Non-Synonymous, Synonymous, and Non-Coding Nucleotide Variants Converge on Mitotic, RNA Processing, and PRC1 Dysregulation During Retinoblastoma Progression

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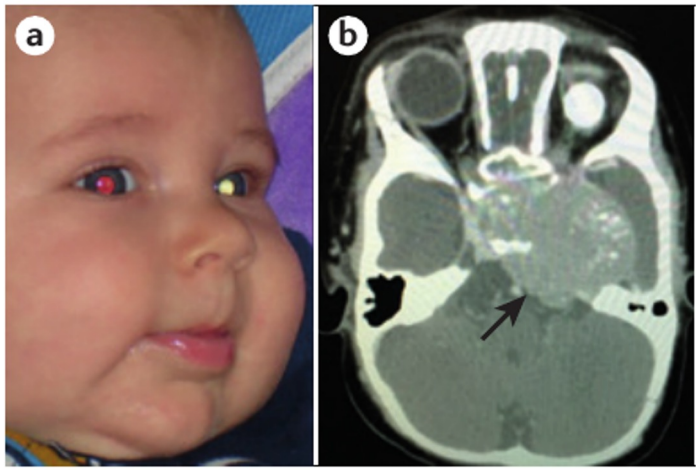
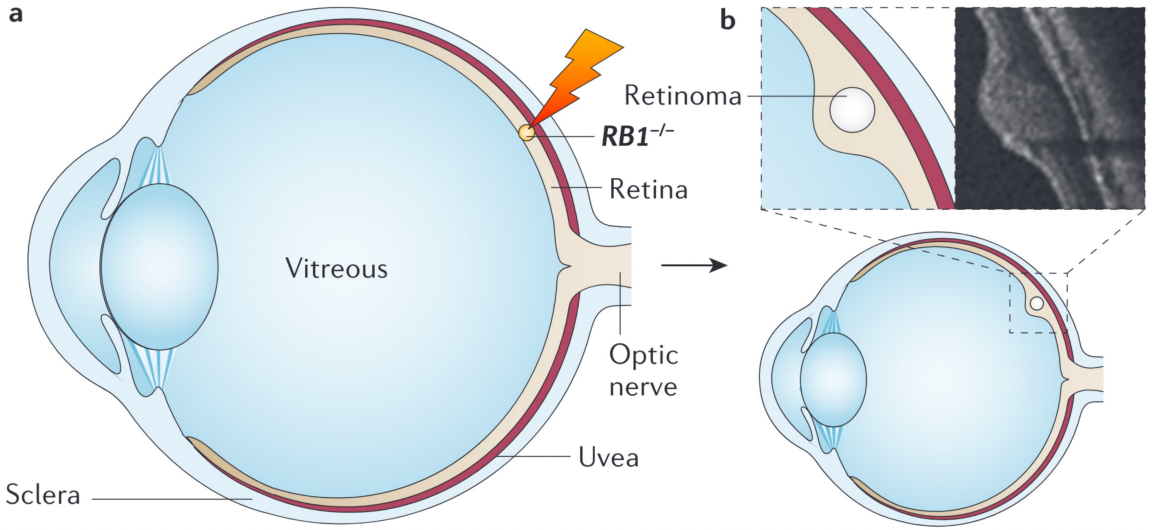
Cobrinik Laboratory

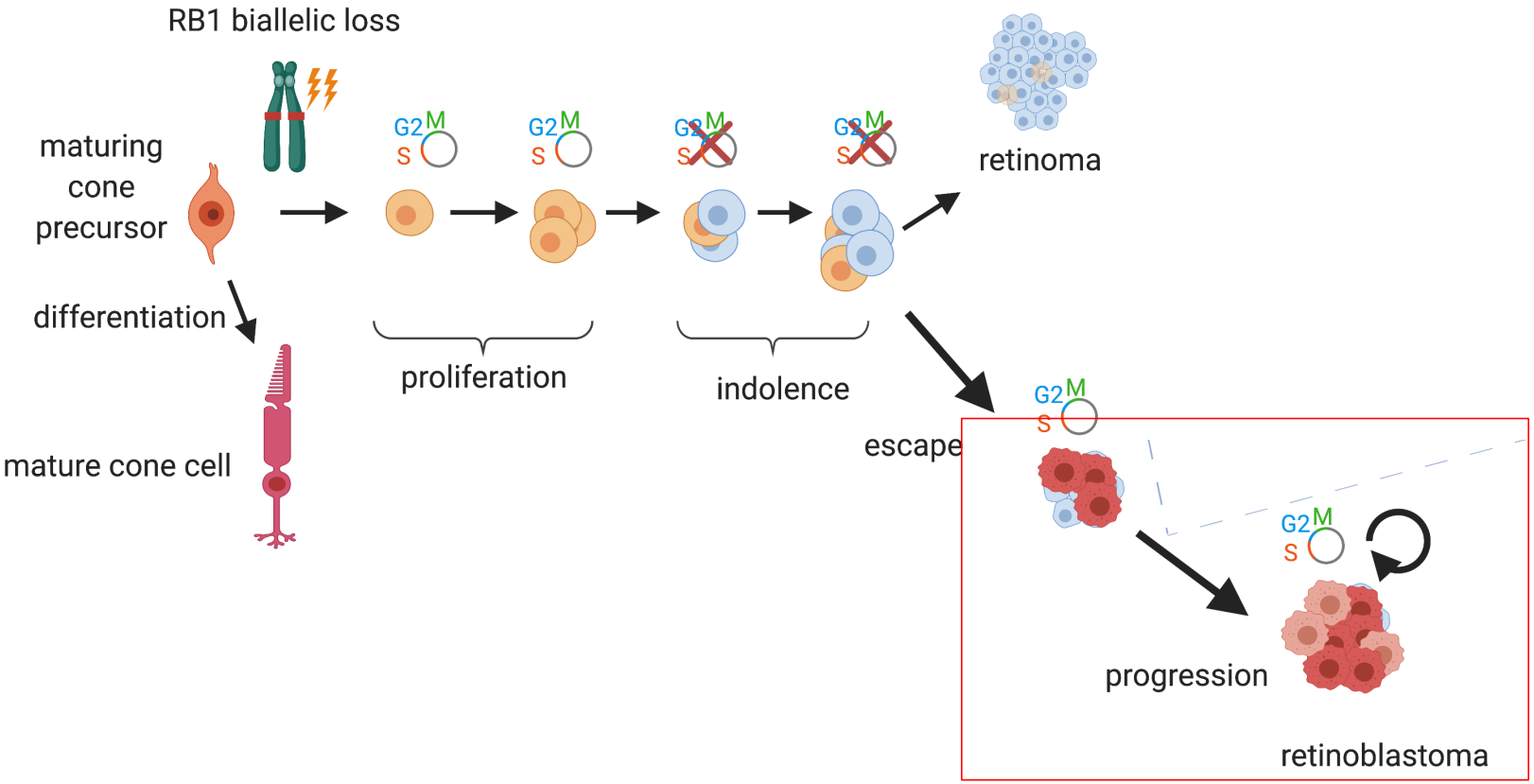
Retinoblastoma arises during retinal development





