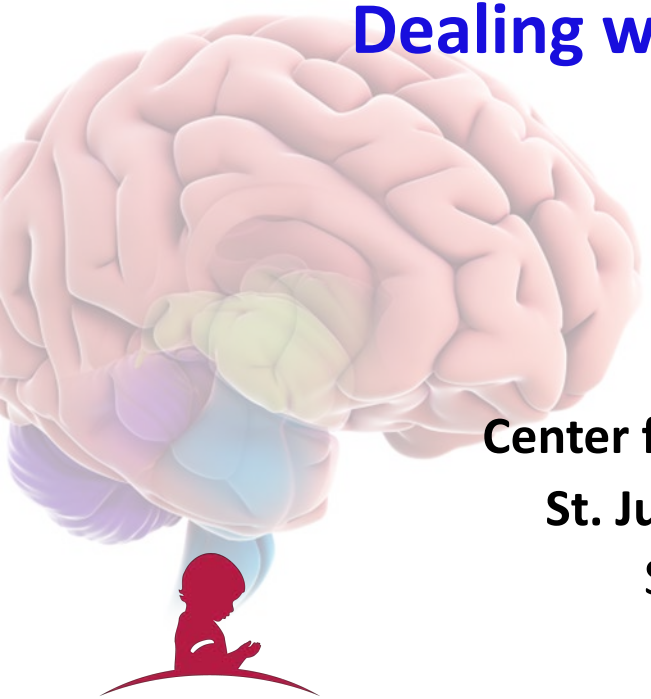


Dealing with Damage: Genome Integrity and Neurologic disease

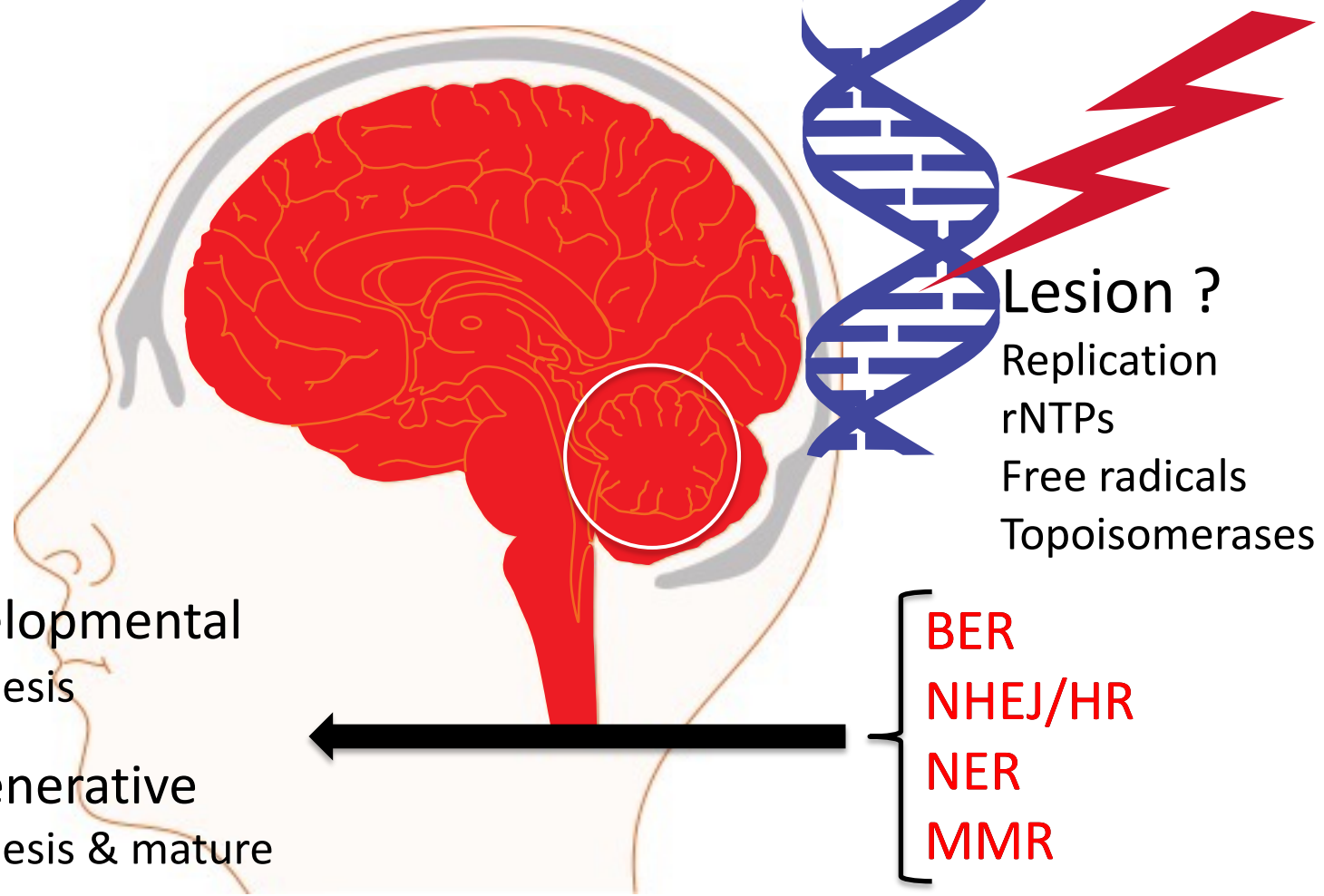
Peter J. McKinnon, PhD.
Center for Pediatric Neurological Disease Research
St. Jude Translational Neuroscience Initiative
St. Jude Children's Research Hospital
Memphis, USA

St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder
Finding cures. Saving children.

NIA/NIH: July 14th, 2020

Overview:

- Neurons are a life-long investment for an organism.
- Genome integrity is essential for normal brain function.
- Multitude neurologic diseases arise from defective DDR.
- Much is known about DNA damage in replicating cells, but little is known about differentiated neural cells.
- Suitable models are needed to address gaps in understanding.



Lesion ?

Replication

rNTPs

Free radicals

Topoisomerases etc

Neurodevelopmental

- Neurogenesis

Neurodegenerative

- Neurogenesis & mature

BER

NHEJ/HR

NER

MMR

Overview:

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Neurologic disease (ataxia) linked to defective DDR

BER

Syndrome (strand break defect)	Defective gene	Nervous system phenotype: Ataxia
A-T Ataxia telangiectasia	**ATM** kinase	**Neurodegeneration**
AOA1 Ataxia, oculomotor apraxia 1	**APTX** Nucleotide hydrolase (5' end-repair)	**Neurodegeneration**
SCAN1 Spinocerebellar ataxia, axonal neuropathy 1	**TDP1** Phosphodiesterase (3' end-repair)	**Neurodegeneration**
AOA4/MCSZ Ataxia, oculomotor apraxia 4 Microcephaly with seizures	**PNKP** Kinase/phosphatase (SSB end repair, NHEJ)	**Neurodegeneration** **/microcephaly**
XRCC1 (SCA26) Ataxia, oculomotor apraxia 5	**XRCC1** Scaffold protein (assembles BER)	**Neurodegeneration**

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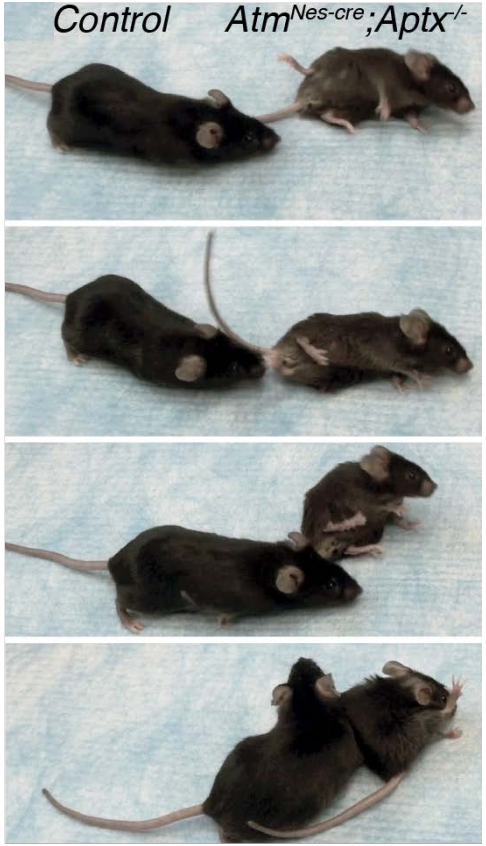
BER

- A-T & AOA1 have similar pathology
- Single mutants don't develop overt neuropathology
- Compound mutants to elevate genotoxic stress

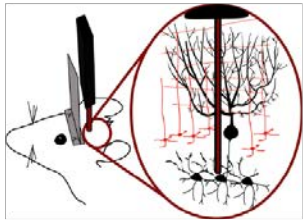
ATM/APTX dKO progressively develop profound ataxia.

[Controls]
-WT or single mutants

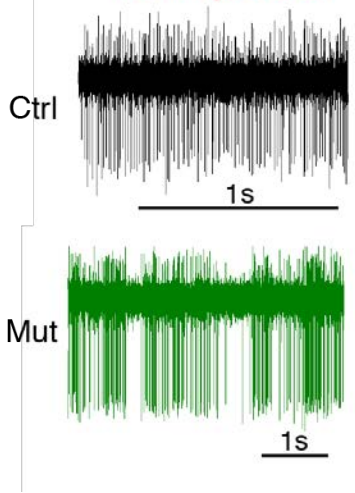


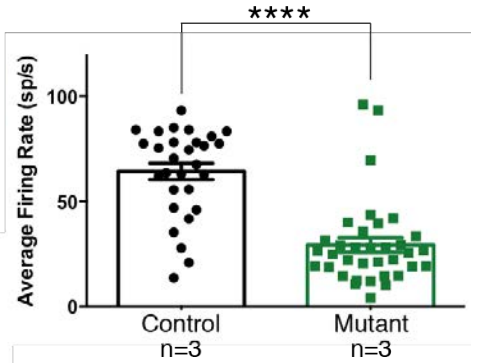
12 months old

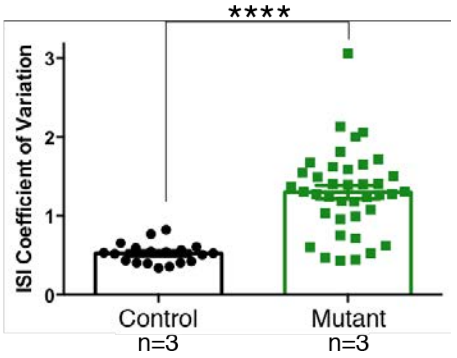
In vivo awake recordings, *Atm*$^{Nes\text{-}cre}$;*Aptx*$^{-/-}$ mice



Purkinje cells



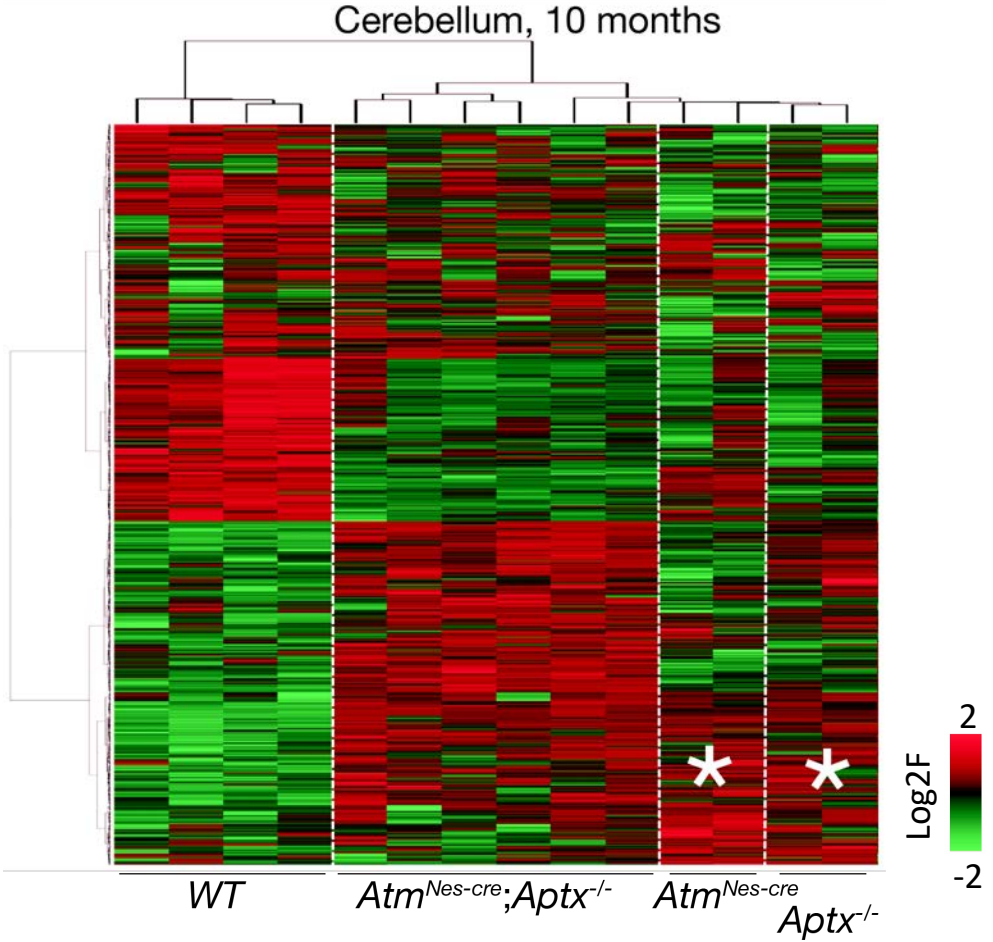




Kamran Khodakah/Einstein SOM, NYC

Gene expression alterations in the mutant cerebellum

- RNA-seq to assess gene expression changes that may underpin phenotype in double-mutants
- Comparative expression of top 1,000 expressed genes per genotype



