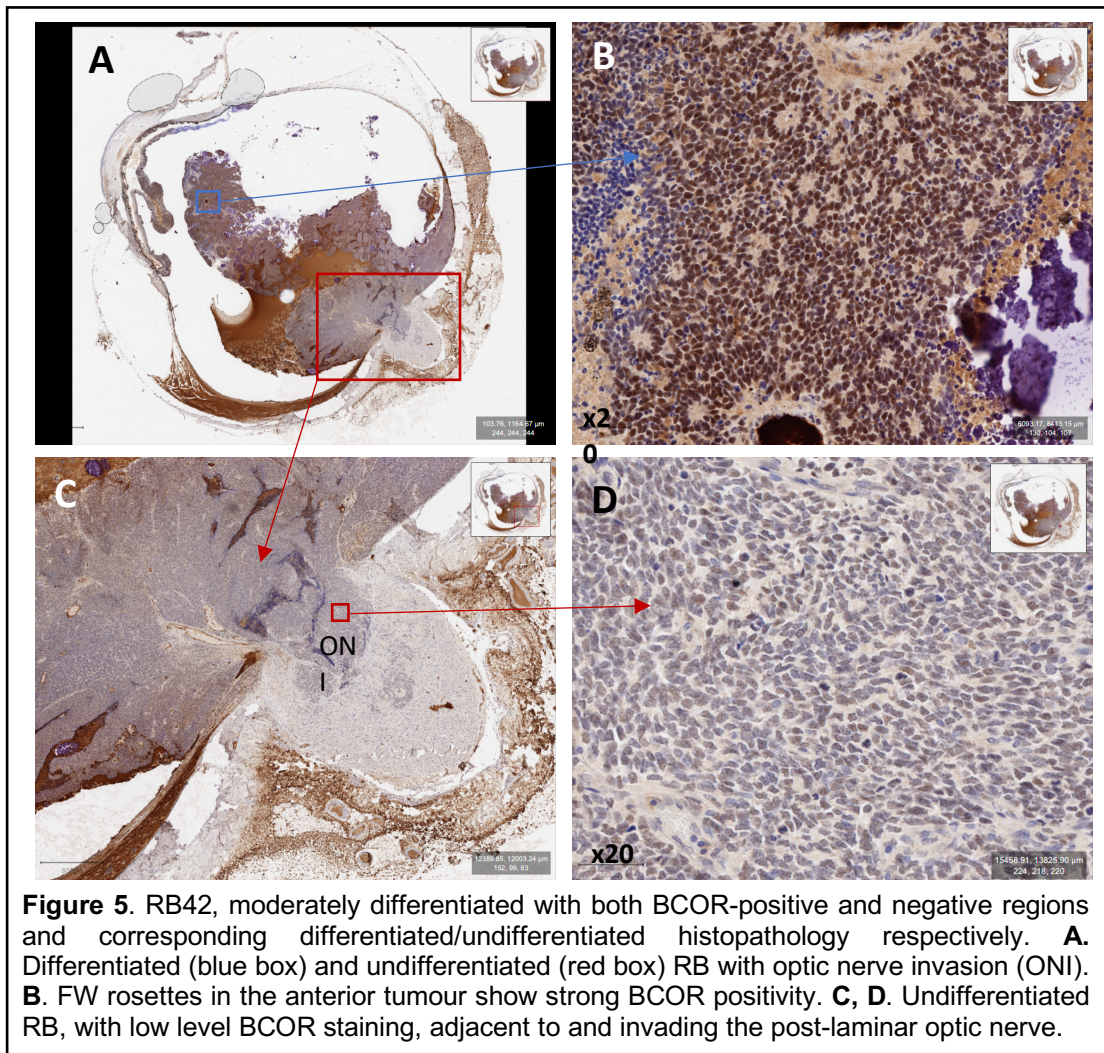
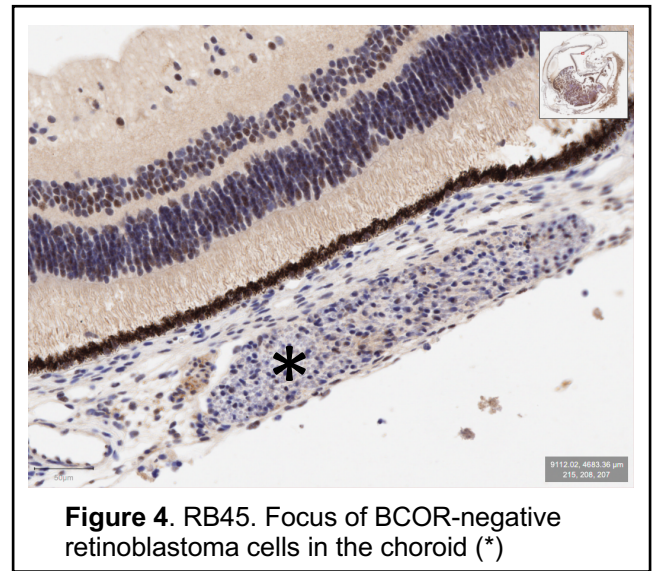
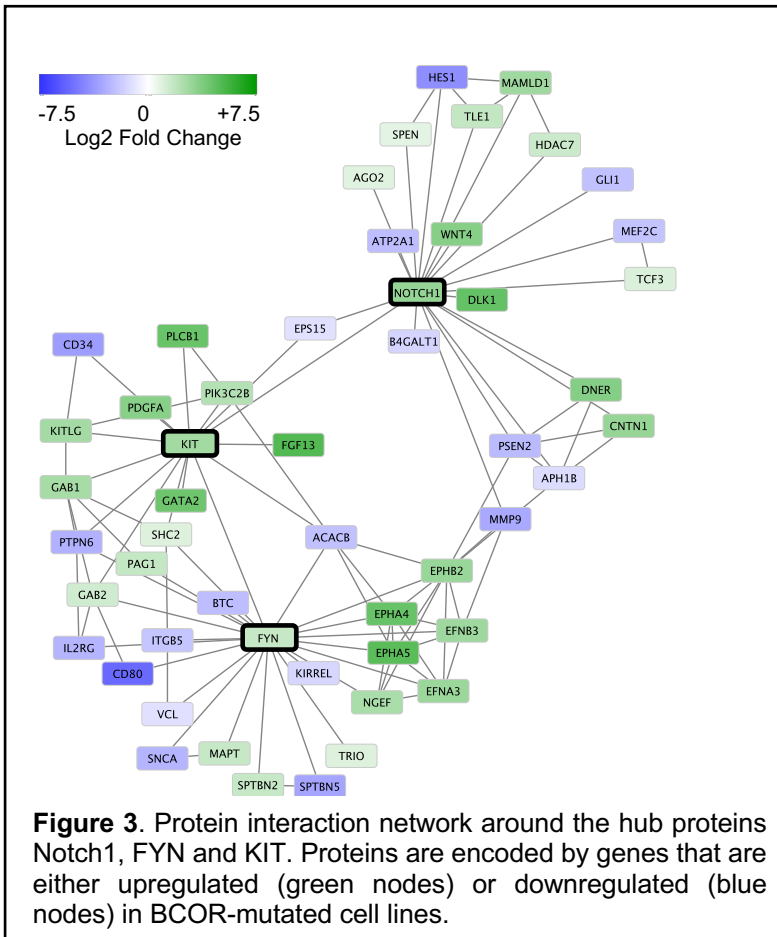
RB47 - Deletion (46kb) Exons 2, 3 & partial 4 (inverted/direct repeat)
CCATCTGACTTATAAACAACCTCAGATTAACCTTTGCCCACTTTAAACTTGACATAGG NG_008880 Intron 1A
|||||
CCATCTGACTTATAAACAACCTCAGATTAAGCTCAAAGCGACAAGCTTGCTACGTAGAC RB47
AACCCCAACCTGGAATCAAGGGAAGACTGTTGTCAAAGCGACAAGCTTGCTACGTAGAC NG_008880 Exon 4