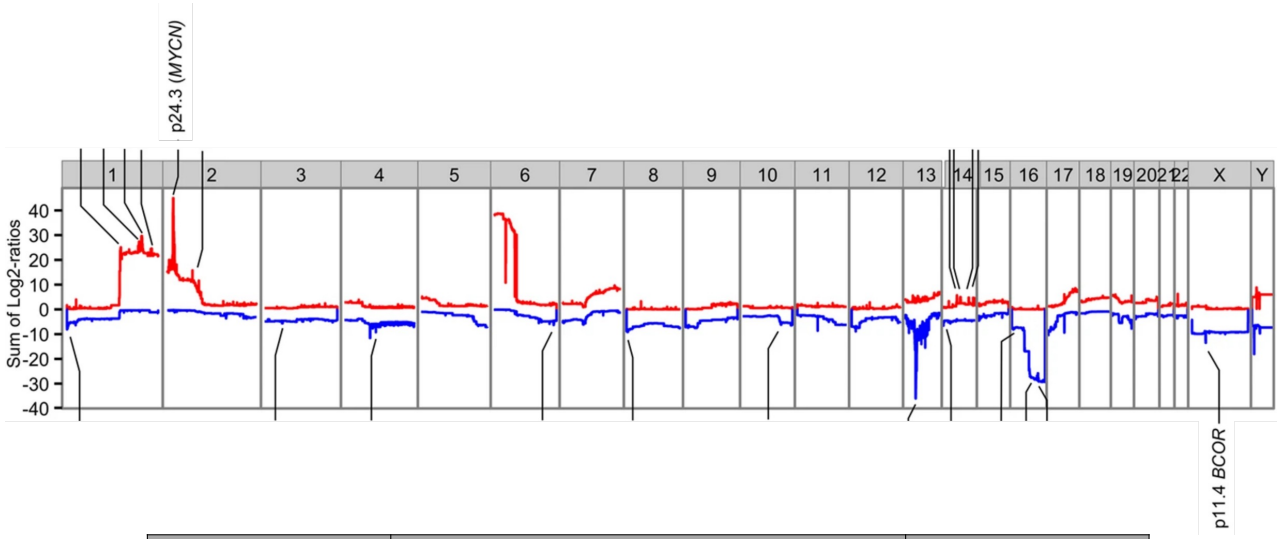
Dimaras, H., Corson, T.W., Cobrinik, D. et al. 2015 Retinoblastoma. Nat Rev Dis Primers *1*, 15021.

Tumor progression occurs via the accumulation of SCNAs and SNVs



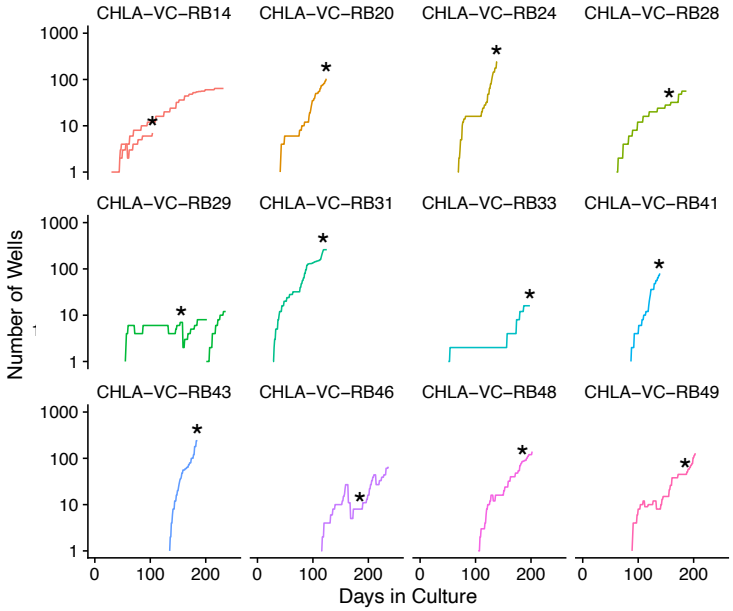
Adapted from Singh, H.P. et al. 2018 Proc Natl Acad Sci

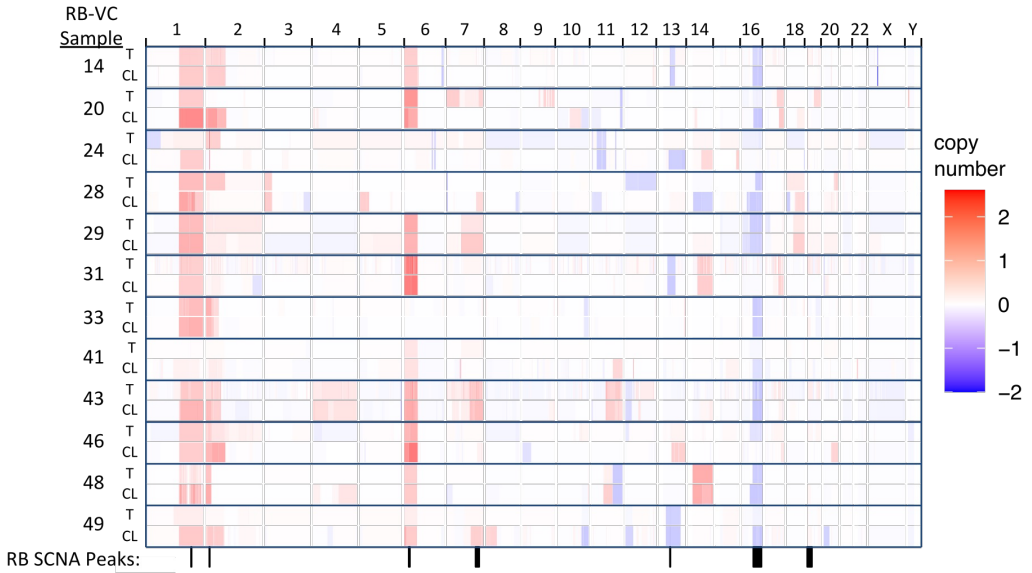
Some prior evidence of recurrent mutations



Gene	Type	VAF
BCOR	stopgain SNV	0.92
BCOR	nonsynonymous SNV	0.46
BCOR	frameshift deletion	0.5
BCOR	stopgain SNV	0.38
BCOR	frameshift deletion	0.19
CREBBP	nonsynonymous SNV	0.17
CREBBP	nonframeshift deletion	0.27

Cell Lines

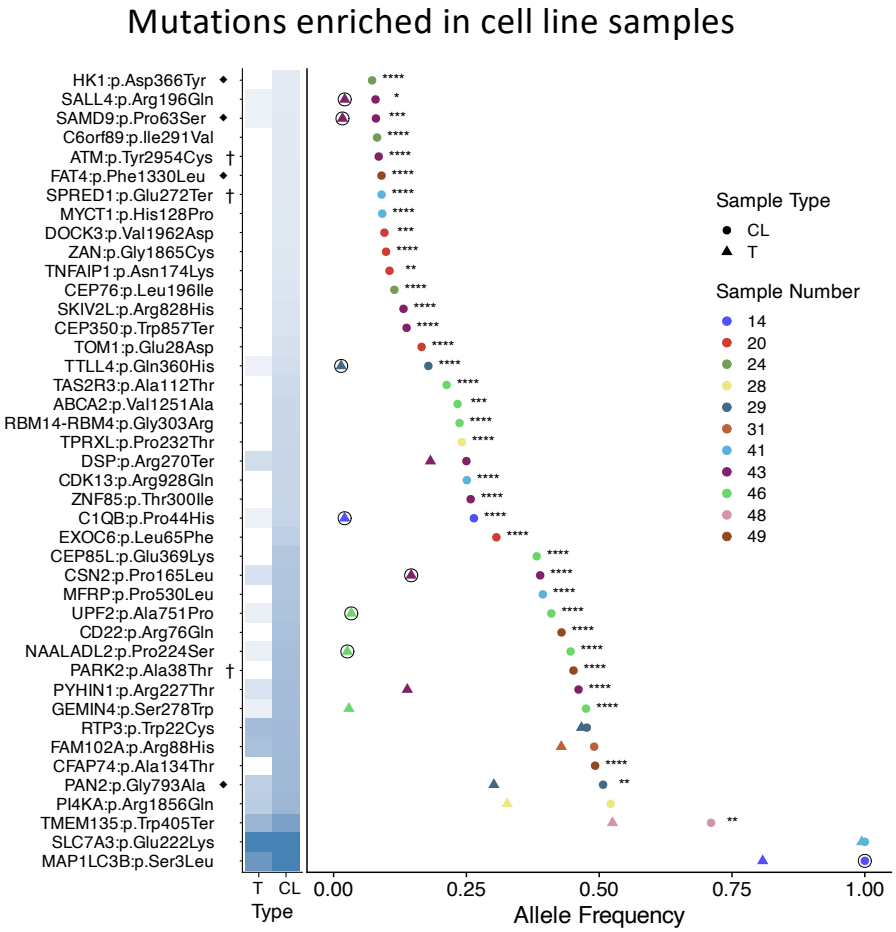

CHLA–VC–RB14
CHLA–VC–RB20
CHLA–VC–RB24
CHLA–VC–RB28
CHLA–VC–RB29
CHLA–VC–RB31
CHLA–VC–RB33
CHLA–VC–RB41
CHLA–VC–RB43
CHLA–VC–RB46
CHLA–VC–RB48
CHLA–VC–RB49
Number of Wells
1000
100
10
1
0
100
200
Days in Culture