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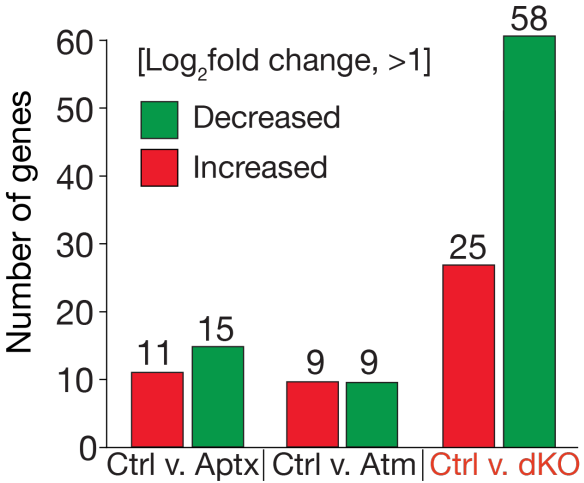
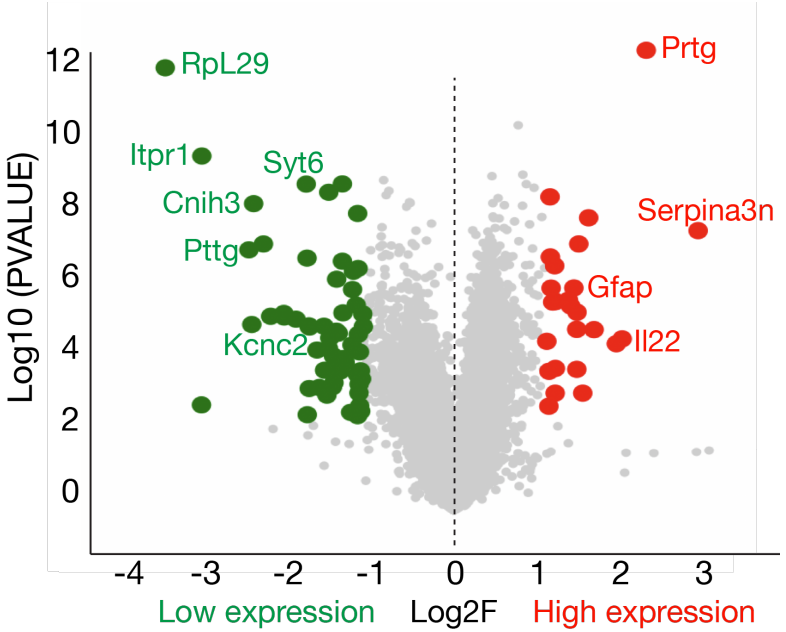
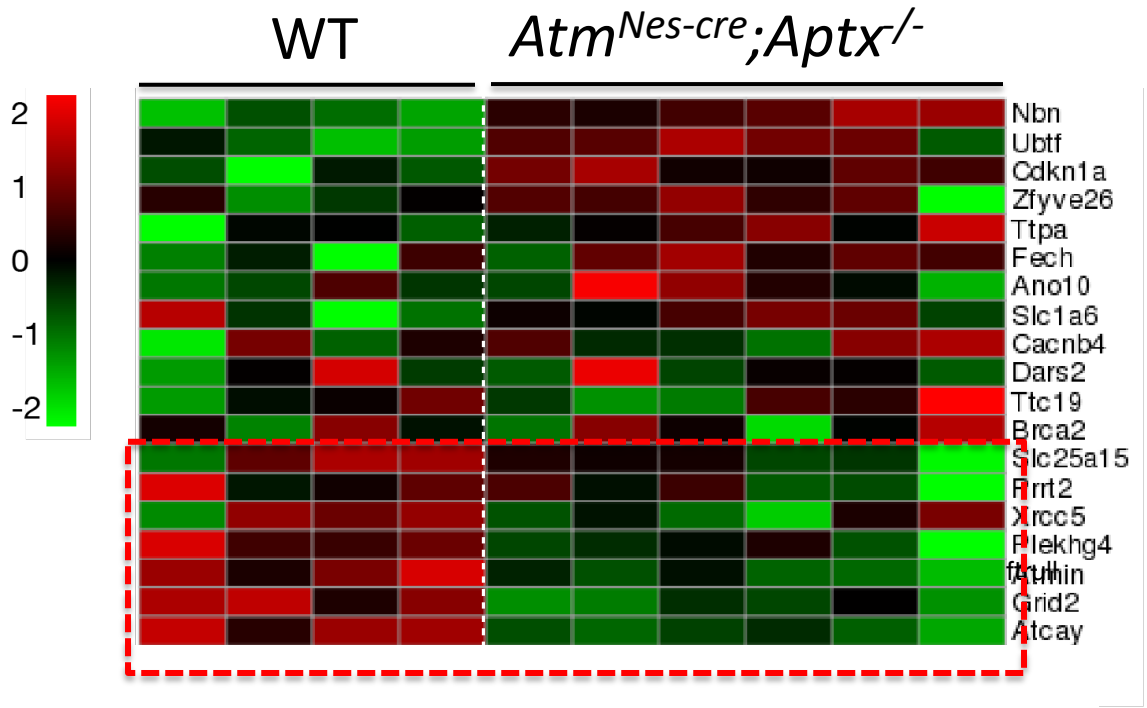




Ataxia-related gene expression

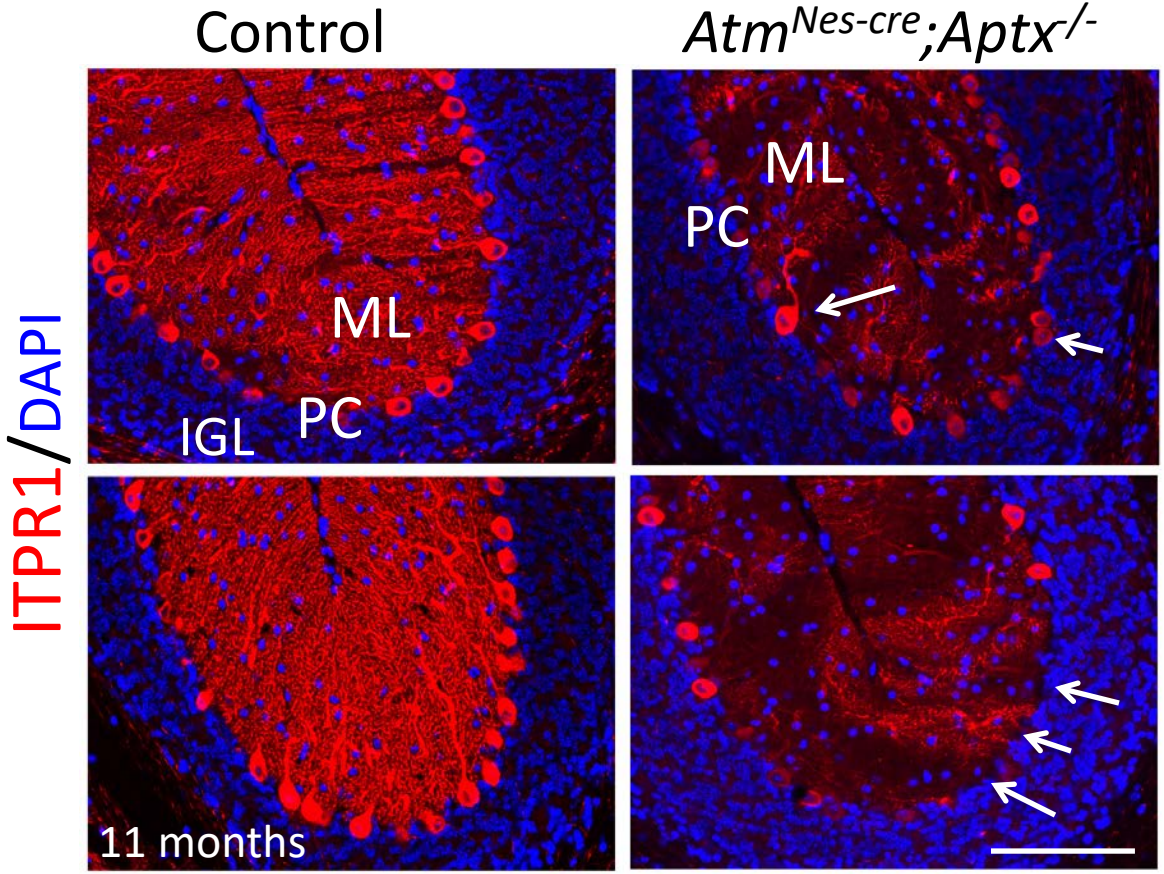
- The DISEASE database [*Pletscher-Frankild*, 2015] identifies genes whose loss is linked to ataxia:



Key cerebellar genes affected in the mutants:

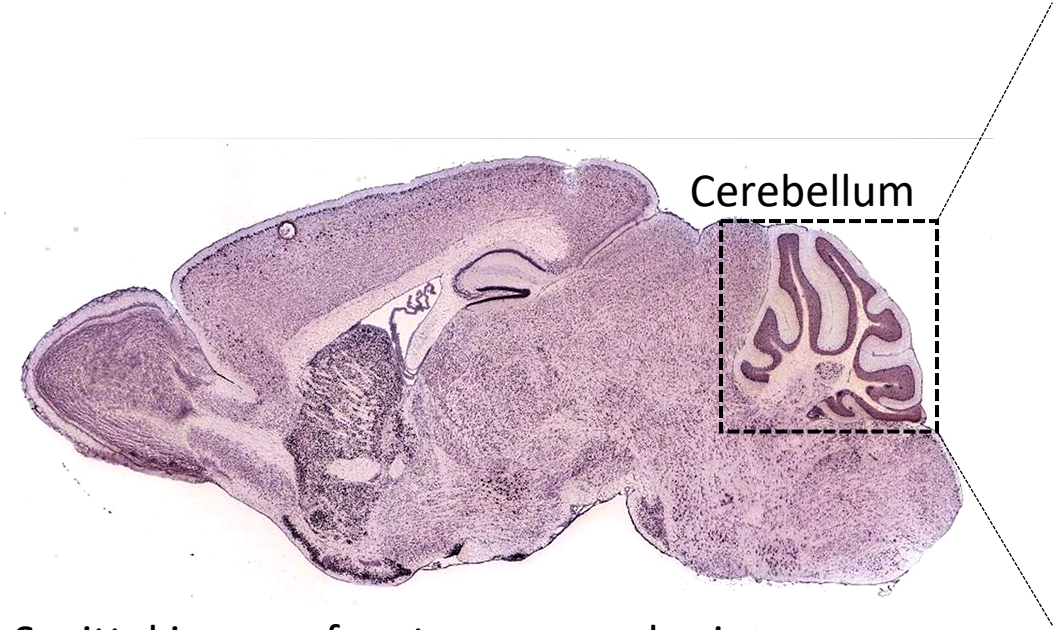
1. ITPR1- (inositol 1,4,5-trisphosphate receptor 1) [SCA15]
2. GRID2-(glutamate receptor, ionotropic, delta 2) [cerebellar ataxia]
3. BEAN1--Brain-expressed protein with Nedd4 homolog [SCA31]

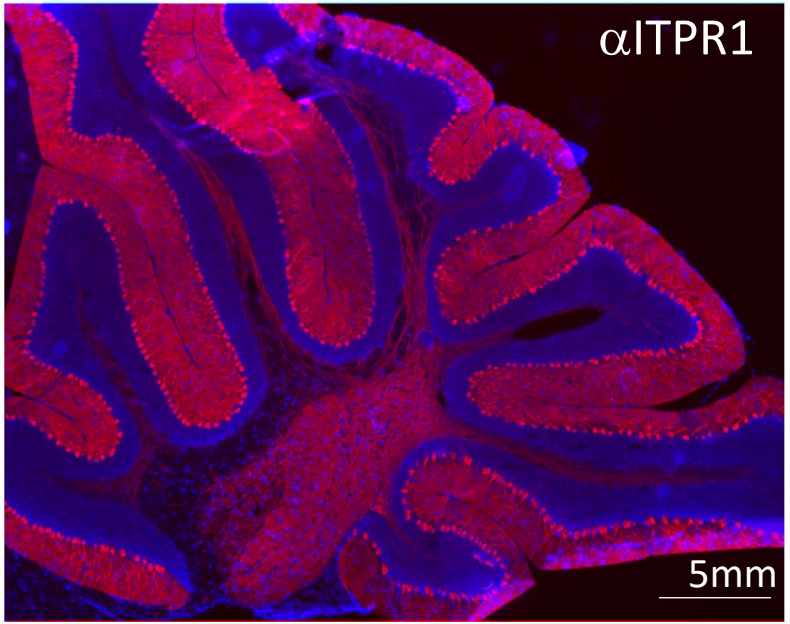
Purkinje cell homeostasis is perturbed in the dKO cerebellum



- Purkinje neurons show a loss of ITPR1 expression.
- ITPR1 regulates Ca^{++} release via IP_3
- Loss causes SCA15/29
- Disrupted cerebellar homeostasis is progressive >2-3 months.