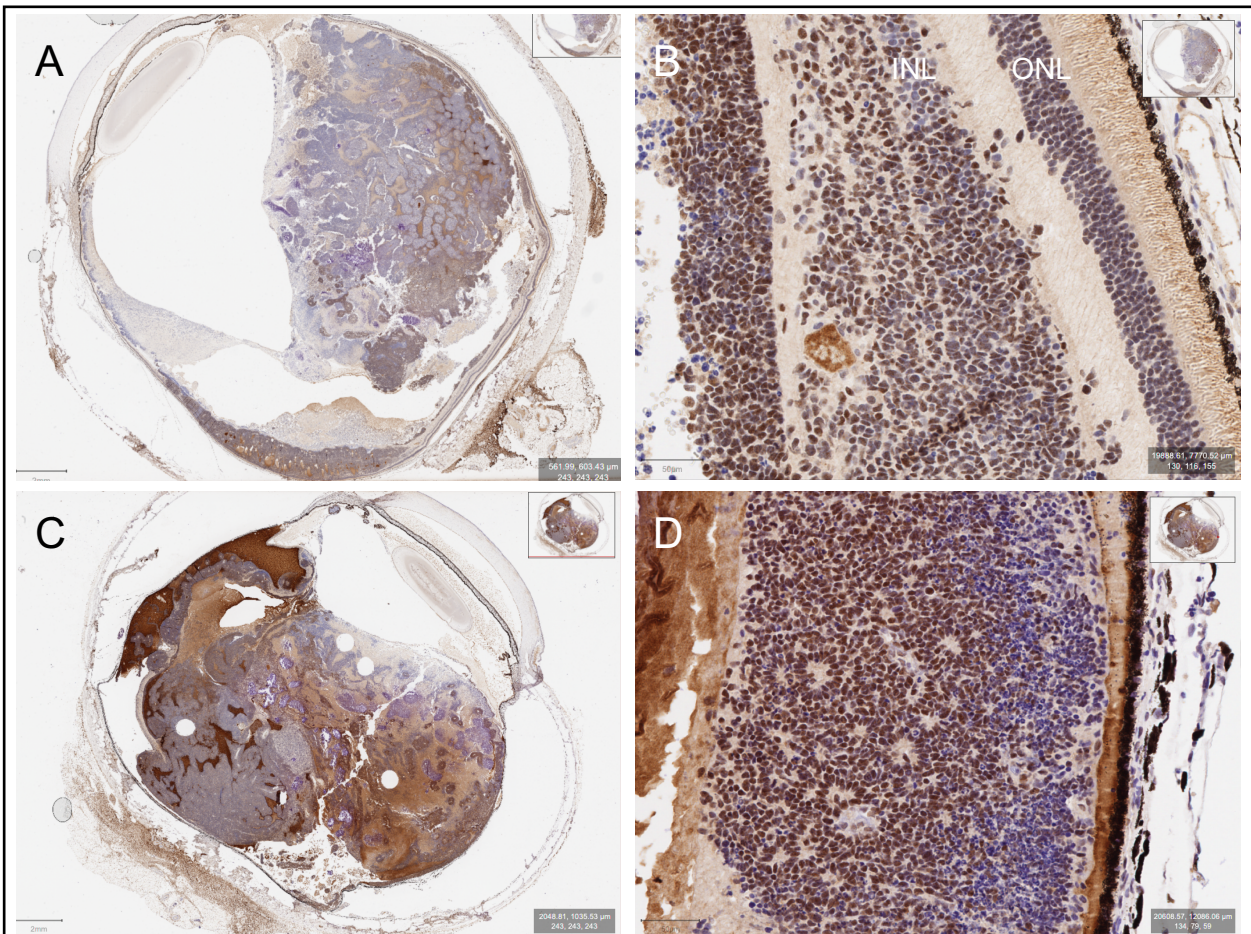
RB36 - Deletion (853bp) Exons 9 & 10 (interrupted homology; G-rich sequence)
CAGCCAACTGCGCACCAGCCTCCAGCCGCTGCAAAACAGCAGAAAATTAAGAAAAC NG_008880 Exon 9
|||||
CAGCCAACTGCGCACCAGCCTCCAGGCC...GGGCTGCAGGCTGGGGGCGGGCCTGCGGACTGTGCTGGGCGAGGGAGGG RB36
TGAGGTGTGCTCCCTGTGGGGCCTCCTGCGGCTGCAGGCTGGGGGCGGGCCTGCGGA NG_008880 Intron 10

RB57 - Deletion (62kb) Exons 1B - 10 (2 direct repeats & inverted repeat)