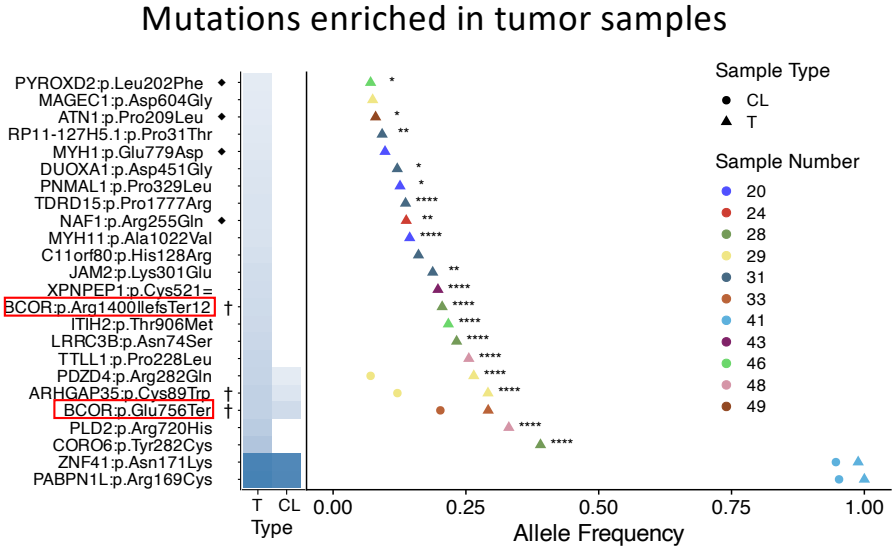

RB-VC
Sample
1 2 3 4 5 6 7 8 9 10 11 12 13 14 16 18 20 22 X Y
14
20
24
28
29
31
33
41
43
46
48
49
T
CL
RB SCNA Peaks:
copy
number
2
1
0
−1
−2

Preliminary study: whole exome sequencing

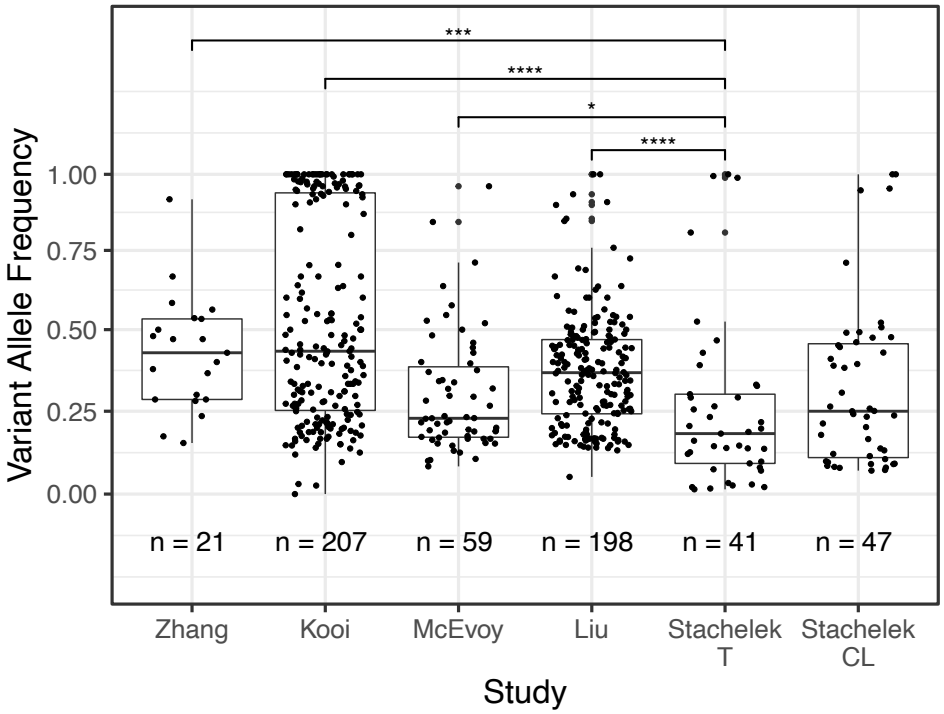
- 12 matched tumor, cell line, and normal samples
- Somatic variants in tumor (T) and cell line (CL) by comparison to normal
- Somatic copy number alterations (SCNAs)
- Patterns in SNVs and SCNAs across patients and sample types

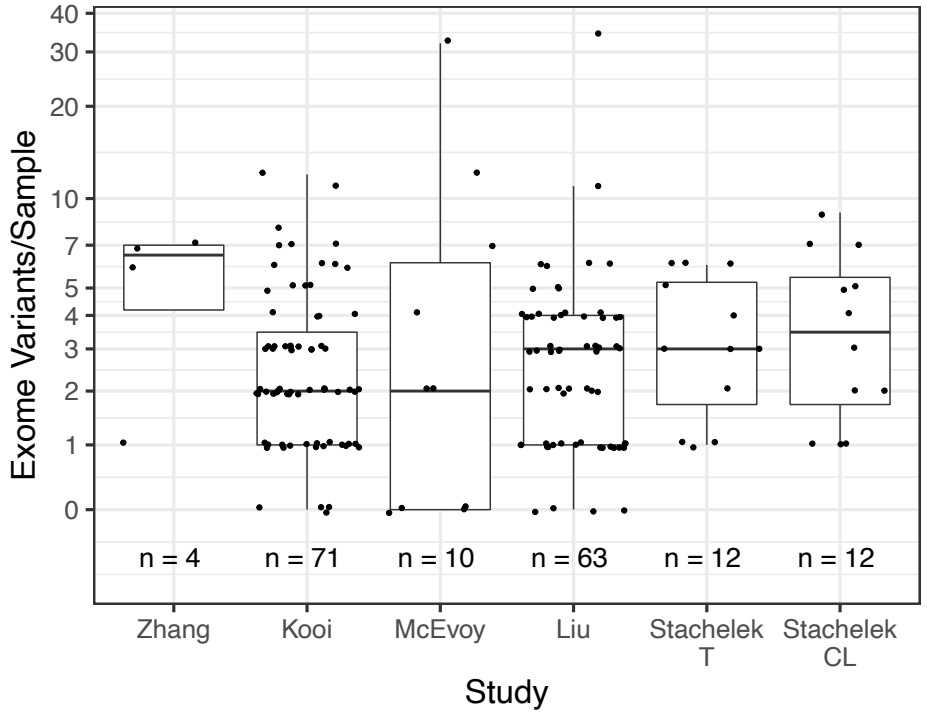
Identified snvs/indels in 12 WES tumor and cell line samples