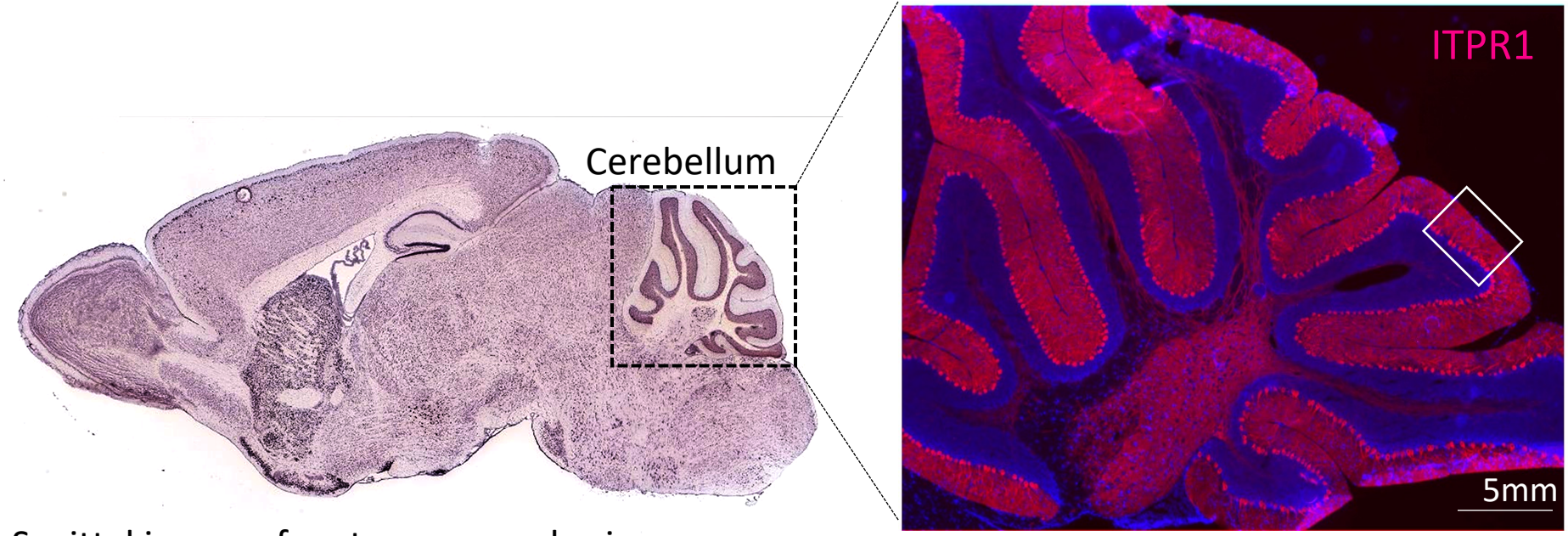
Normal cerebellar histology





Sagittal image of mature mouse brain.

Normal cerebellar histology



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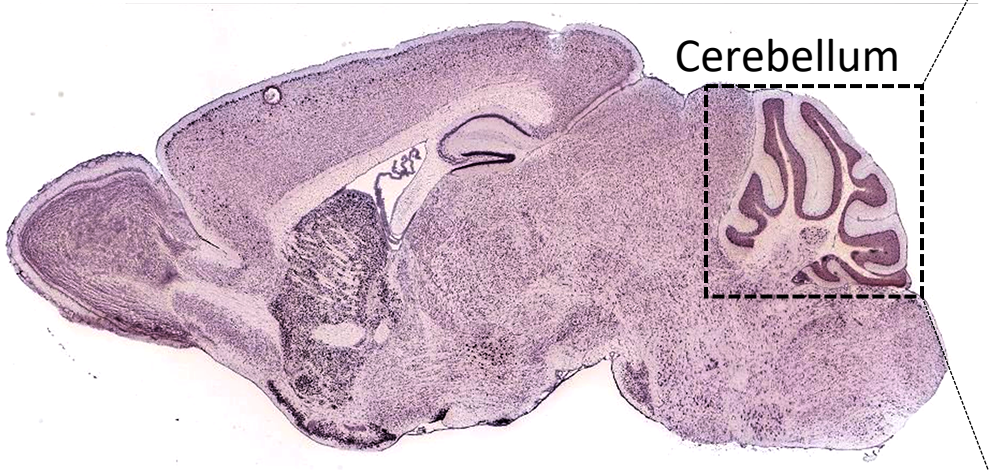

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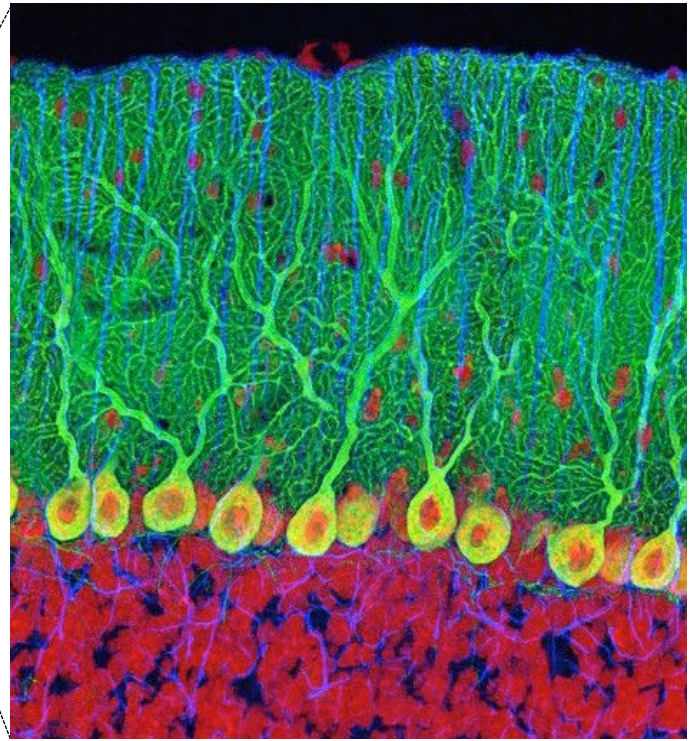

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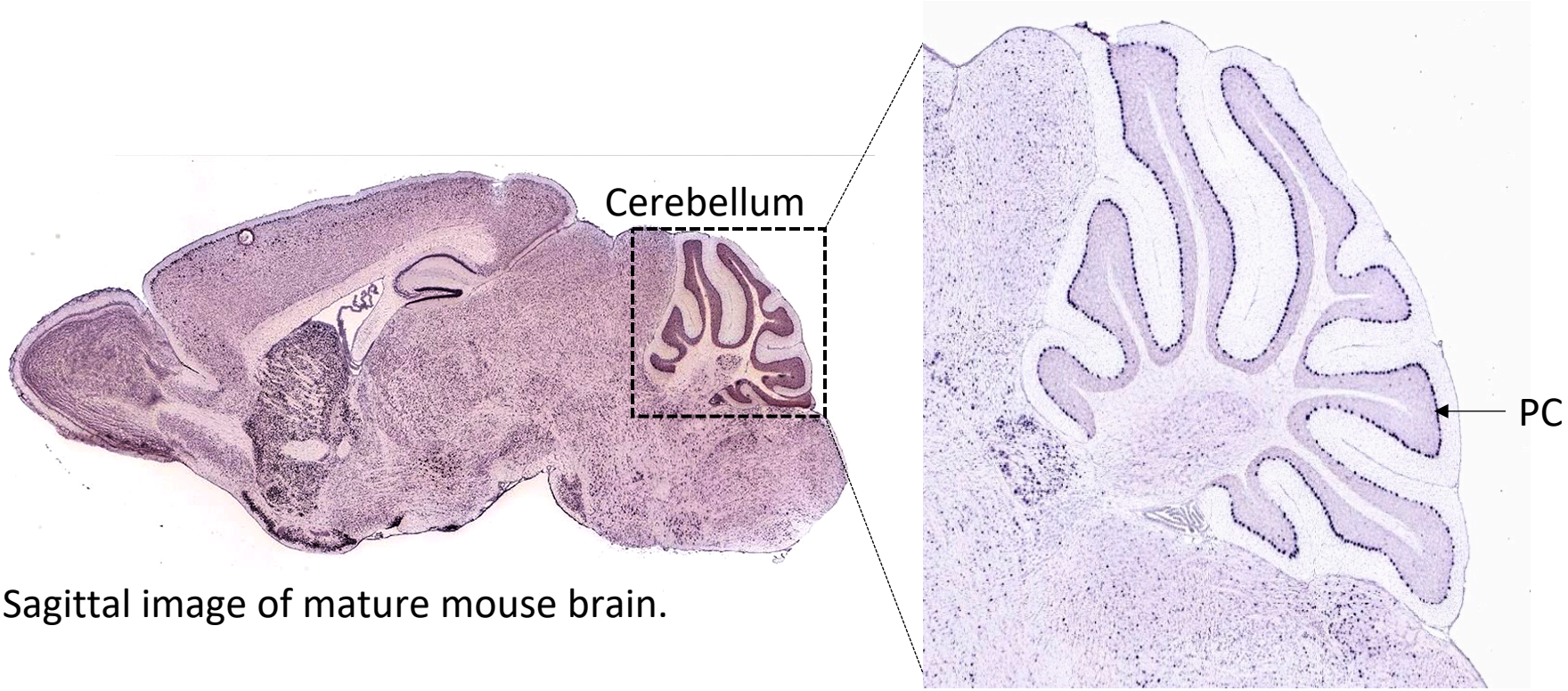


Sagittal image of mature mouse brain.

What is the basis for transcriptional disruption associated with reduced DNA repair capacity?