TCTGATGTGGCCAAAAGCTTCCATCATGCTAAGCAGTTCAAAACAGTCCCGCTCGTCAGG NG_008880 Intron 1A
|||||
TCTGATGTGGCCAAAAGCTTCCATCATGCTTTTGTCTATTACATACCAGCTTGTTCCTTAT RB57
ACTAGTAGTATTTTTGGTTGTATGTGACTTTGTCTATTACATACCAGCTTGTTCCTTAT NG_008880 Intron 10

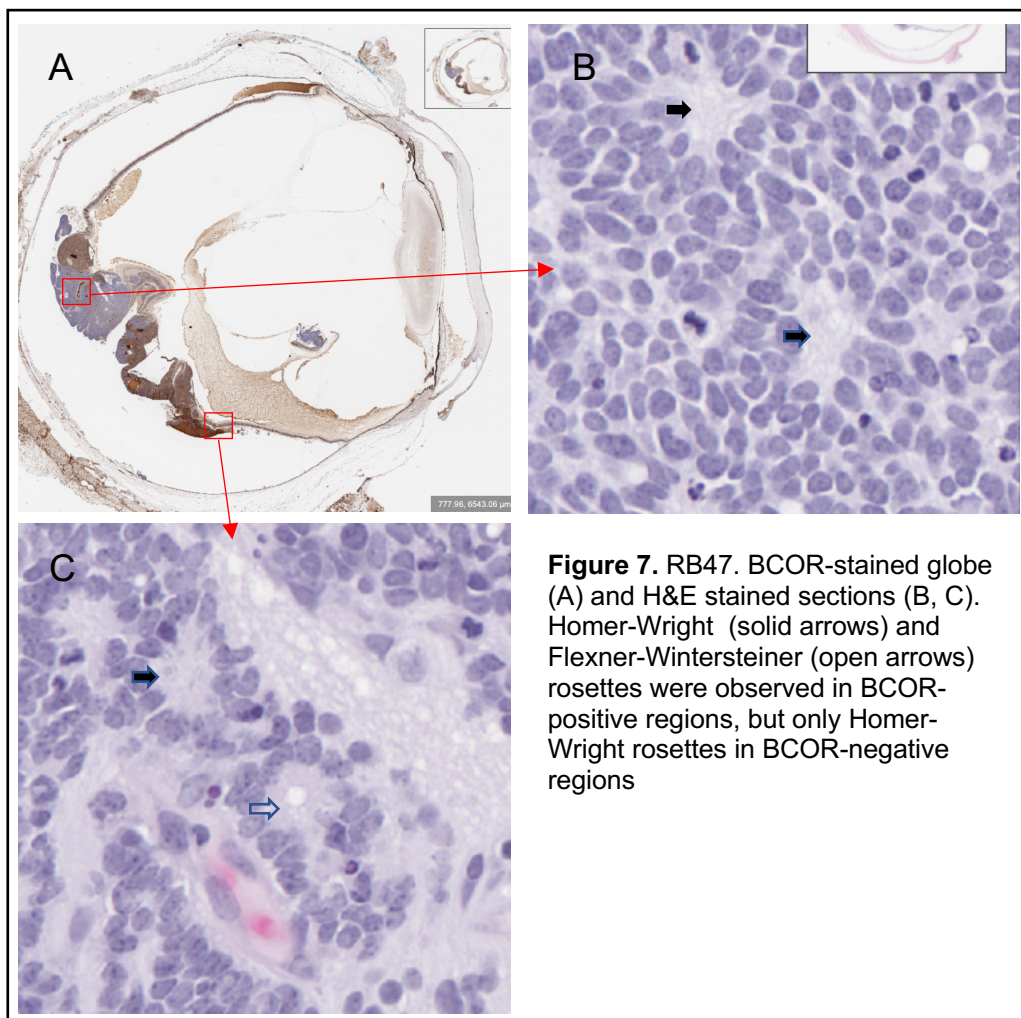
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**Figure 2.** Sequence context of BCOR intragenic break-points.





**Figure 6.** Retinoblastomas RB49 and RB33 are mostly BCOR-negative, but contain BCOR-positive tumour cells within the inner nuclear layer and the subretinal region (A, B: BCH-49; C, D: BCH-33).



**Figure 7.** RB47. BCOR-stained globe (A) and H&E stained sections (B, C). Homer-Wright (solid arrows) and Flexner-Wintersteiner (open arrows) rosettes were observed in BCOR-positive regions, but only Homer-Wright rosettes in BCOR-negative regions