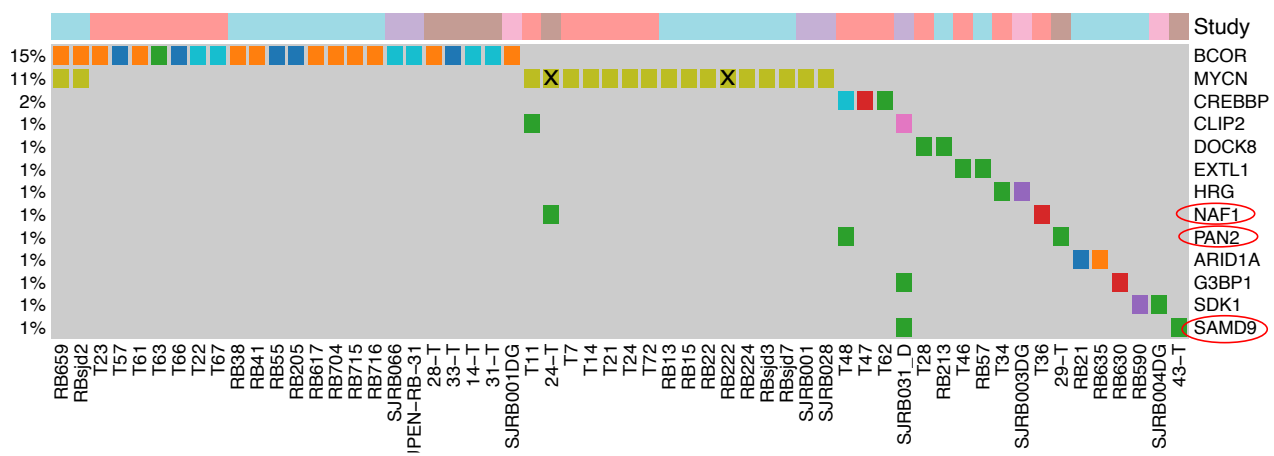


Subclonal variants common across WES/WGS studies





Recurrently mutated genes secondary to RB1



Alterations

- stop_gained
- frameshift_variant
- missense_variant
- inframe_indel
- synonymous_variant
- three_prime_UTR
- focal_amplification
- focal_deletion
- other_mutation
- fusion
- truncating_driver

Study

- Kooi et al.
- Liu et al.
- McEvoy et al.
- Stachelek et al.
- Zhang et al.

Afshar, et al. (2019) Ophthalmology.
Gröbner, S.N. et al. (2018) Nature
Kooi et al. (2016) Sci Rep
Liu et al. (2021) Nat Commun
McEvoy et al. (2014) Oncotarget
Zhang et al. (2012) Nature

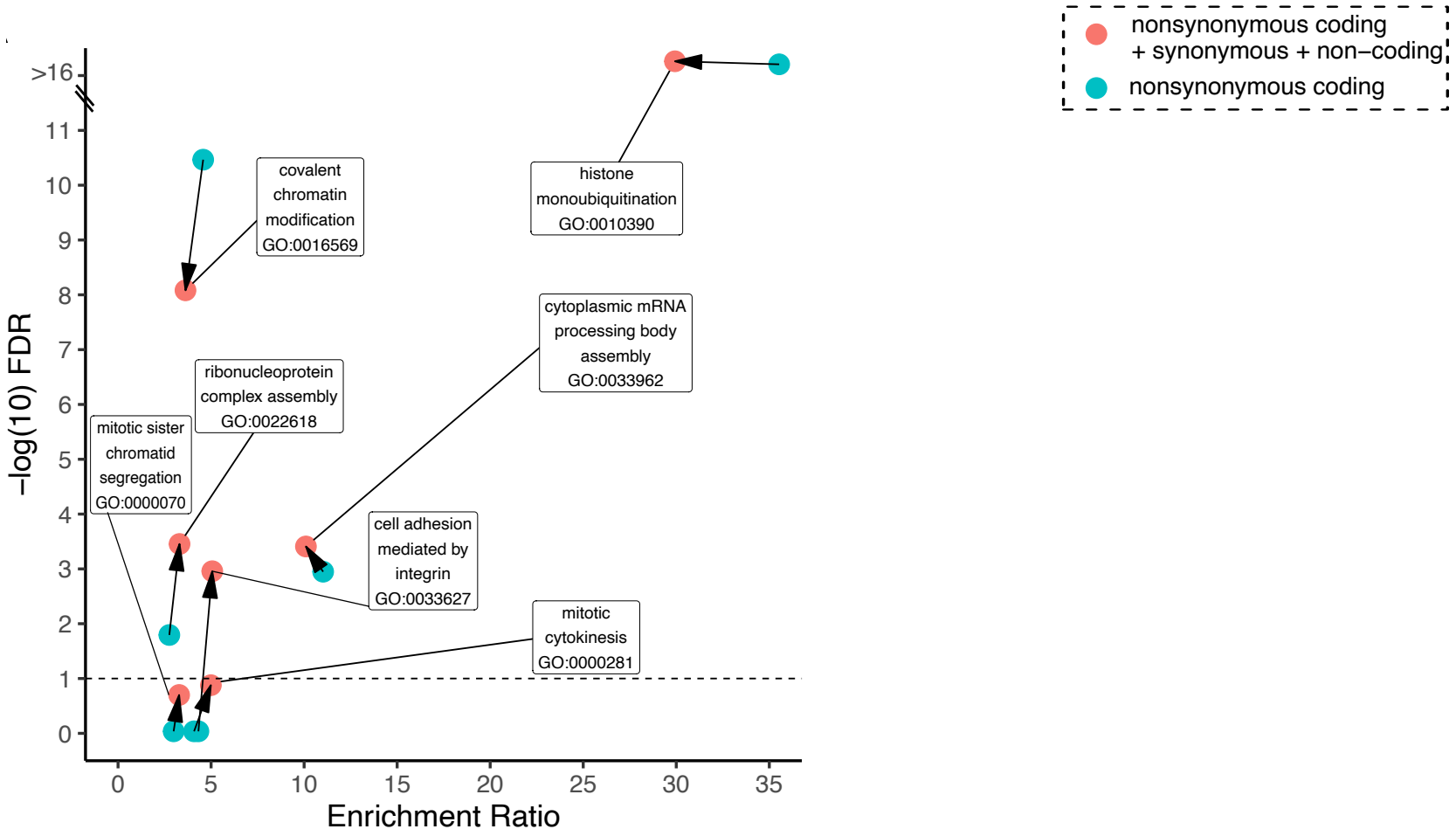
Ontologies over-represented among somatically variants in 161 retinoblastoma tumors

geneSet	Ontology Description	FDR	Variant Genes in Retinoblastoma Tumors
GO:0010390	Histone monoubiquitination	< 1E-16	***BCOR* (18);** *DDB1; RNF20; PCGF3*
GO:0016569	Covalent chromatin modification	8.29E-09	***BCOR* (18); *CREBBP* (2);** *BRCA2; BRMS1; CHD1; DDB1; EHMT1; EYA1; HDAC10; HIST1H1E; KAT6A; KDM8; NSD1; PADI4; PRKCA; RNF20; SUPT6H; TAF1; TAF1L; TAF9; PCGF3, HDAC9*
GO:0022618	Ribonucleoprotein complex assembly	3.51E-04	***G3BP1* (2); *NAF1* (2); *PAN2* (2);** *CNOT1; CNOT2; EIF3E; EIF4B; GEMIN4; NLE1; TAF9; CELF3; DYNC1H1; GEMIN8; RPF2; USP4*
GO:0033962	Cytoplasmic mRNA processing body assembly	3.90E-04	***PAN2* (2);** *CNOT1; CNOT2; DYNC1H1*
GO:0033627	Cell adhesion mediated by integrin	1.10E-03	***HRG* (2);** *CRK; ITGB4; ITGB6; ITGBL1; ACER2; ITGB2*
GO:0000281	Mitotic cytokinesis	0.132	*BRCA2; NUP62; SEPT10; SPTBN1; STAMBP; UNC119; ANK3; INCENP; MYH10; ZFYVE26*
GO:0000070	Mitotic sister chromatid segregation	0.199	*BOD1; BUB1B; DYNC1LI1; HIRA; KIF14; NUP62; PIBF1; PRC1; CDC14B (2); INCENP; RAB11A; TTN*

Bold, recurrently altered (number of recurrences)
Red, non-protein-altering variant.