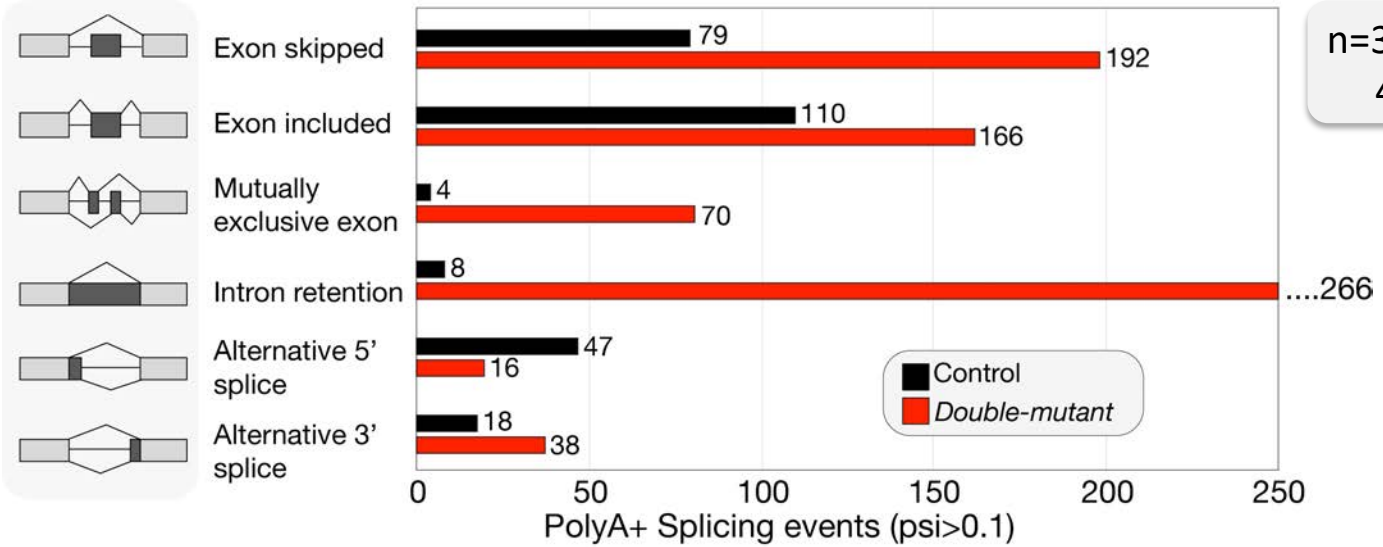
mRNA-seq analysis identifies aberrant splicing in the ataxia mutants

- PolyA$^+$ libraries for mRNA structural analysis.
- MATS (multivariate analysis of transcript splicing) [*Shen et. al., 2014, NAR*] & Intron retention score [*Bai et. al., 2013, PNAS*] to identify splicing patterns in control vs. mutant cerebellum.

Tim Shaw, PhD; Dept. Computational Biology

Aberrant splicing occurs in the DNA repair mutants

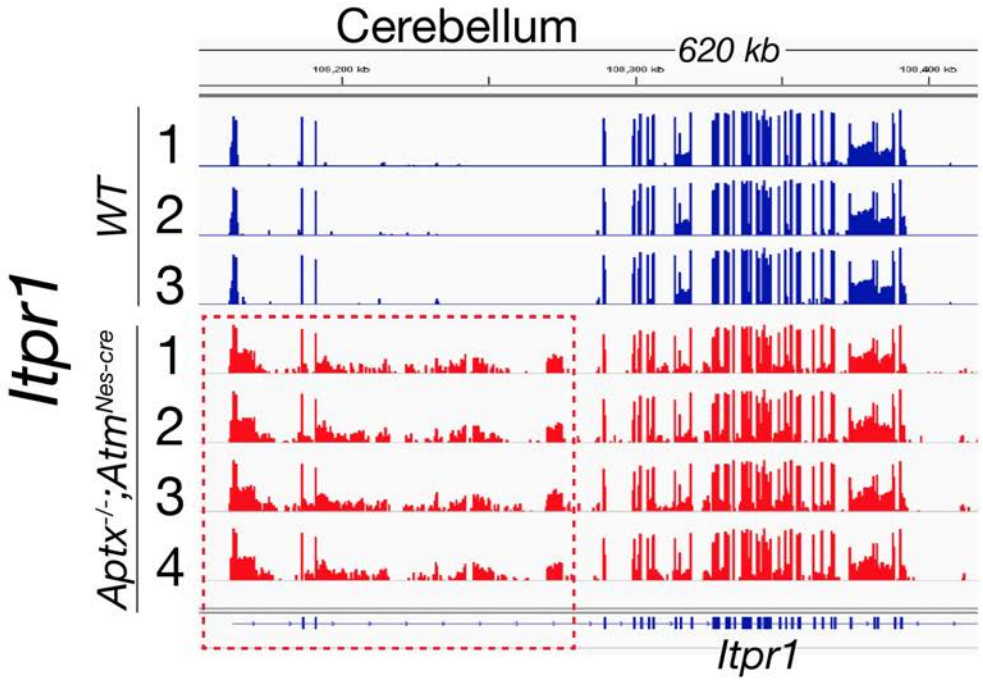
*Multivariate analysis of transcript splicing (MATS)/Intron retention score



n=3x WT,
4x dKO, @ 12m

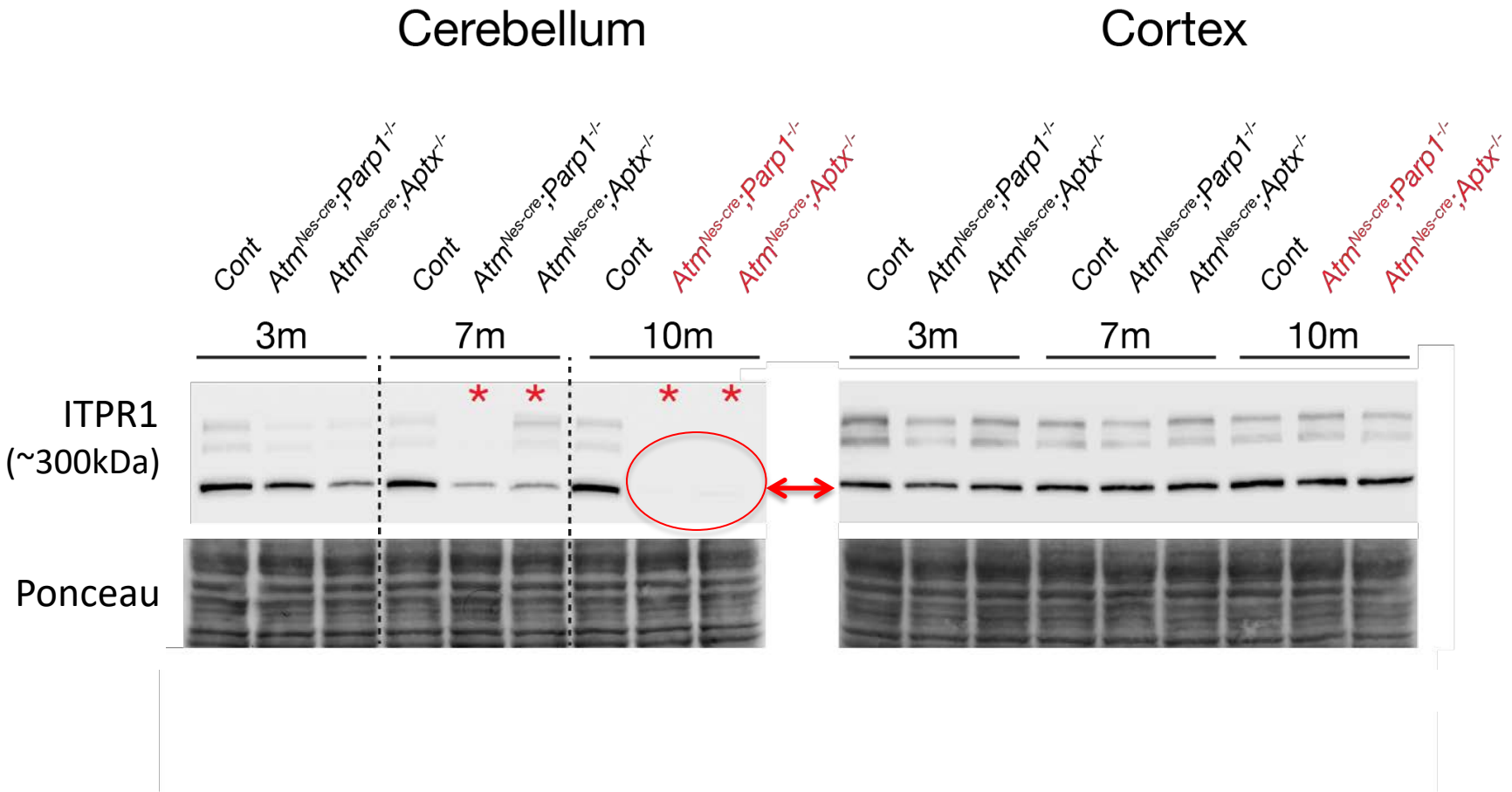
- 17.6% of genes are potentially affected (2,222/ 12,959 individual messages).
- A unique set of ~750 genes in all double-mutant cerebellum (after normalization of splicing variants).

Aberrant splicing occurs in the DNA repair mutants



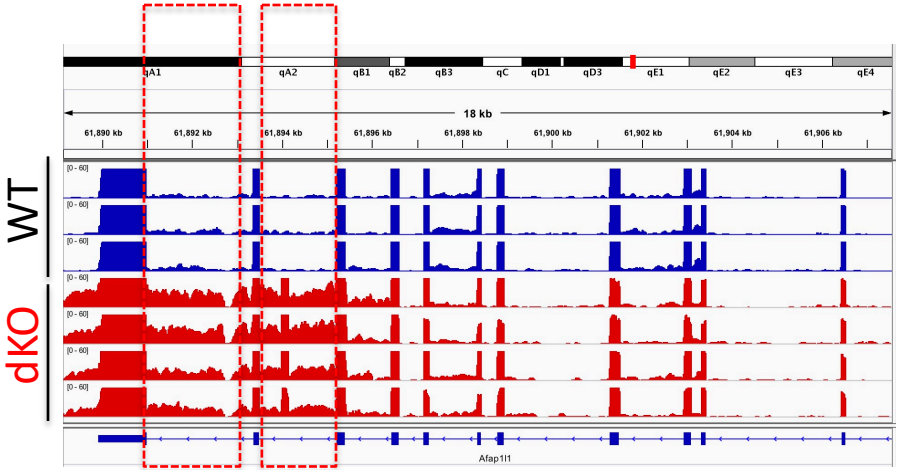
- Note recurrent intron retention pattern.

ITPR1 is progressively reduced the dKO cerebellum

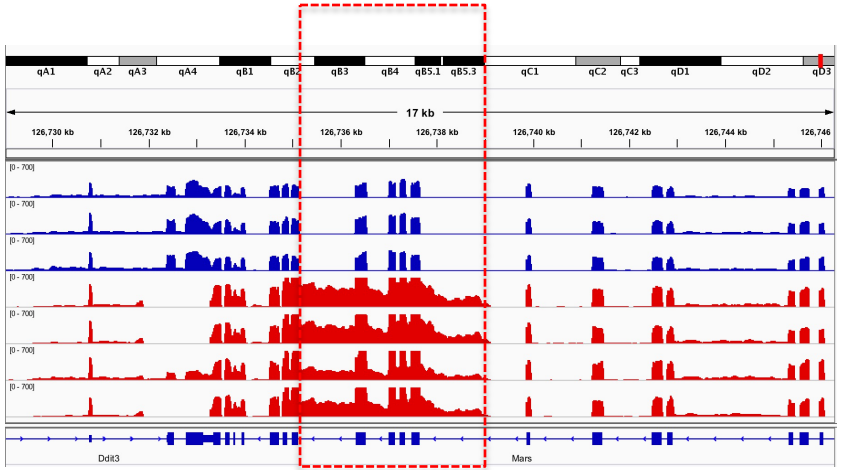


Aberrant splicing occurs in the DNA repair mutants

[Intron retention is a common event]



Afap1l1

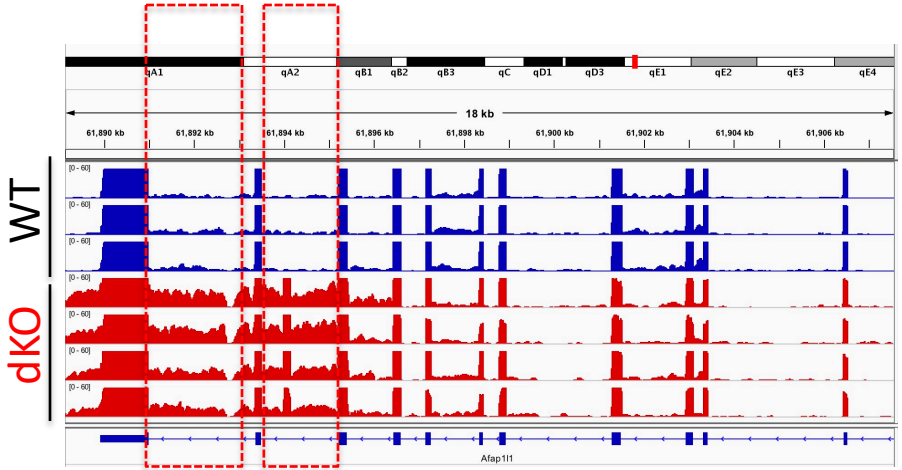


Ddit3

- dKO cerebellum shows recurrent intron retention patterns

Aberrant splicing occurs in the DNA repair mutants

[Intron retention is a common event]



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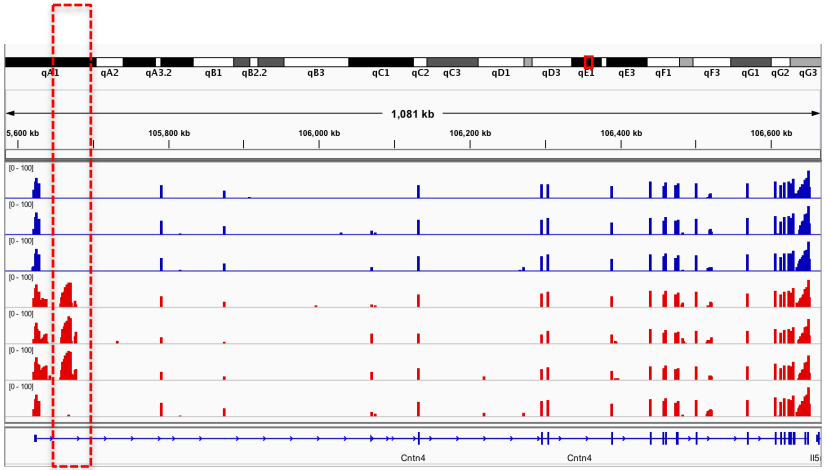


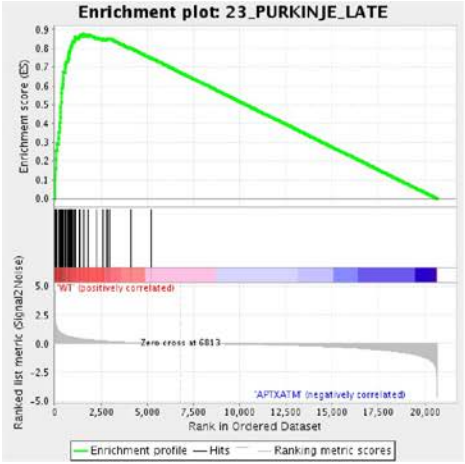
Cntn4

- *Cntn4* in one dKO cerebellum shows stochastic event.

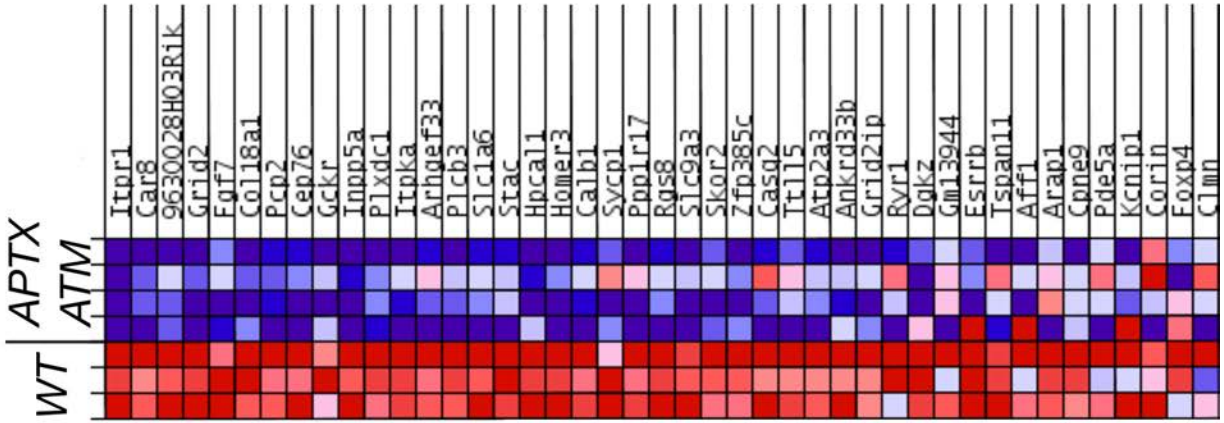
Aberrant splicing is associated with Purkinje cells

- Although aberrant splicing events are frequent, analysis does not support broad general splicing abnormalities in cerebellar transcripts
- (KS test of the distribution of splicing deficiency score between WT and dKO cerebellum is p=0.2125)

GSEA: Purkinje cells

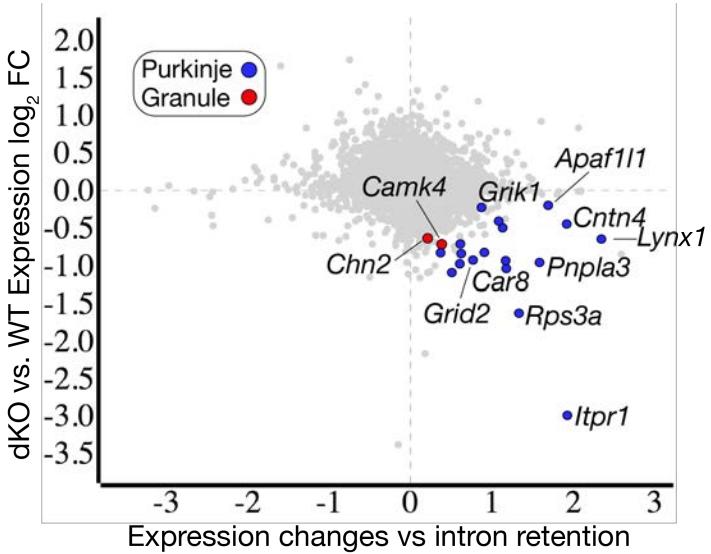


P<0.0001



Rosenberg et al., Science (2018)

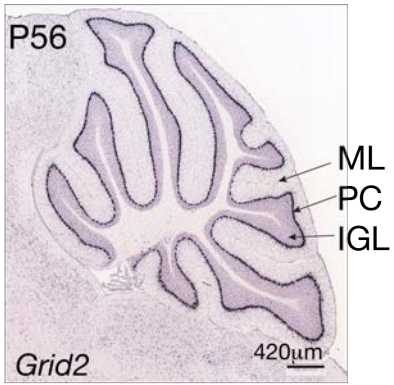
Intron retention associated with Purkinje cell genes

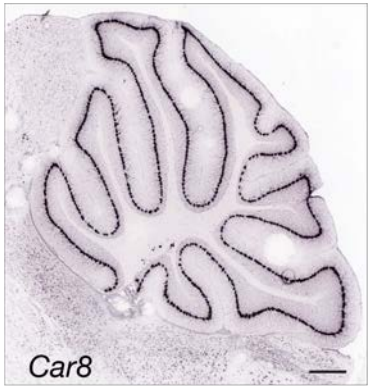


Gene	PC expression	GN expression
Car8	+++++	-
Clec3b	++	-
Cep76	+++++	-
Cntn4	+++	-
Fgf7	+++++	-
Grik1	+++++	-
Itpr1	+++++	-
Kcnip4	++++	-
Lynx1	++++	-
Ndufs5	+++	-
Pnpla3	++++	-
Rps3a	+	-
Sbk1	+++++	-
Tdg	++	-
Them4	++++	-
Ubash3b	++++	-
Camk4	-	++++
Caro2b	-	+++
Chn2	-	+++++

**Gene list: Rosenberg et al., Science (2018)*

Purkinje neurons





In situ hybridization: Allen Brain Atlas

DNA repair-deficient mice develop progressive ataxia, which is associated with the loss of critical cerebellar gene expression.

Many of these genes underpin ataxia syndromes in humans.

- Alternative splicing targets Purkinje cells in the cerebellum
- Intron retention is a common event
- RNA Pol II occupancy at TSS/PPP sites reduced (often associated with splicing abnormalities, particularly intron retention)

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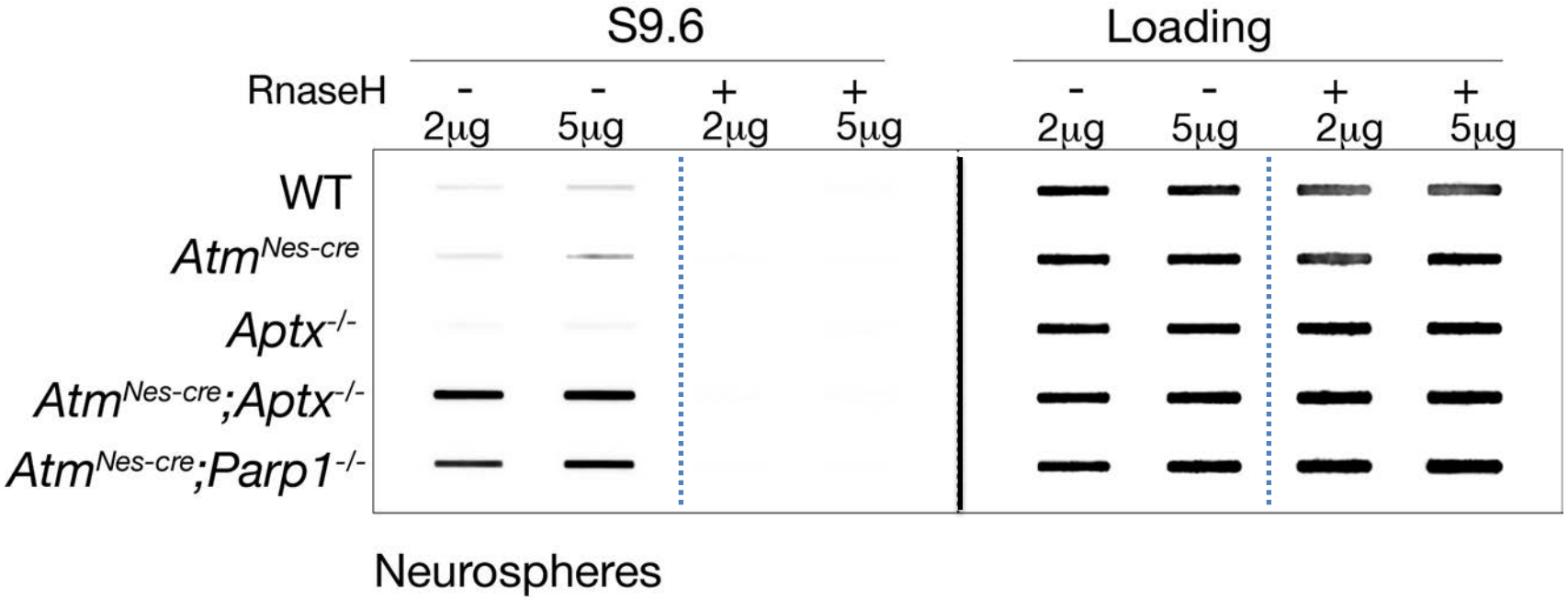
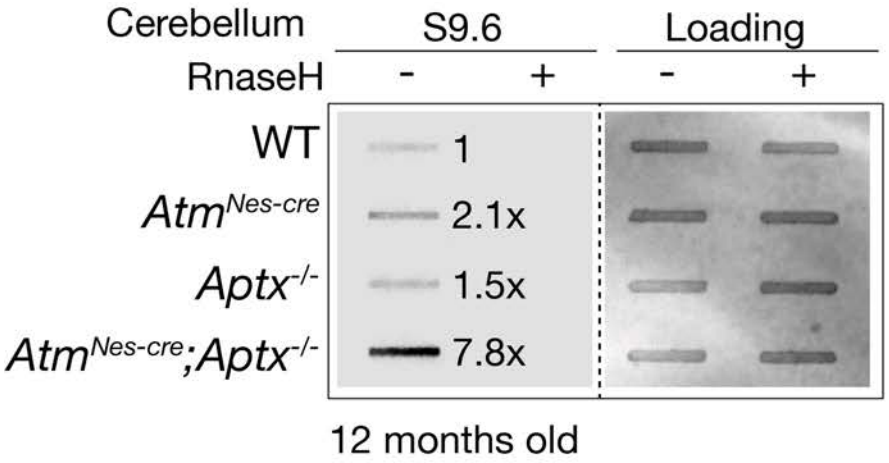
- Intron retention common event
- RNA Pol II occupancy at TSS/PPP sites reduced (often associated with splicing abnormalities, particularly intron retention)
- Other events contributing to transcription disruption: R-Loops?