Underlined, genes included in UCSF500.
Double underlined, genes included in UCSF500 and MSK-IMPACT

Ontologies reflect contribution of synonymous and noncoding variants



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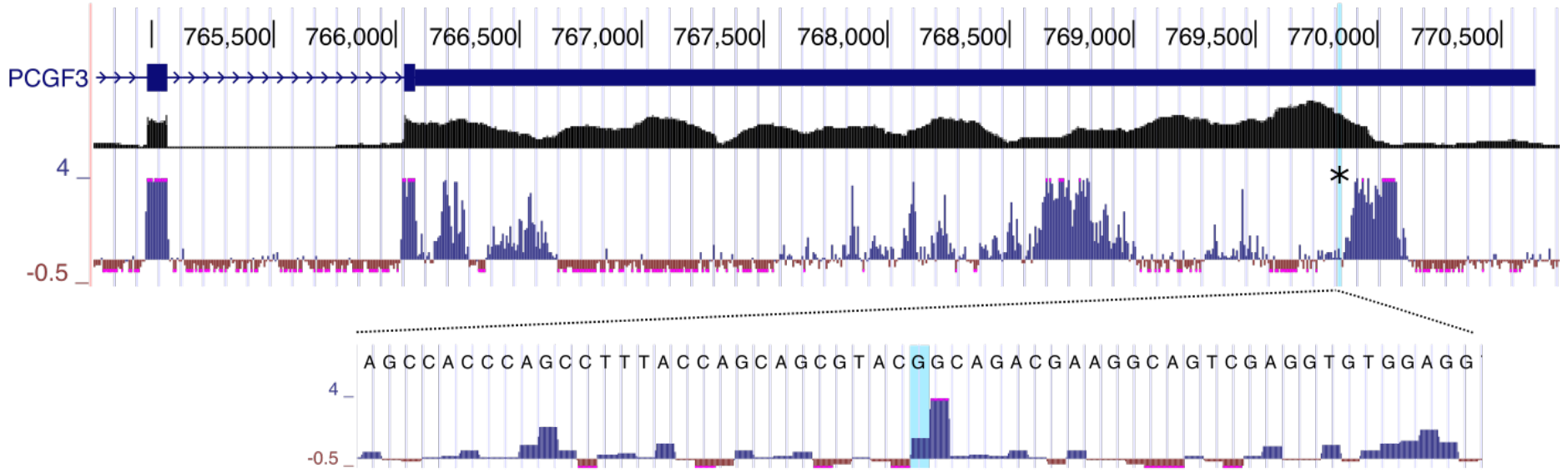
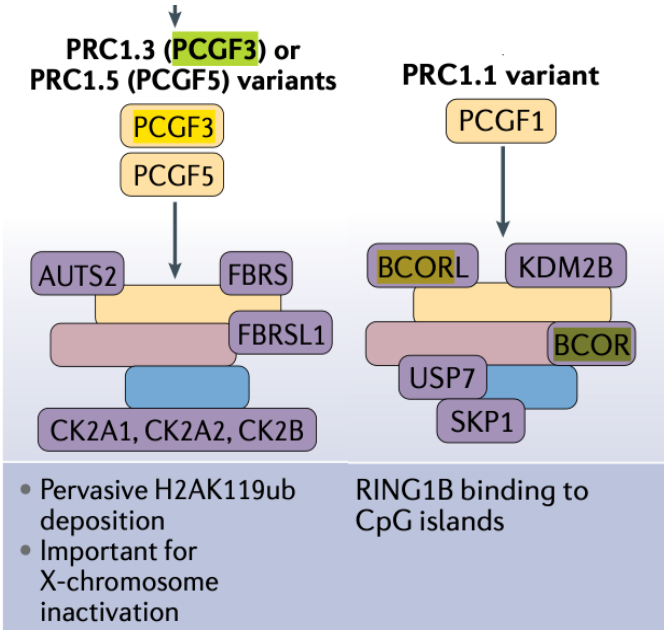
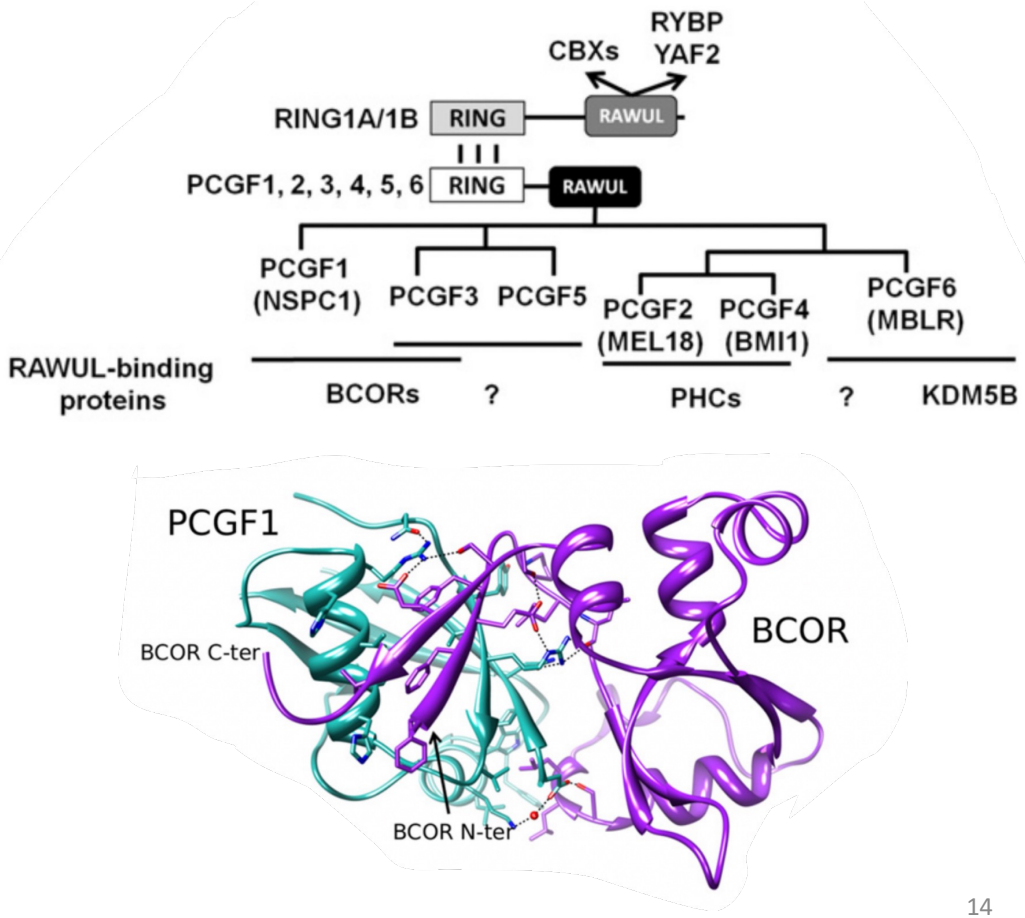
PCGF3 noncoding variant in 3’ UTR is expressed in retinoblastoma and highly conserved



Covalent chromatin modification: PCGF3

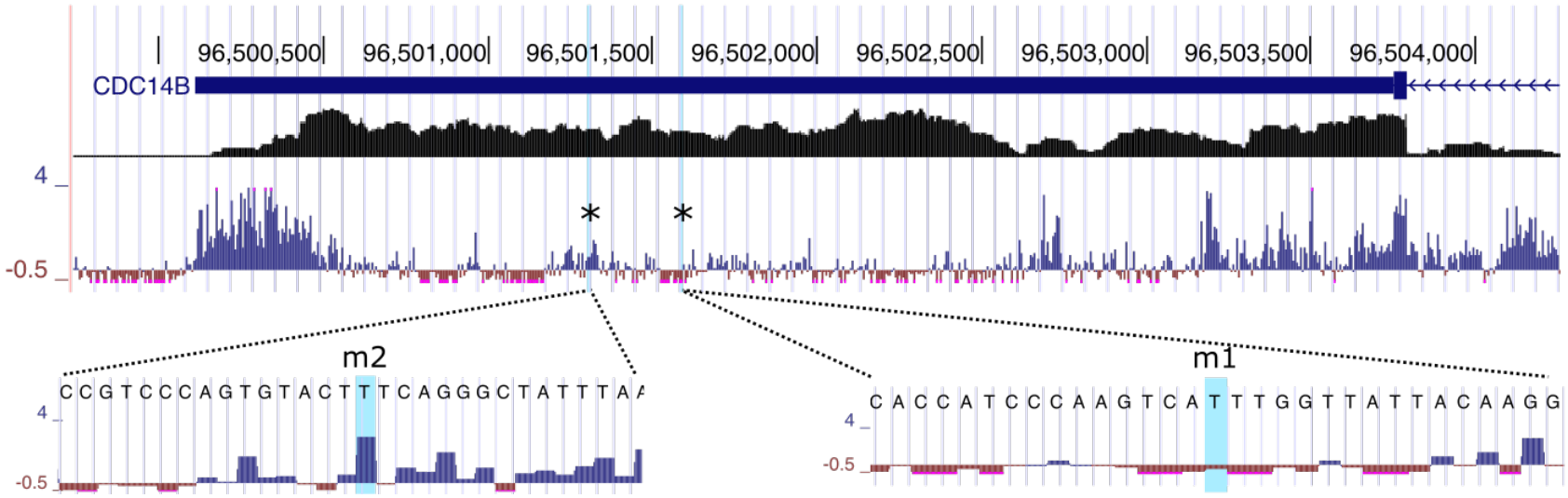


Piunti et al. 2021

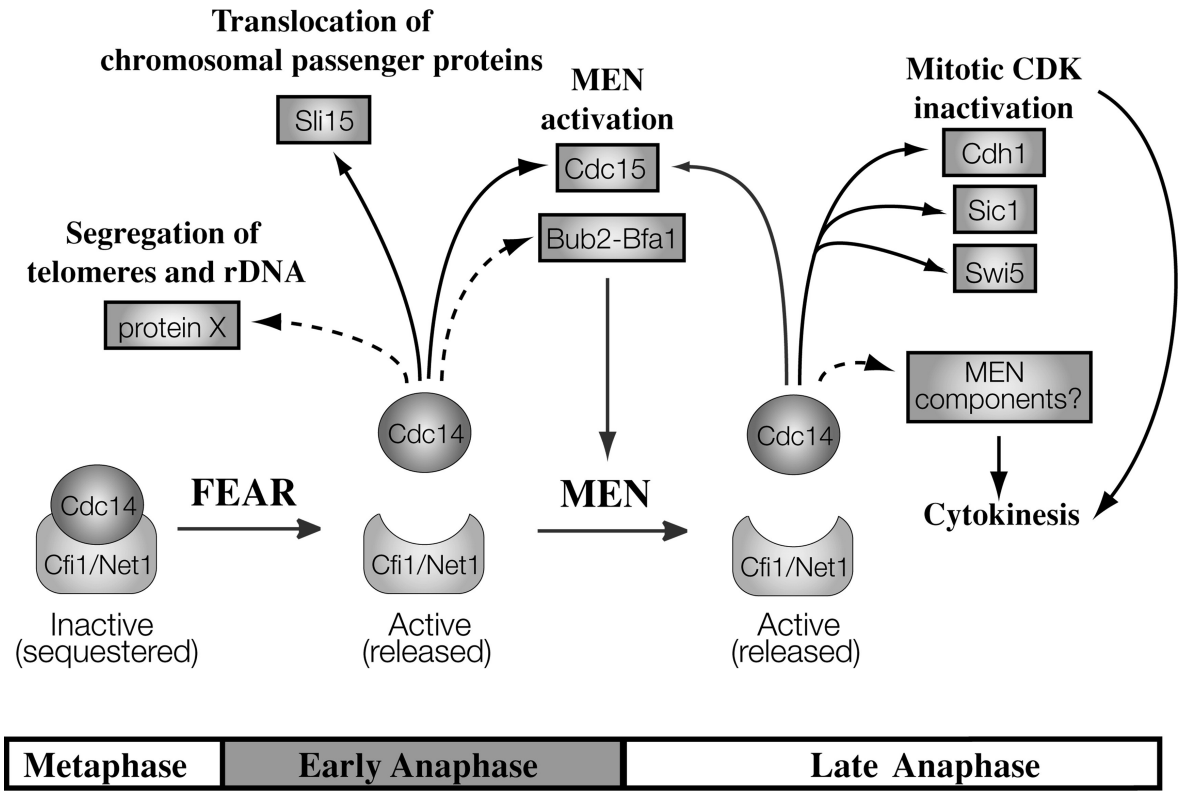


Junco et al. 2013

CDC14B noncoding variant in 3’ UTR is expressed in retinoblastoma and moderately conserved

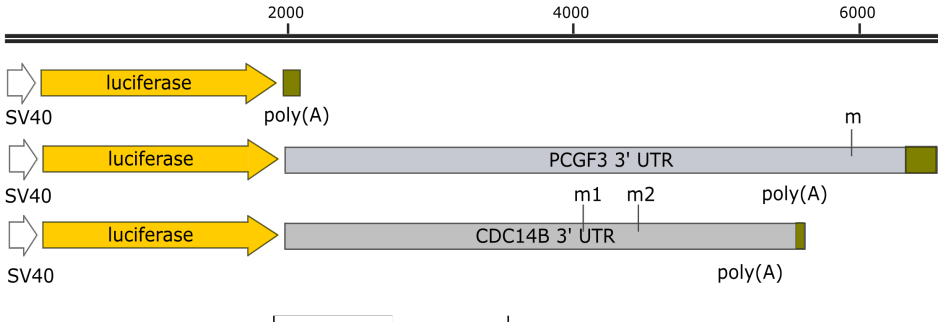


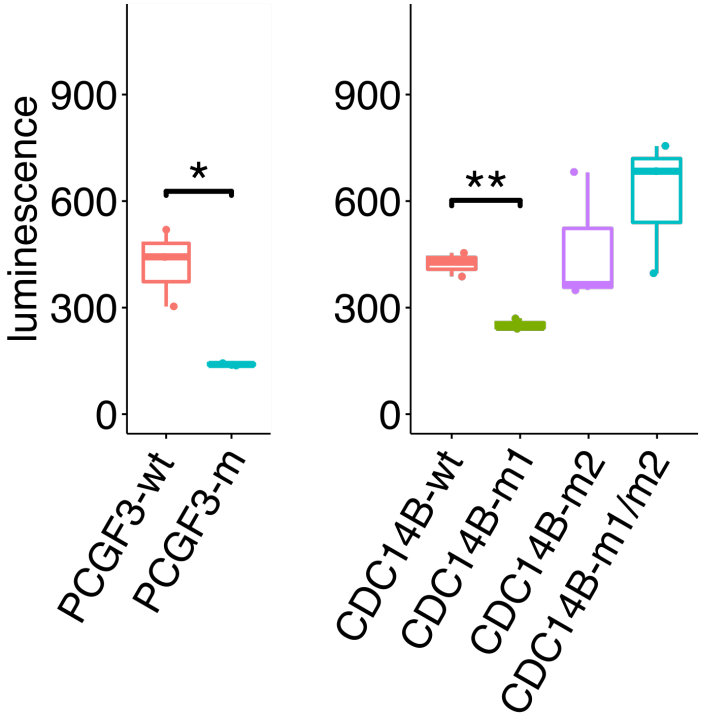
Mitotic sister chromatid segregation: CDC14B



Stegmeier and Amon 2004

3’ UTR variants affecting *PCGF3* and *CDC14B* impact RNA stability or translation.





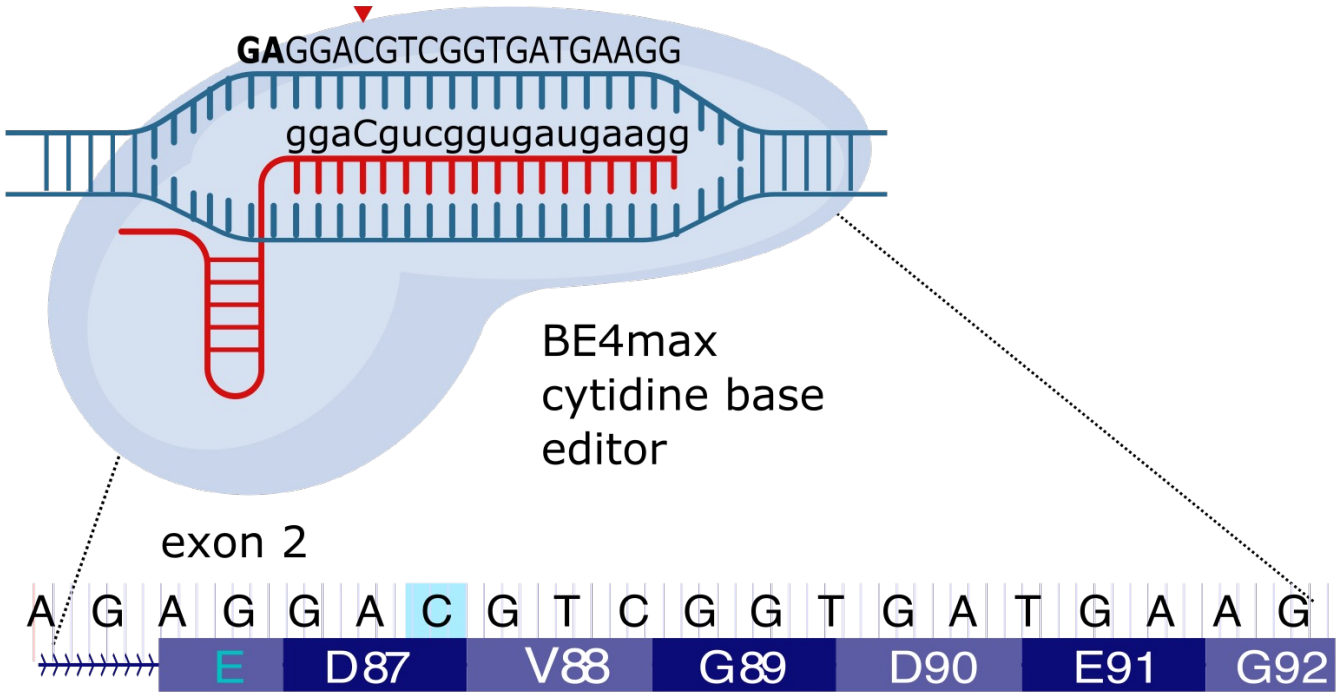
Ribonucleoprotein complex assembly and biogenesis: DYNC1H1

Dynein and kinesin regulate stress-granule and P-body dynamics

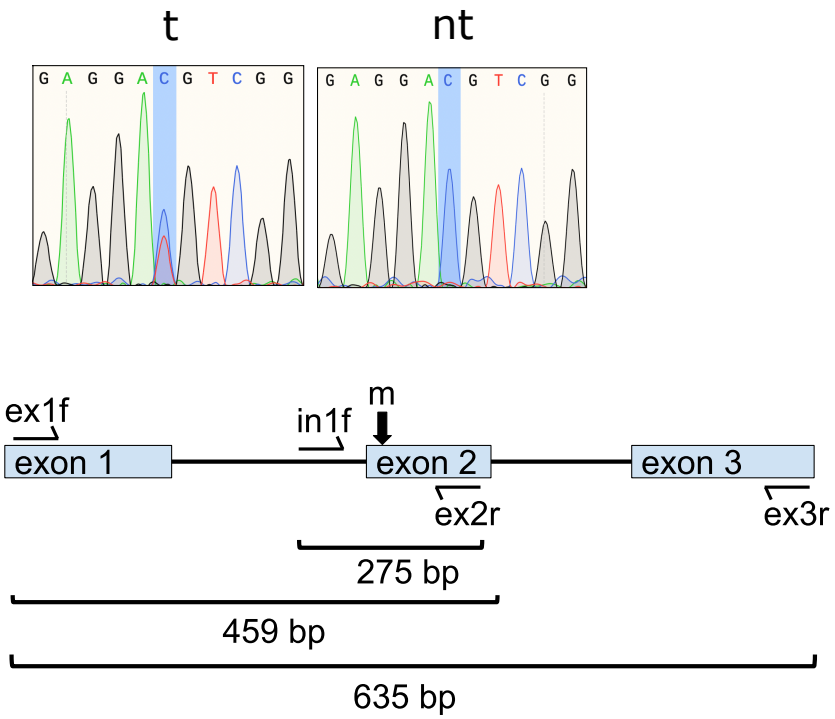
Mariela Loschi[1,2], Claudia C. Leishman[1], Neda Berardone[1] and Graciela L. Boccaccio[1,2,*]

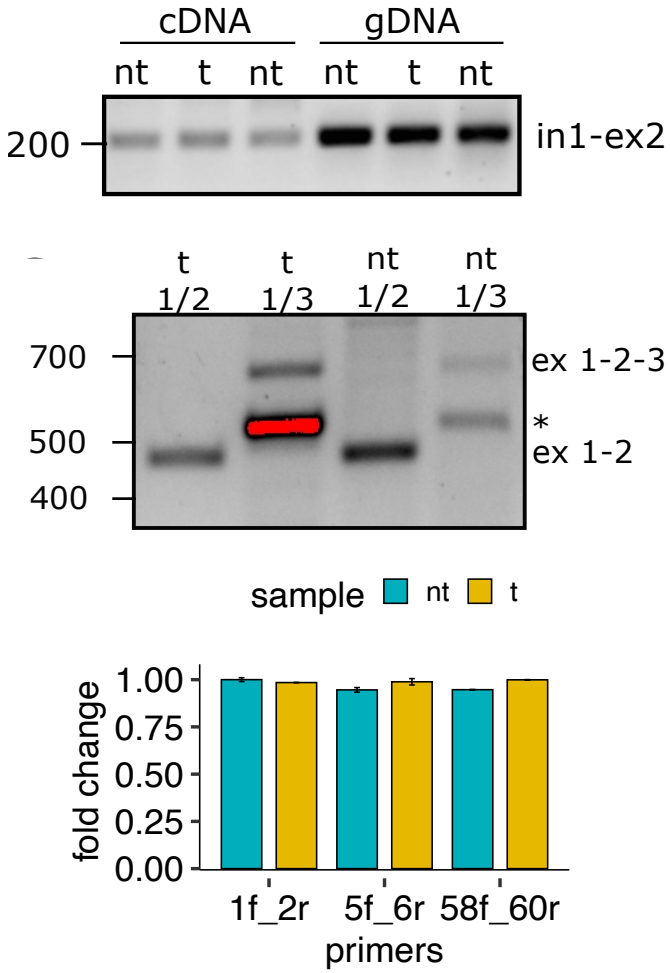
[1]Instituto Leloir, Avenida Patricias Argentinas 435, C1405BWE-Buenos Aires, Argentina
[2]Facultad de Ciencias Exactas y Naturales, University of Buenos Aires and IIBBA-CONICET, C1405BWE-Buenos Aires, Argentina