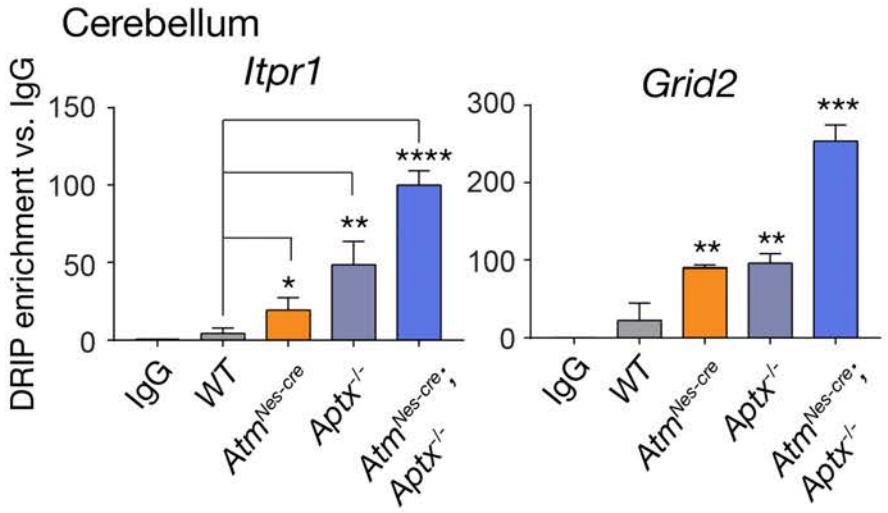
R-Loop formation in ataxia models

- S9.6 antibody recognizes DNA-RNA hybrids (R-Loops)
- DKO neurospheres show high levels of R-Loops (that are sensitive to RNaseH)



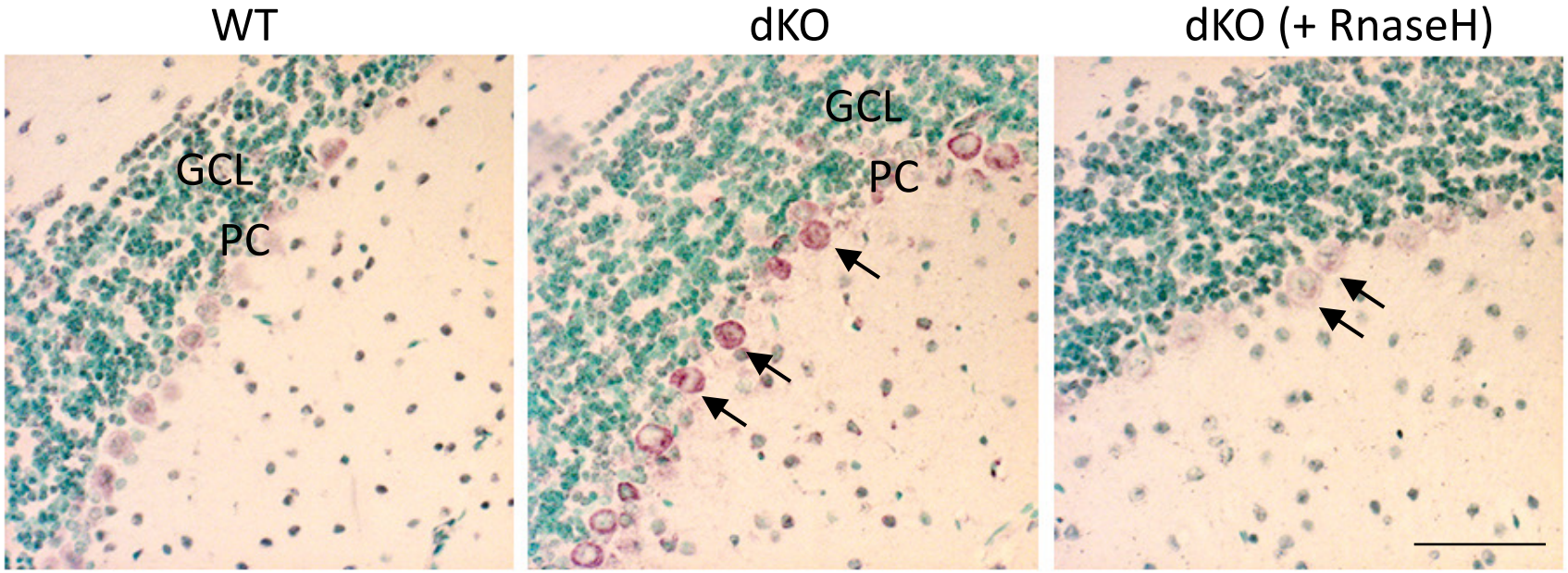
Endogenous R-Loops in the cerebellum of ataxia models





- R-Loops form in the dKO cerebellum
- R-Loops are associated with affected genes

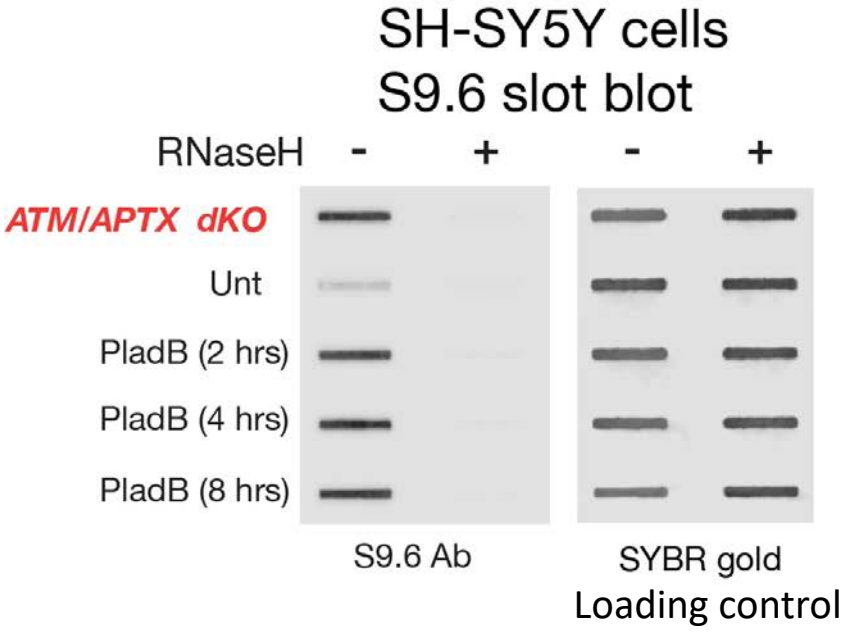
Endogenous R-Loops in the cerebellum of ataxia models

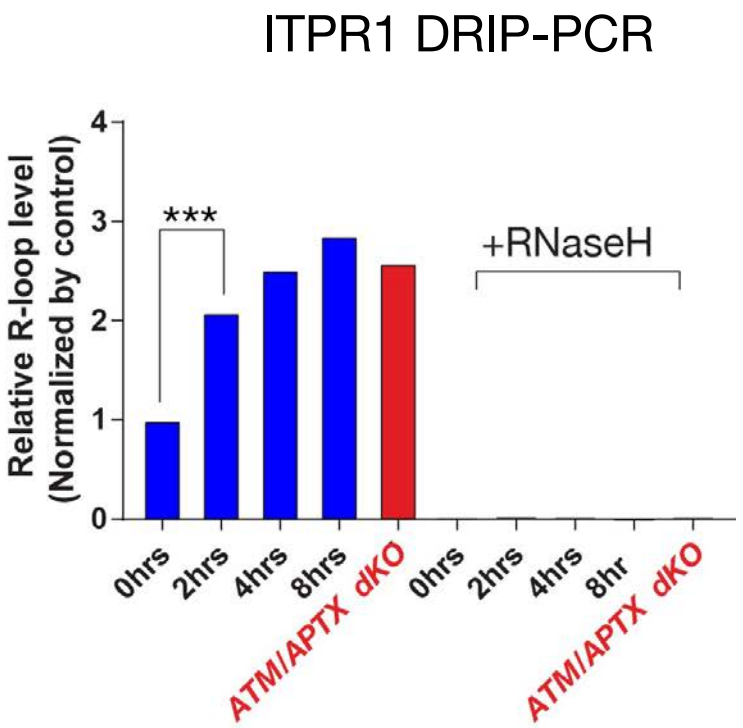


S9.6 IHC

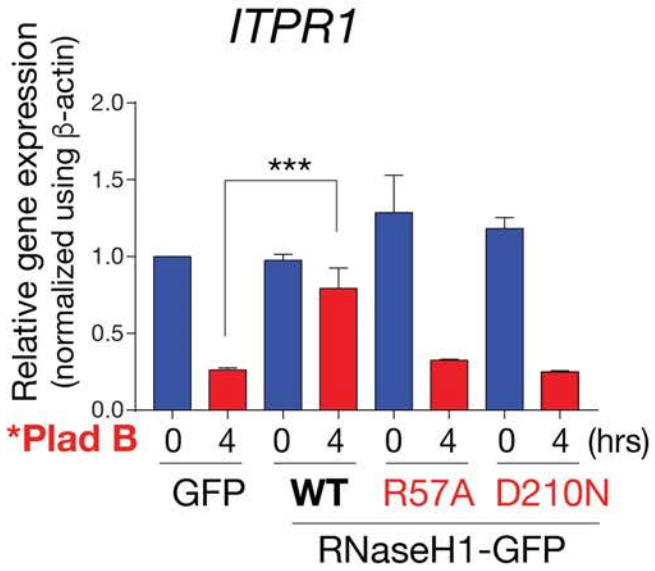
- Endogenous R-Loops form in Purkinje cells of the dKO cerebellum
- R-Loops are associated with affected genes

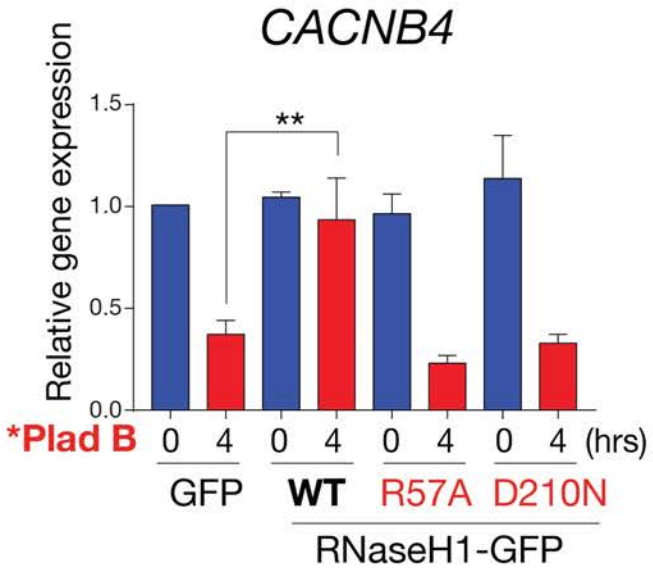
Splicing disruption leads to R-Loops in *ITPR1*

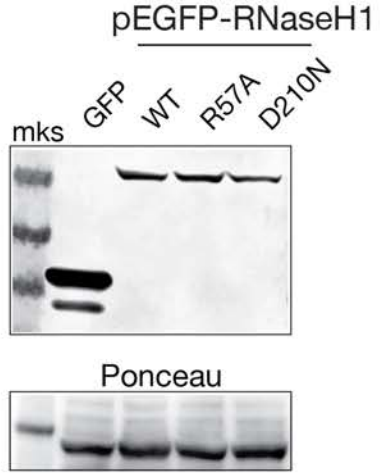




RNaseH1 restores expression after splicing inhibitors

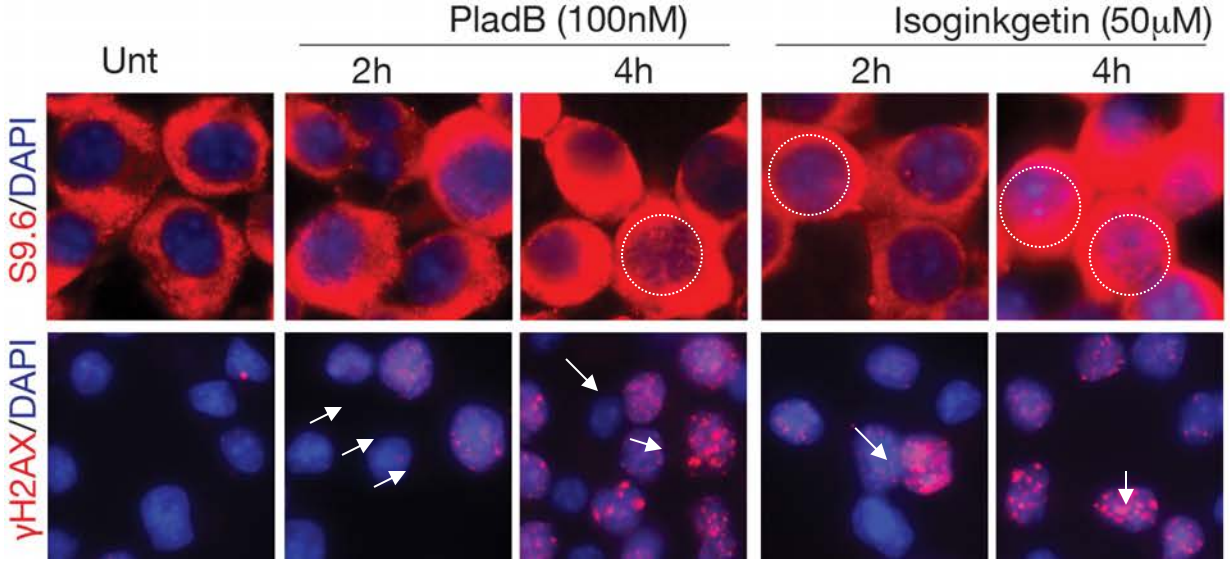




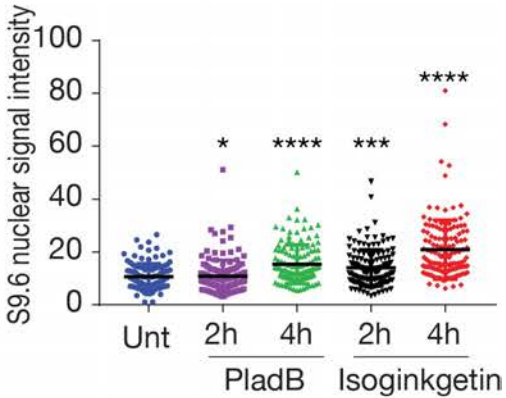


RNaseH1
-catalytic mutant: R57A
-PCNA binding mutant: D210N

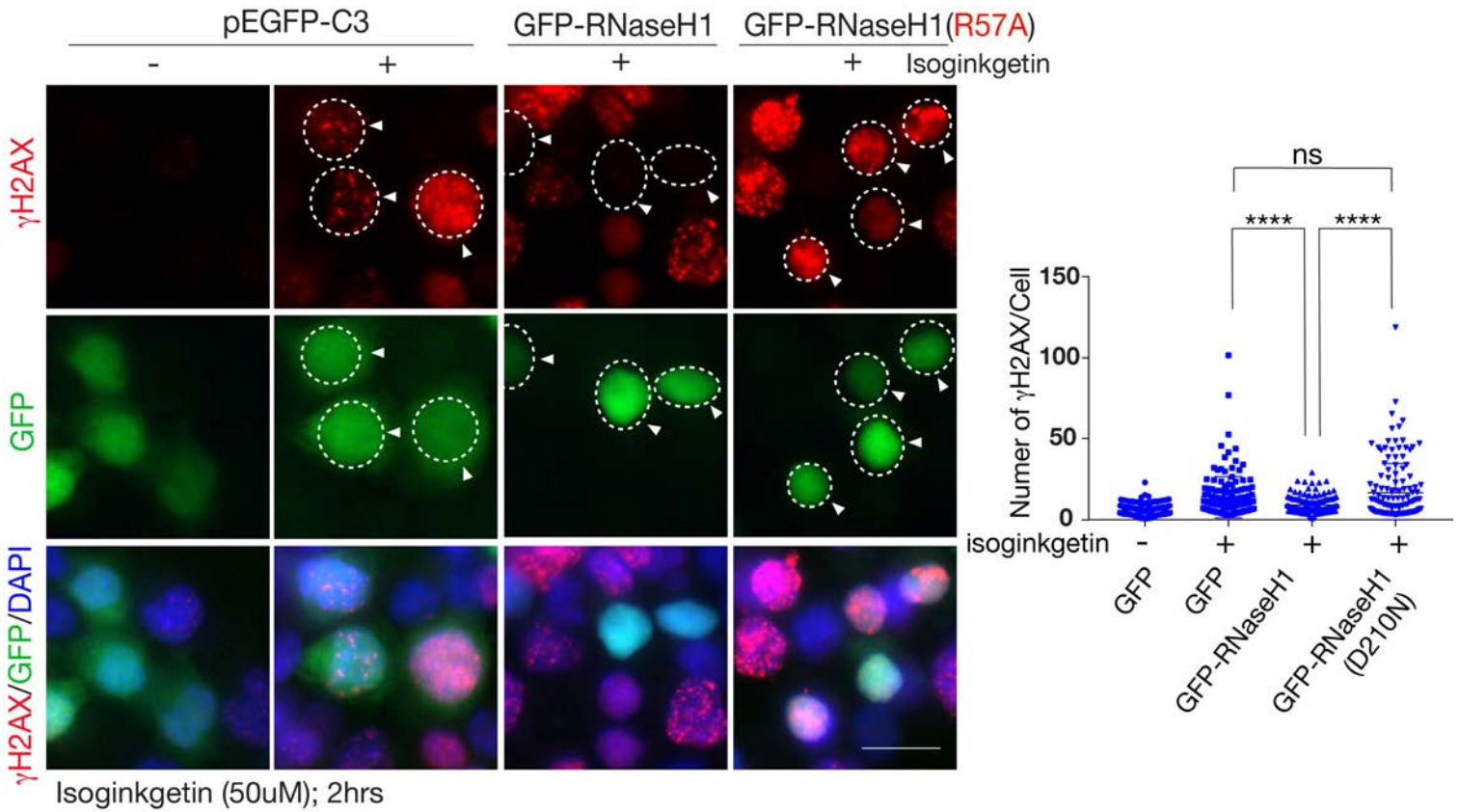
Splicing disruption causes DNA damage and R-Loop formation

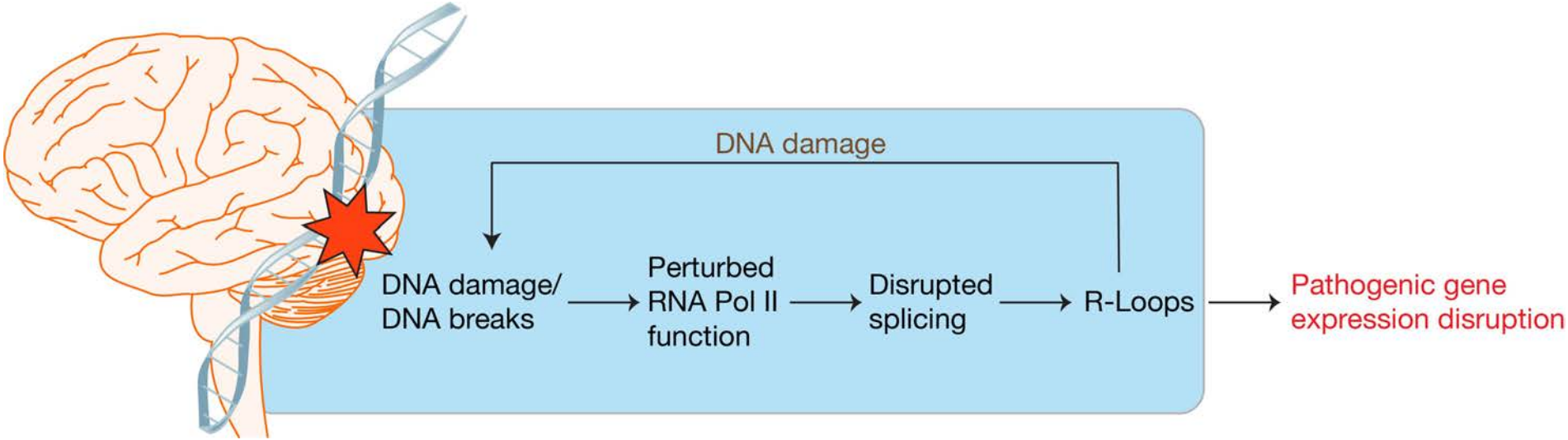


SH-SY5Y cells



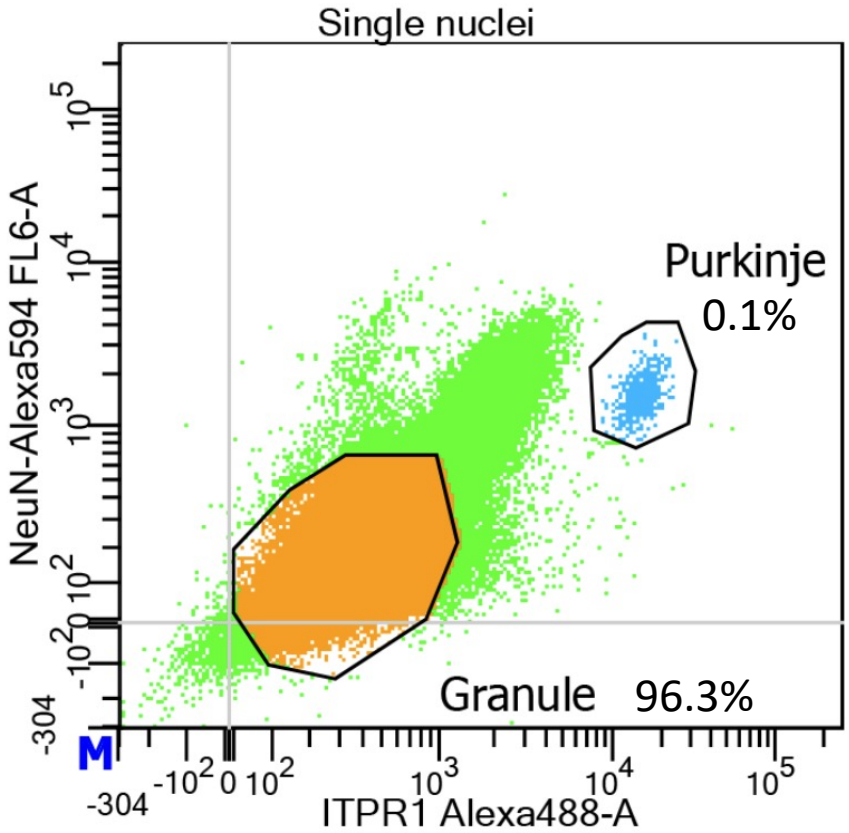
RNaseH1 reduces DNA damage after splicing inhibitors





- Why the cerebellum; why Purkinje cells?

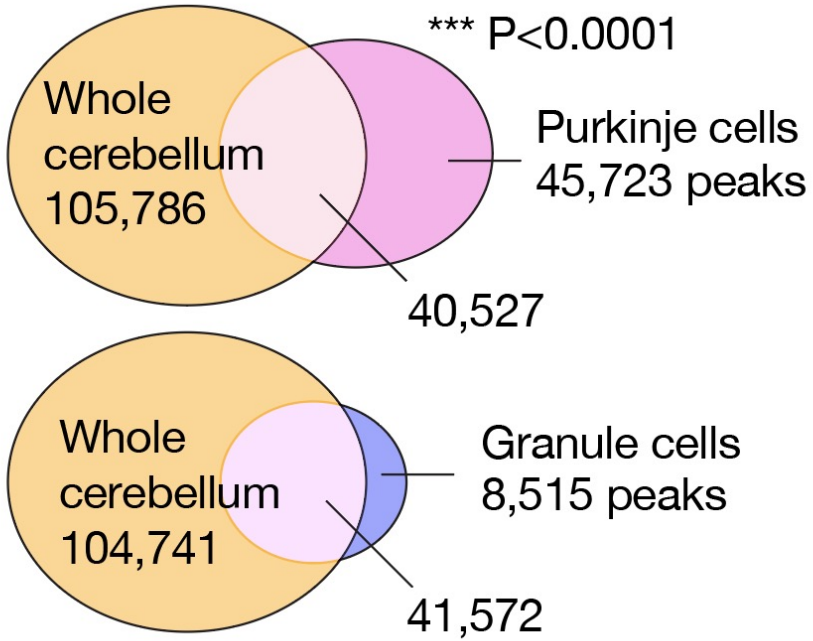
Purification of Purkinje cell nuclei



- Method: *Xu et.al., eLife 2018;7:e37551*
- Pooled frozen WT cerebella, isolate nuclei
- ITPR1 antibody used to identify PCs via flow cytometry (NeuN for GCs)
- Purkinje Cells were ~0.1% of the cerebellar cells