Cytidine base editing of DYNC1H1 C.261C>T

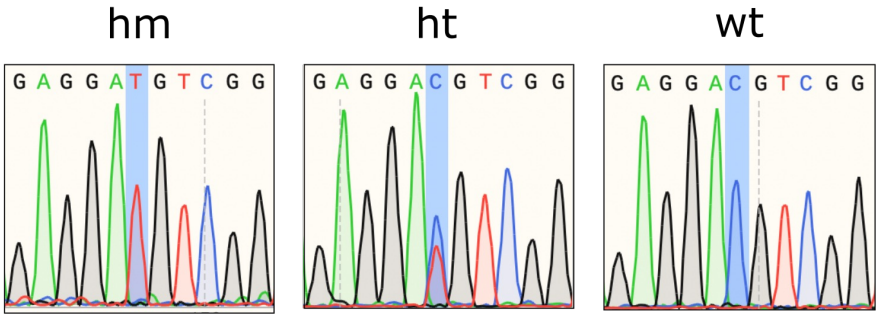


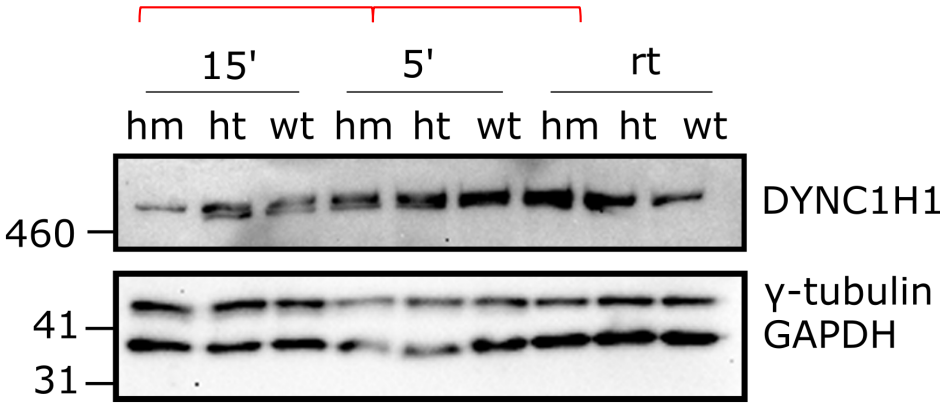
DYNC1H1 C.261C>T does not affect alternative splicing or total RNA abundance





DYNC1H1 C.261C>T variant affects protein stability





Summary

- Identified recurrently mutated genes involved in tumor progression by analysis of 12 tumor exomes and 97 existing tumor WES/WGS samples
- Characterized altered cellular processes that contribute to retinoblastoma progression
- Demonstrated transcriptional and post-transcriptional effects of synonymous and noncoding variants contributing to altered ontologies
- Points the way to targeted studies to define the role of recurrently altered cellular processes in retinoblastoma progression

Acknowledgements

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Heather Davidson

Irsan Kooi

Martin Triska

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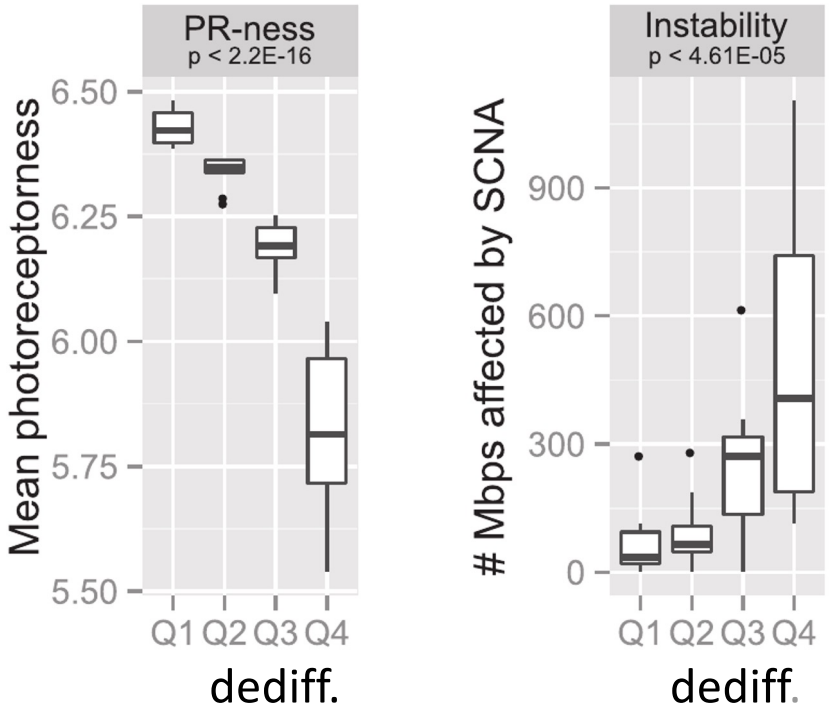
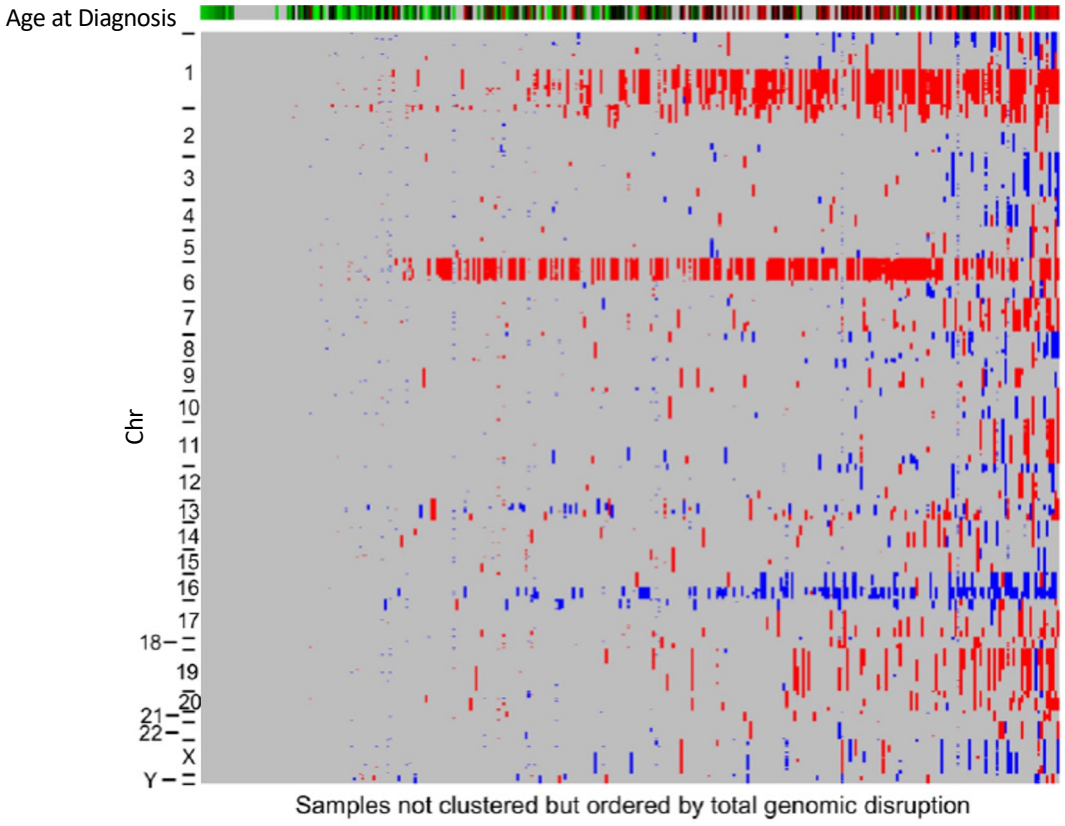
- the Larry and Celia Moh Foundation
- the Neonatal Blindness Research Fund
- the St. Baldrick's Foundation
- NIH R01CA137124 (DC).

SCNA abundance correlates with age at diagnosis





Adapted from Kooi, I.E. *et al.*, 2015. EBioMedicine