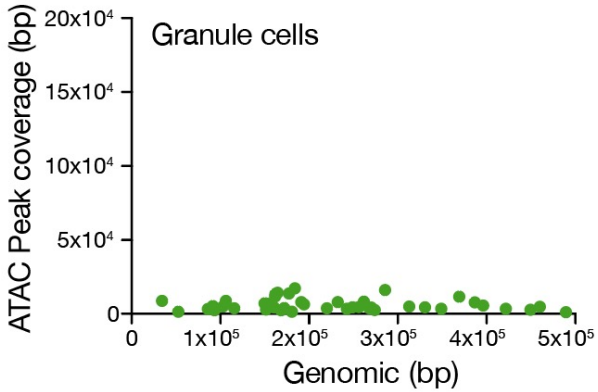
Purkinje cell ATAC-seq

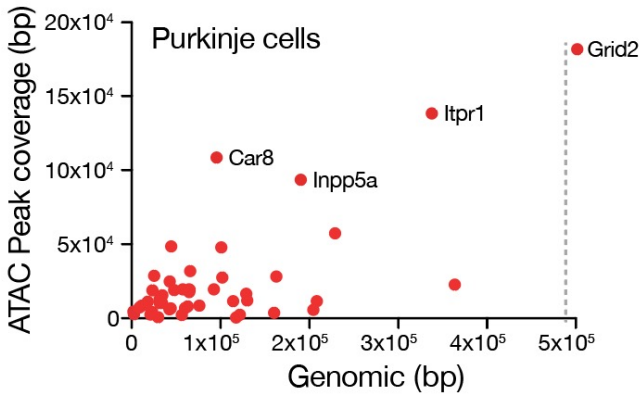
- ATAC-seq: Genome wide Assay for Transposase Accessible Chromatin
- Reveals chromatin accessibility
- Purkinje cells have highly accessible chromatin regions



Purkinje cell ATAC-seq

- ATAC-seq: Genome wide Assay for Transposase Accessible Chromatin
- Reveals chromatin accessibility
- Purkinje cells have highly accessible chromatin regions
- Target genes affected by genotoxic stress show open conformation

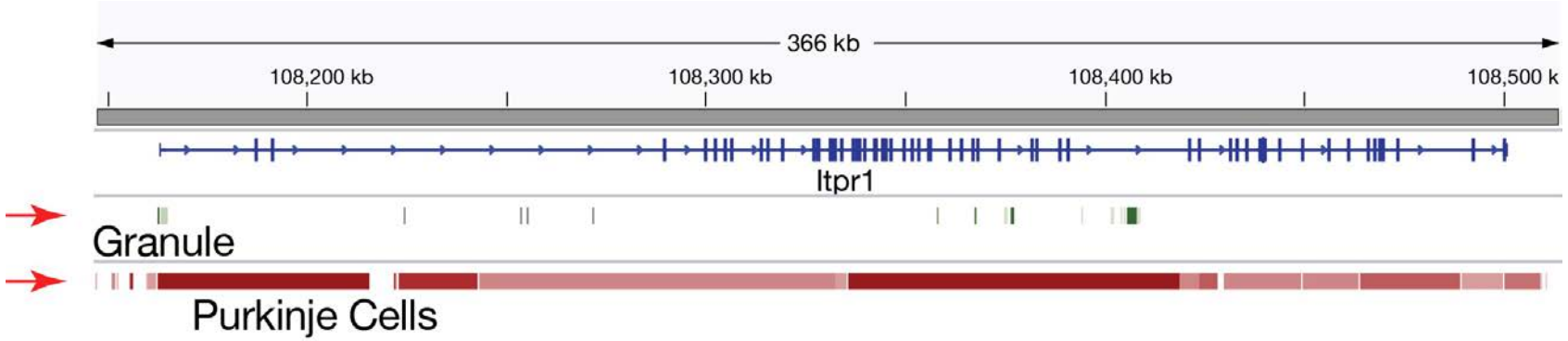




*ATAC-seq of PC/GC genes from ***Rosenberg et al*** data set:

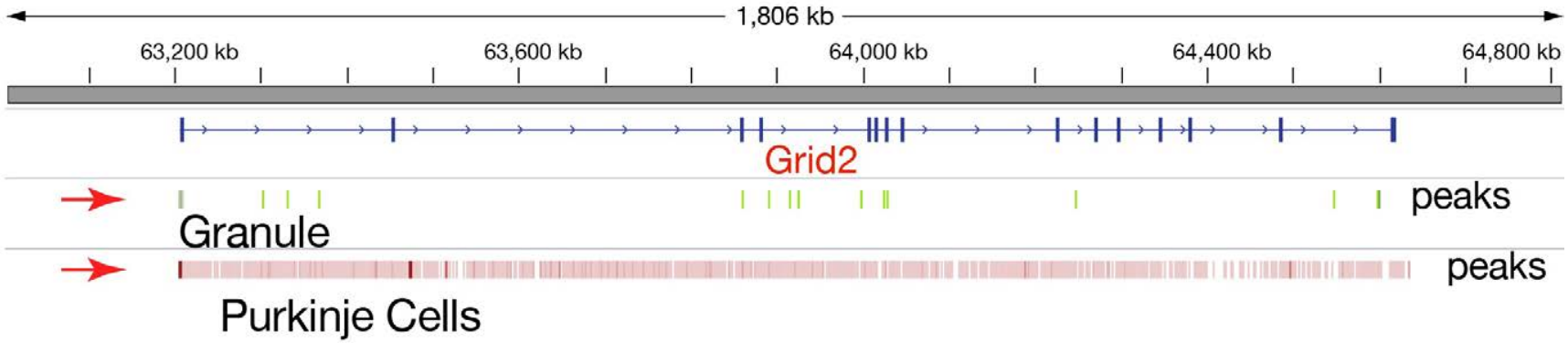
Purkinje cell ATAC-seq

ATAC-seq plots of PC/GC genes from *Rosenberg et al* data set:



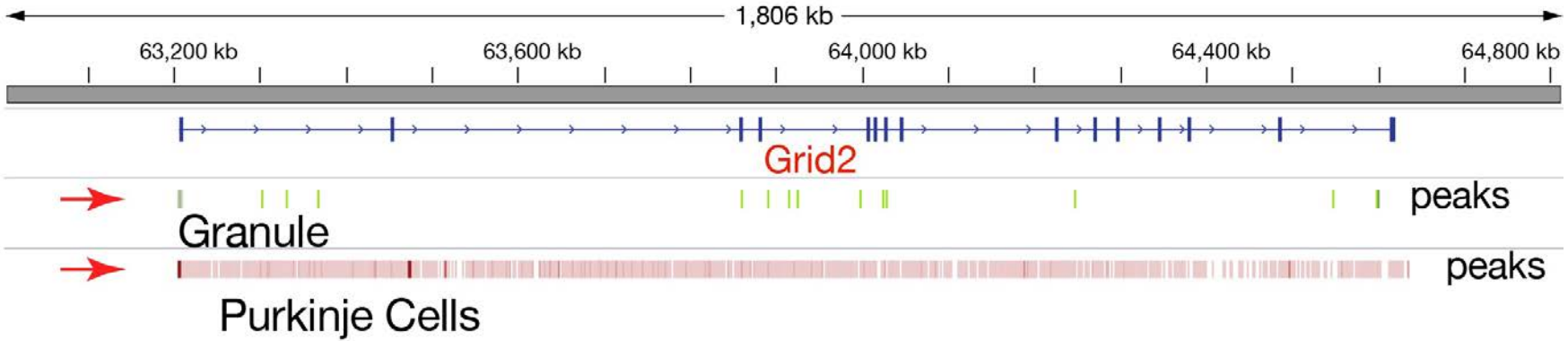
Purkinje cell ATAC-seq

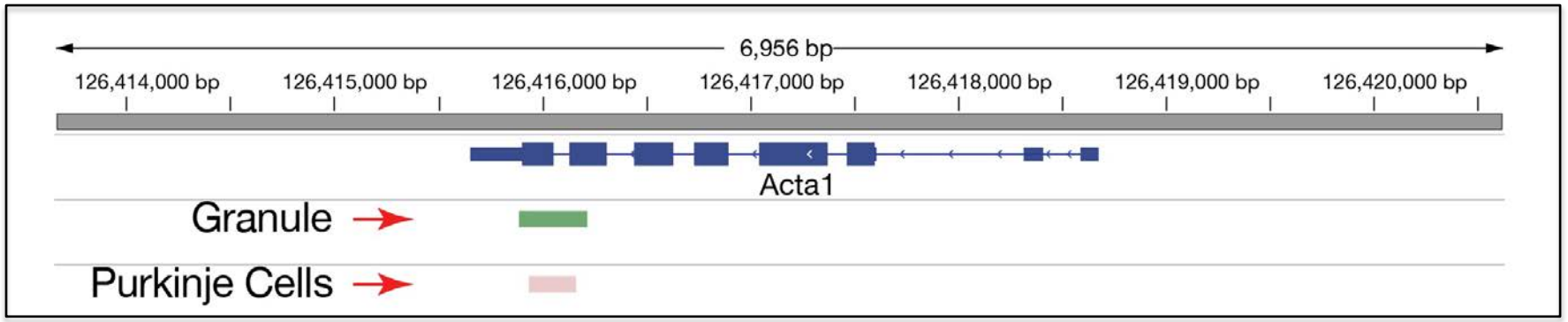
ATAC-seq plots of PC/GC genes from *Rosenberg et al* data set:

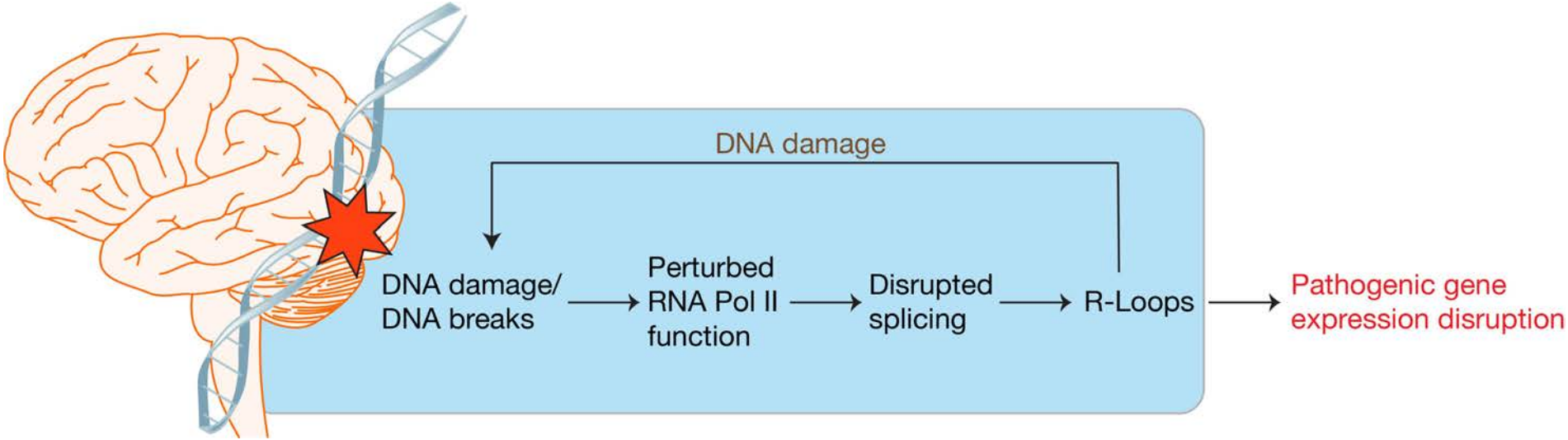


Purkinje cell ATAC-seq

ATAC-seq plots of PC/GC genes from *Rosenberg et al* data set:







- Why the cerebellum; why Purkinje cells

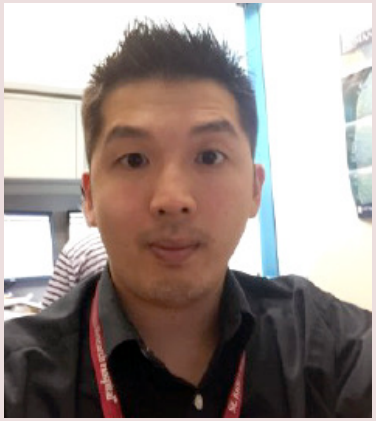
  **Open chromatin predisposes to DNA damage*

  **Chromatin conformation directs genome instability-associated neuropathology*

- Why progressive (temporal component)?
- How is nature of damage important?

All the work was done by…

YoungDon Kwak



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Helen Russell

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Acknowledgements:

Lab members:

- Young Don Kwak
- Helen Russell
- Aditi
- Yang Li
- Lee Pribyl
- Jingfeng Zhao
- Susanna Downing
- Roketa Sloan
- Lavinia Dumitrache
- Yuna Kim

- Sachin Katyal

Einstein School of Medicine,
Kamran Khodakh
Ambika Tewari

Computational Biology
Tim Shaw
Hongjian Jin
Gang Wu
Yiping Fan

St. Jude Cancer Center:
Transgenic Core Unit
Animal Resource Center
Hartwell Biotech Center

Funding:

National Institute of Neurological Disorders and Stroke
National Institutes of Health

National Cancer Institute

St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder