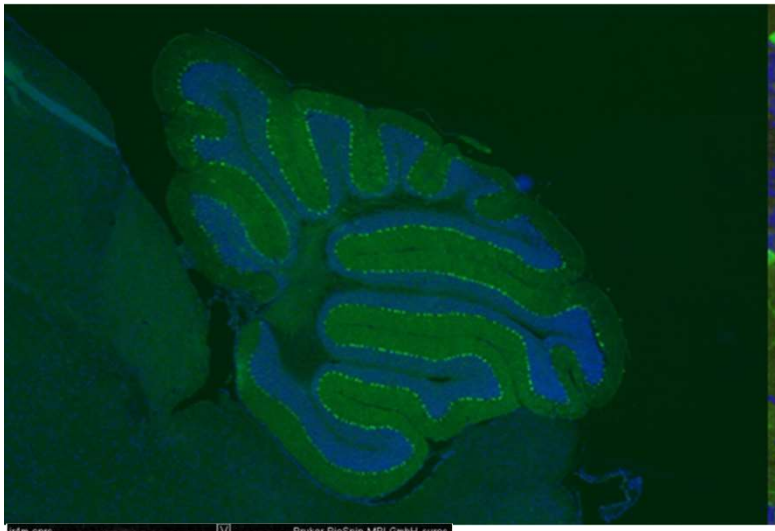
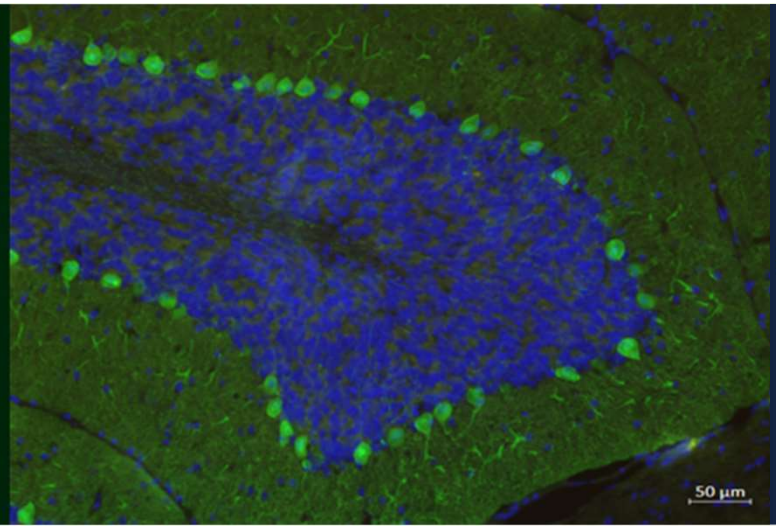
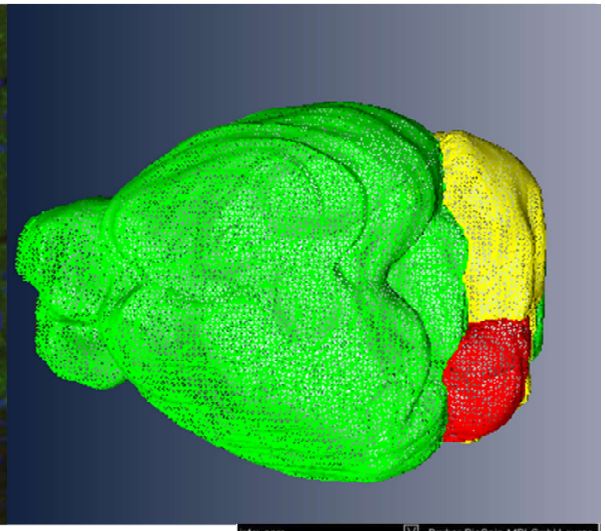
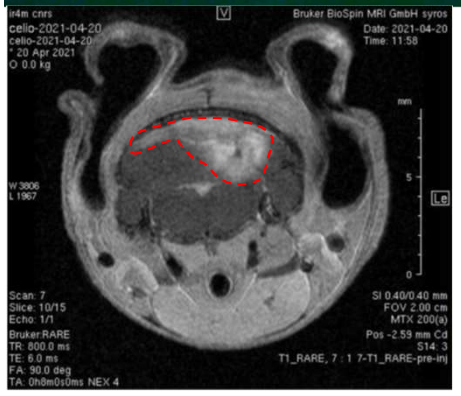
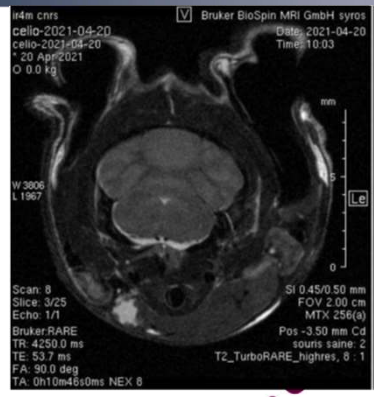
Team: “Signaling and Cancer Progression”











Celio Pouponnot
Team: Signaling and Cancer progression
Institut Curie ORSAY
UMR3347 CNRS/U1021 INSERM/ UPSaclay

institutCurie

cnrs

Inserm
Institut national
de la santé et de la recherche médicale

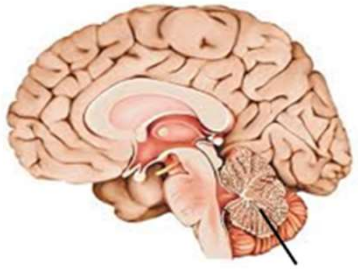
université
PARIS-SACLAY

FACULTÉ
DES SCIENCES
D’ORSAY

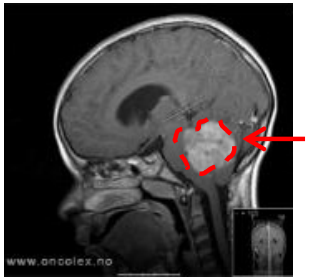
Medulloblastoma : an abnormal identity drives cancer progression

Institut Curie
UMR3347 CNRS/U1021 INSERM

Medulloblastoma (MB)



cerebellum



- Pediatric tumor of the cerebellum (Median age 7 yrs)

Pediatric tumor

Pediatric Cancers

In general rare cancers

- Represent 1 to 2% of the cancers in total

Pediatric Cancers

In general rare cancers

- Represent 1 to 2% of the cancers in total

In industrialized countries, represents 2nd cause of mortality betwen 1 to 15 y

(Less than 1% of death between 0 and 1 year but 20 % between 1 and 14 years)

Pediatric Cancers

In general rare cancers

- 75-80% Cured
  - **Better than for adult cancers. But some cancer are of high risk**

Pediatric Cancers

In general rare cancers

- **75-80% Cured**
  - Better than for adult cancers.

- BUT very important side effects affecting adult life

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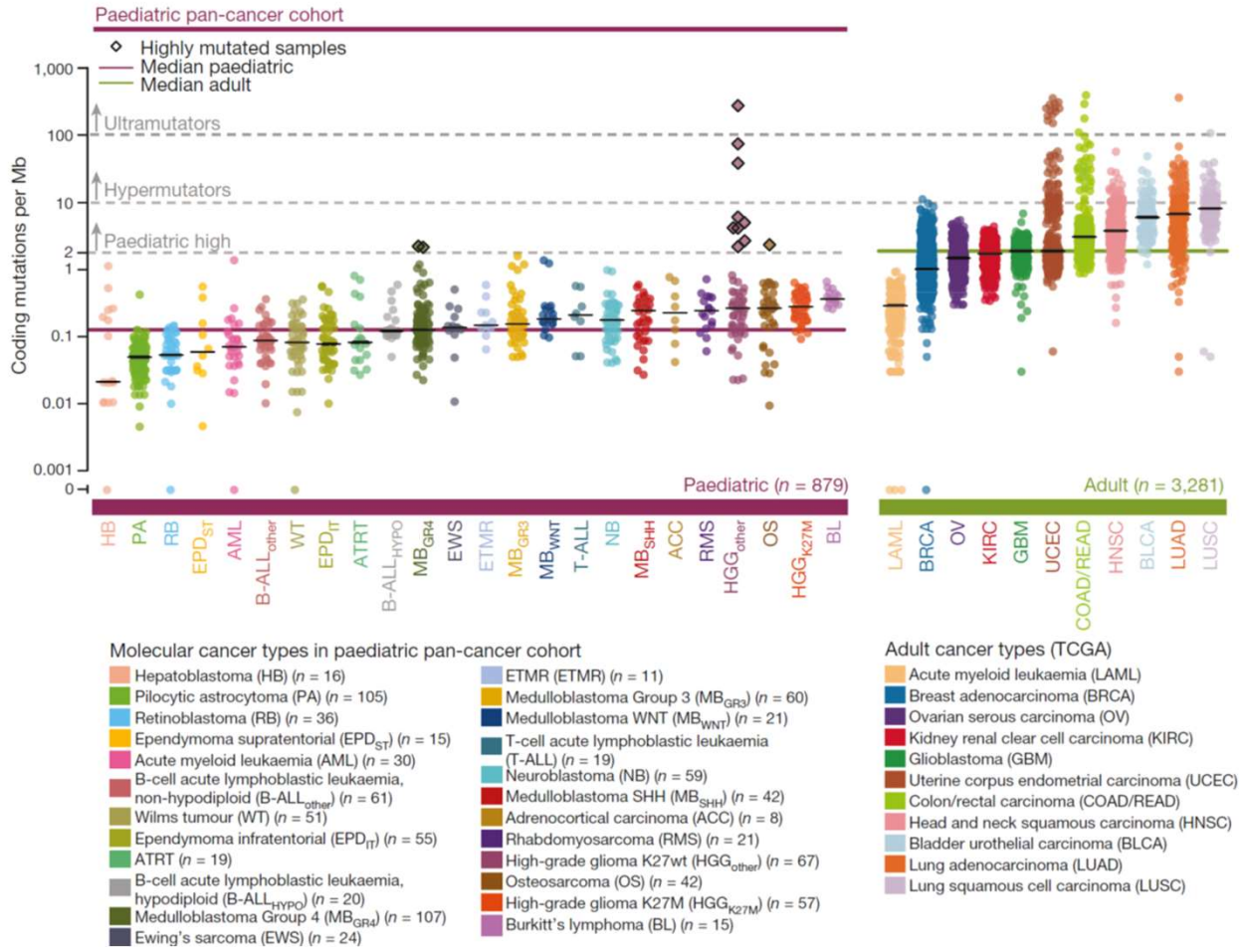

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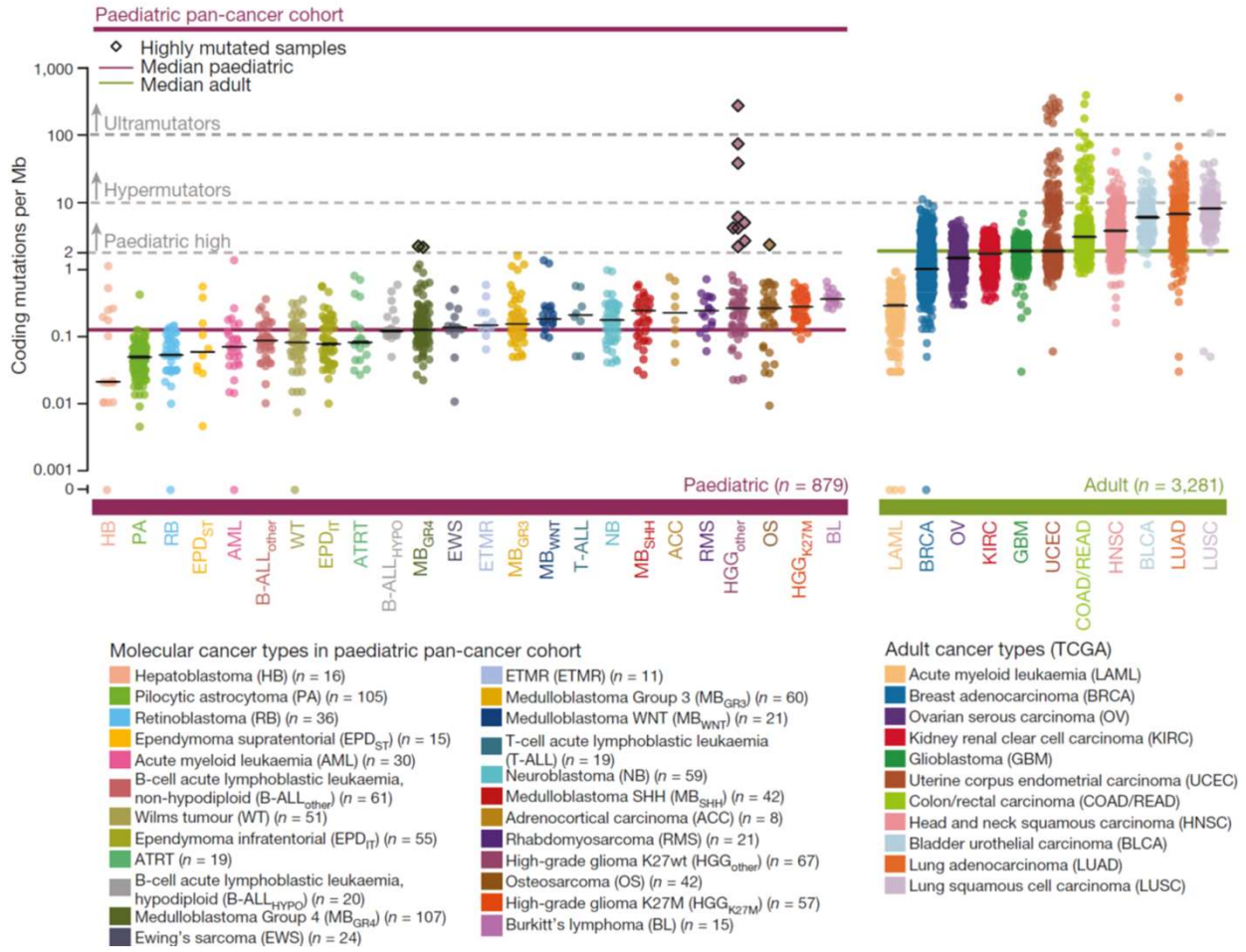

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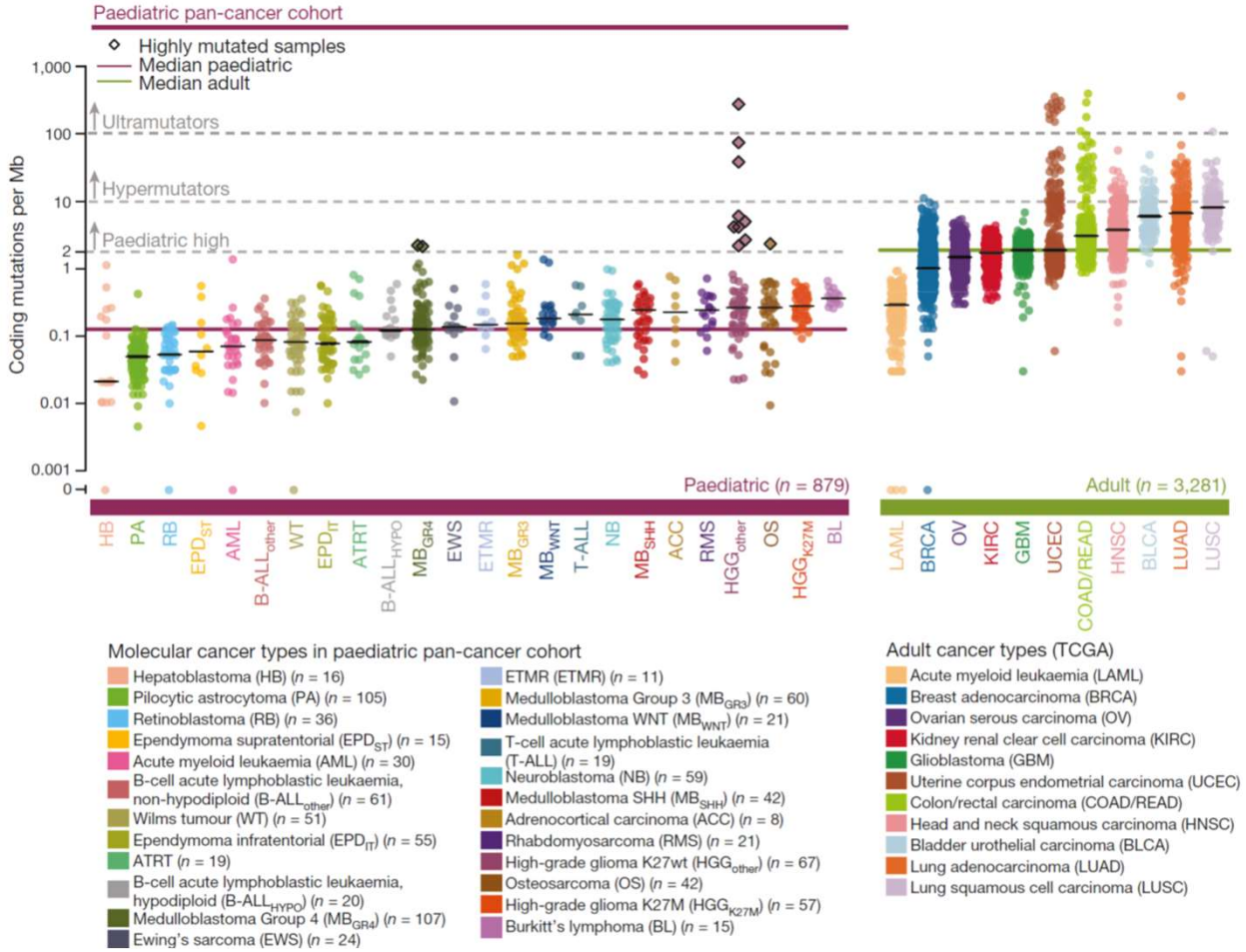

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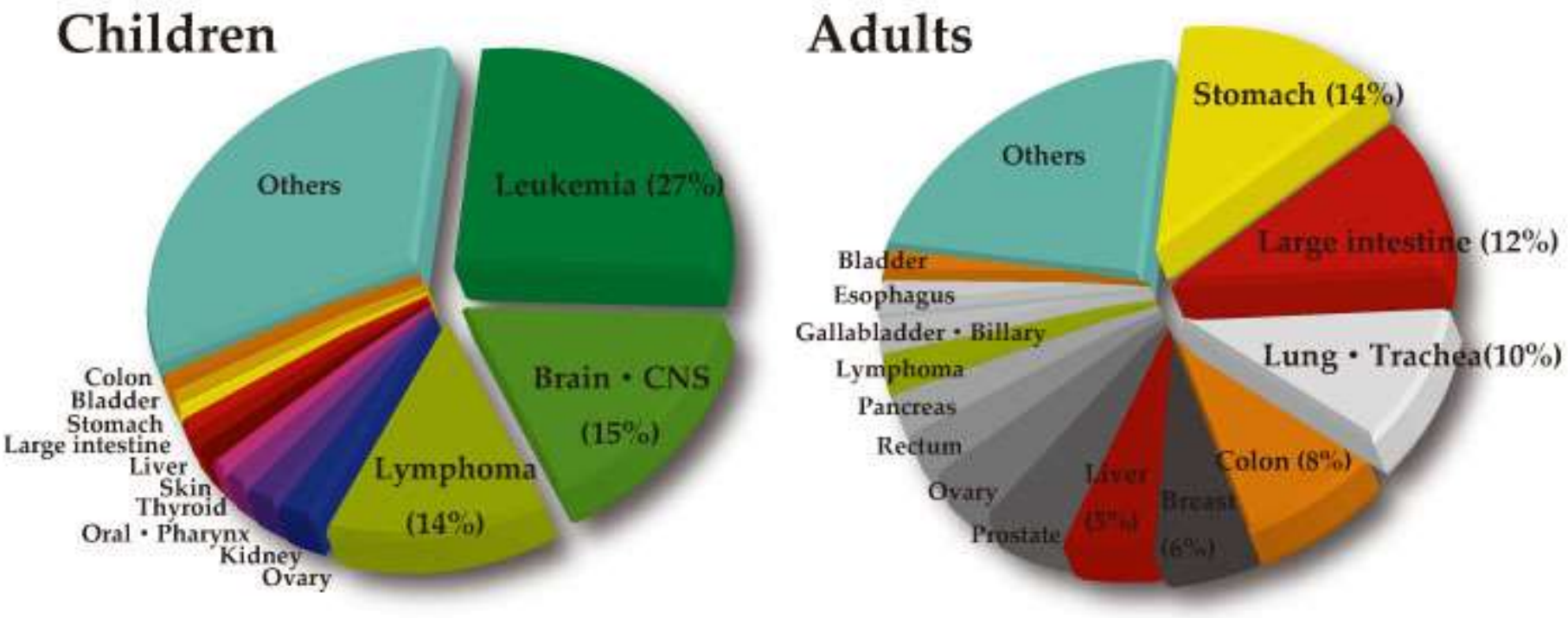

Pediatric Cancers

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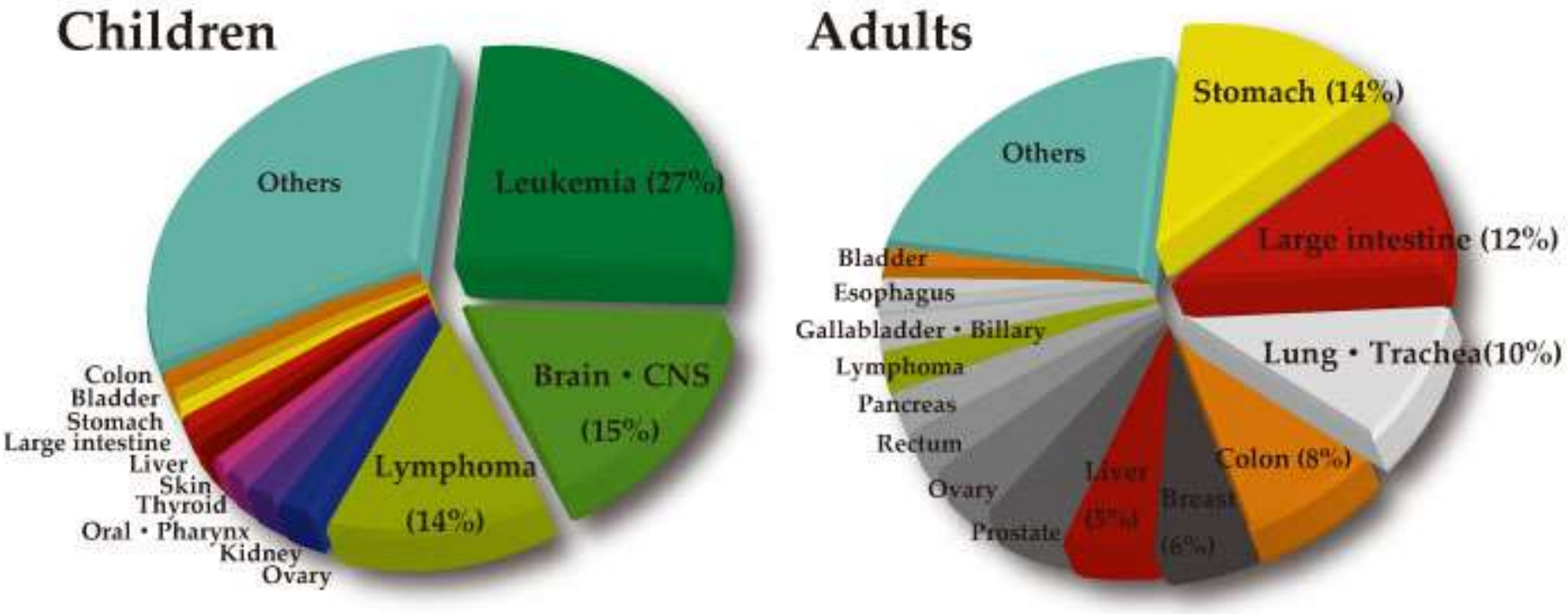
- **Different spectrum**



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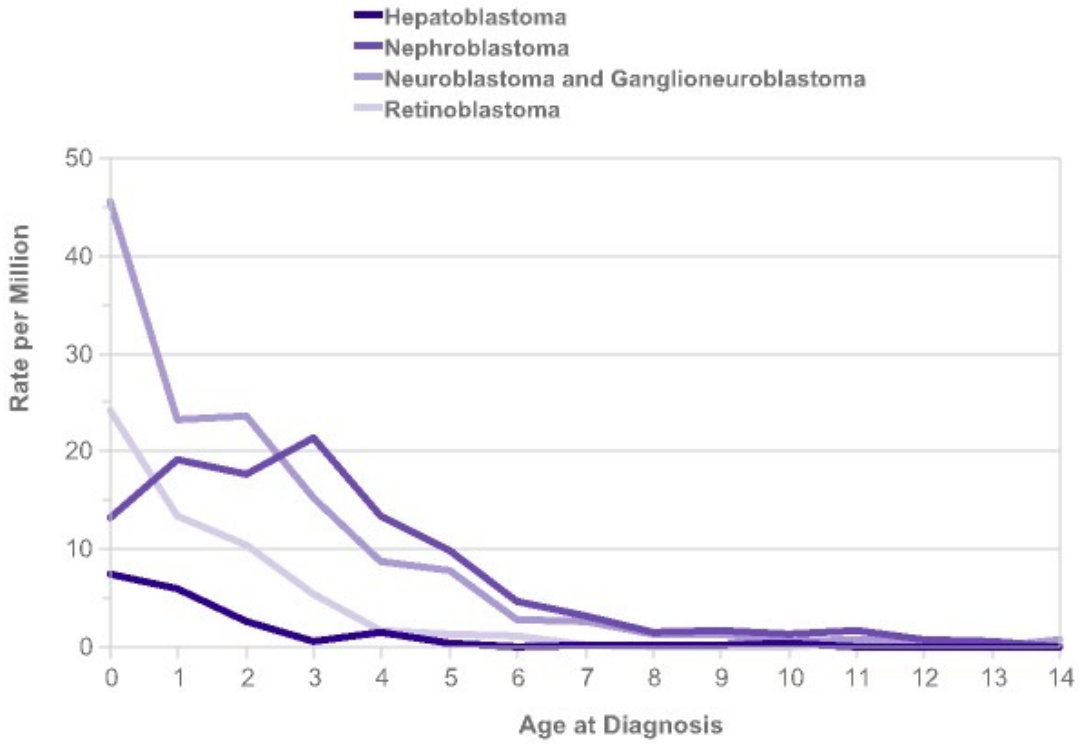

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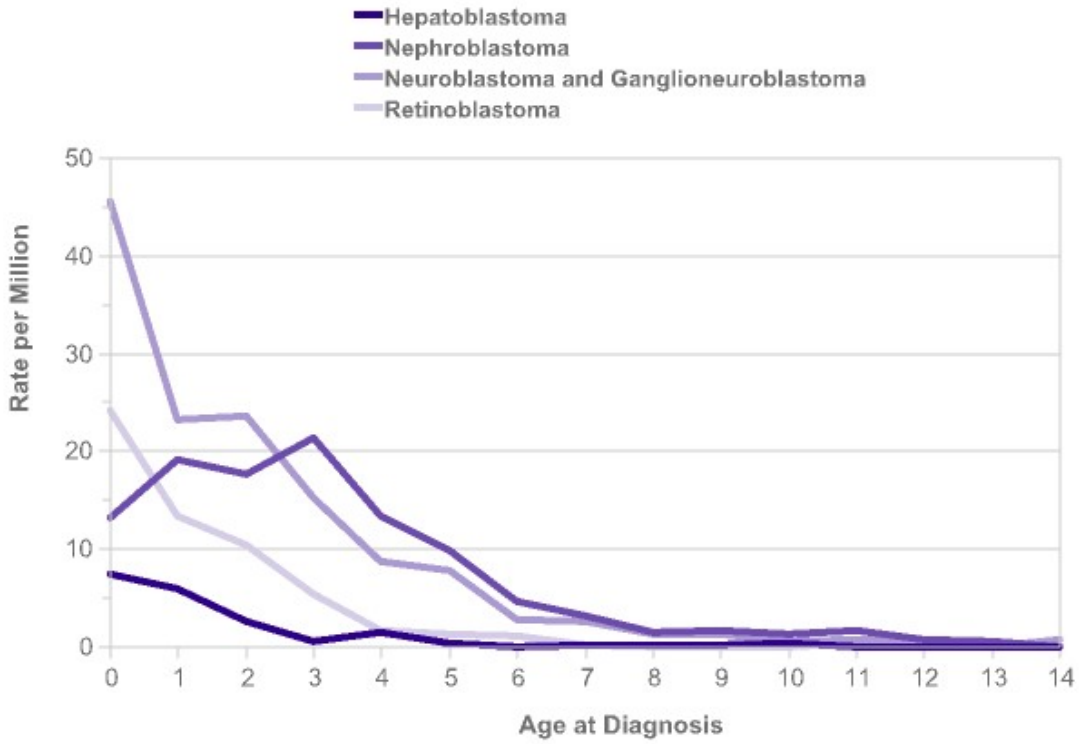
- **In a defined window of time**



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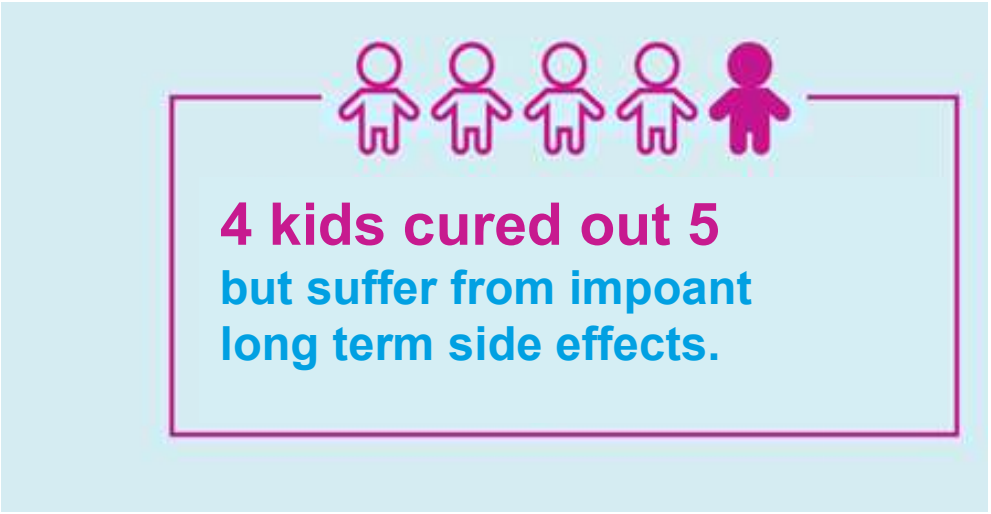

Improve cure rate and decrease side effects

Pediatric Cancers

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- **In a define window of time**

How to explain these differences?

Pediatric Cancers

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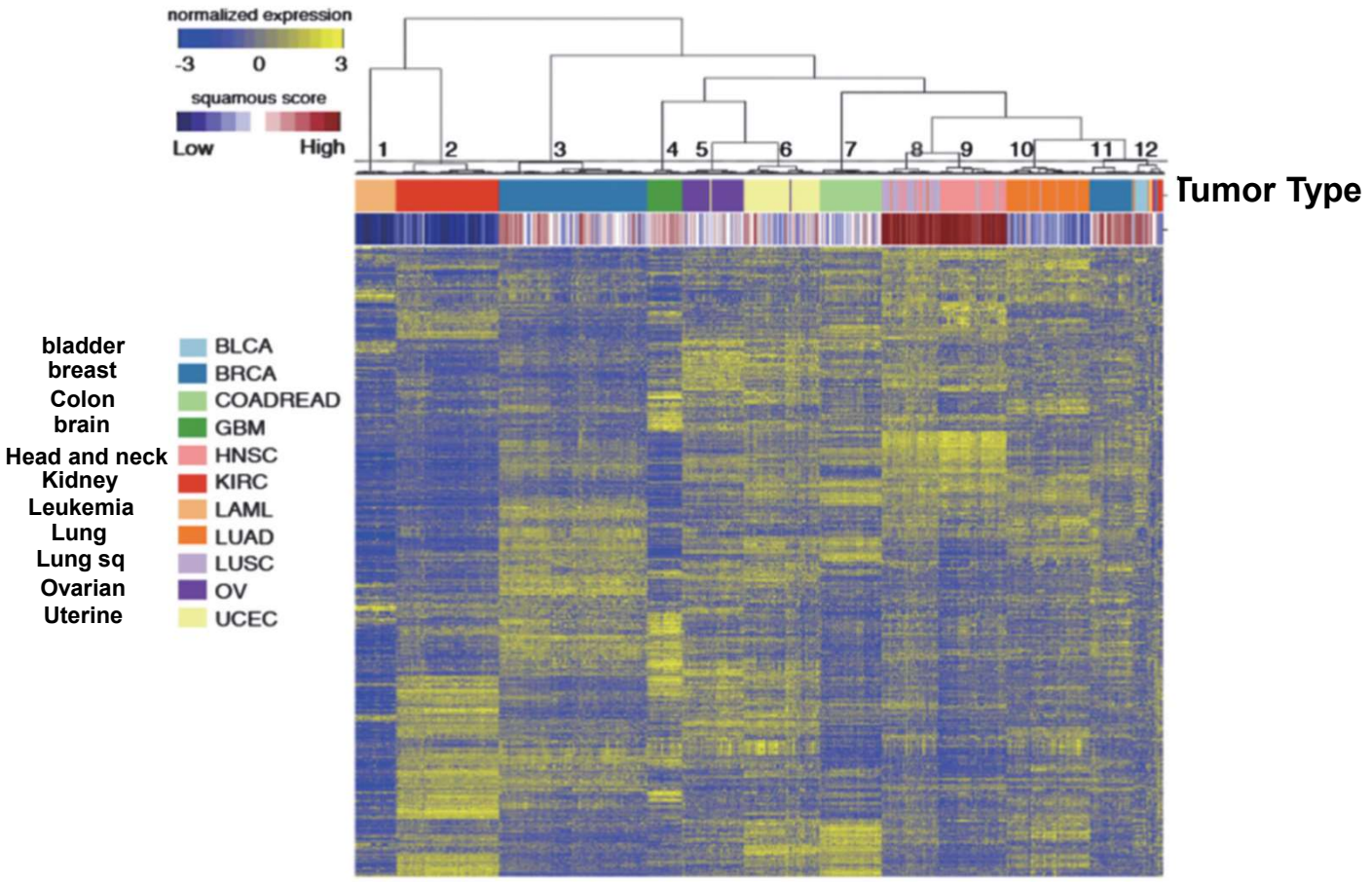
- **In a define window of time**

How to explain these differences?
(tissue context and cell of origin)

Cancers of different locations



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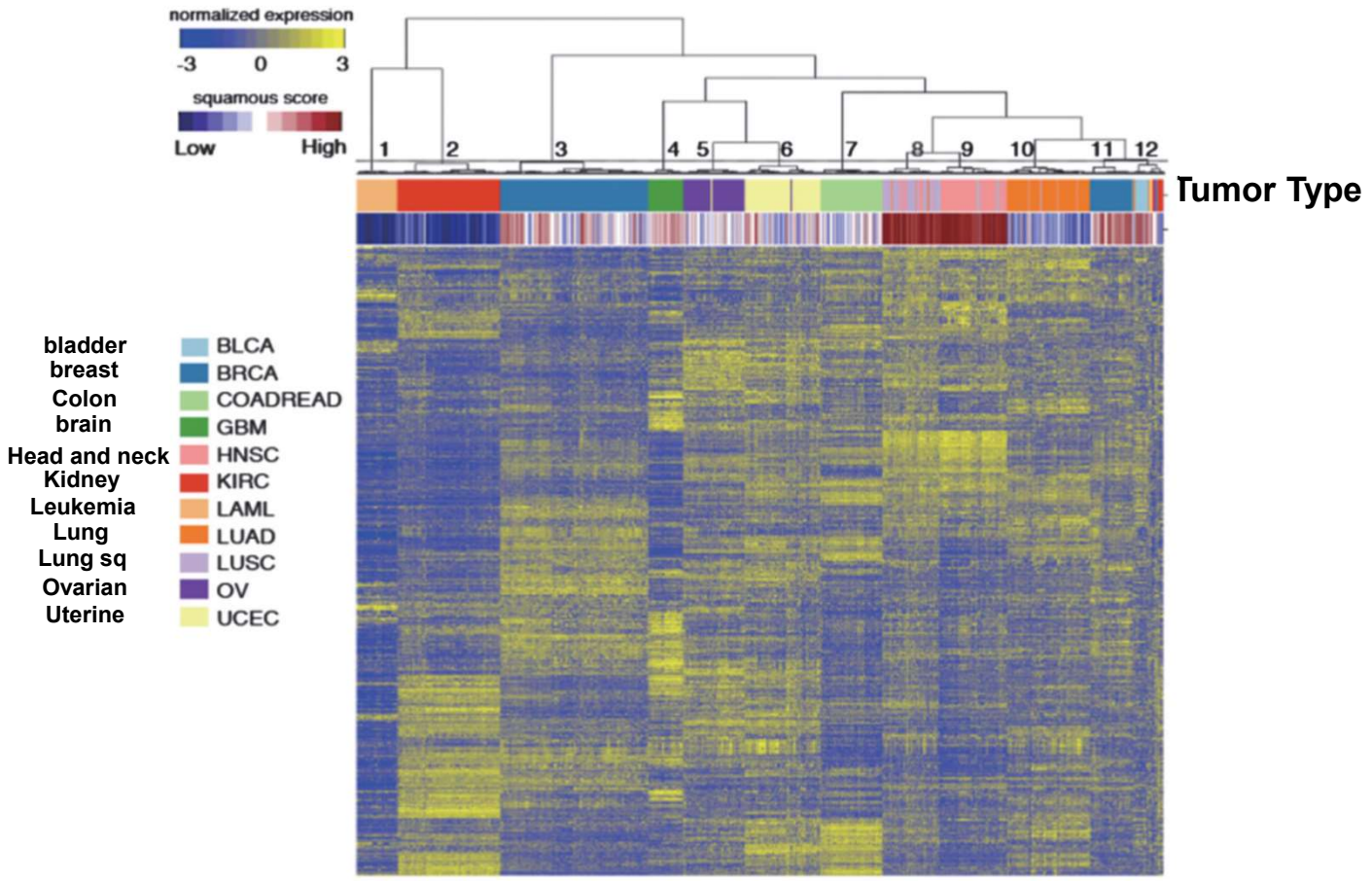


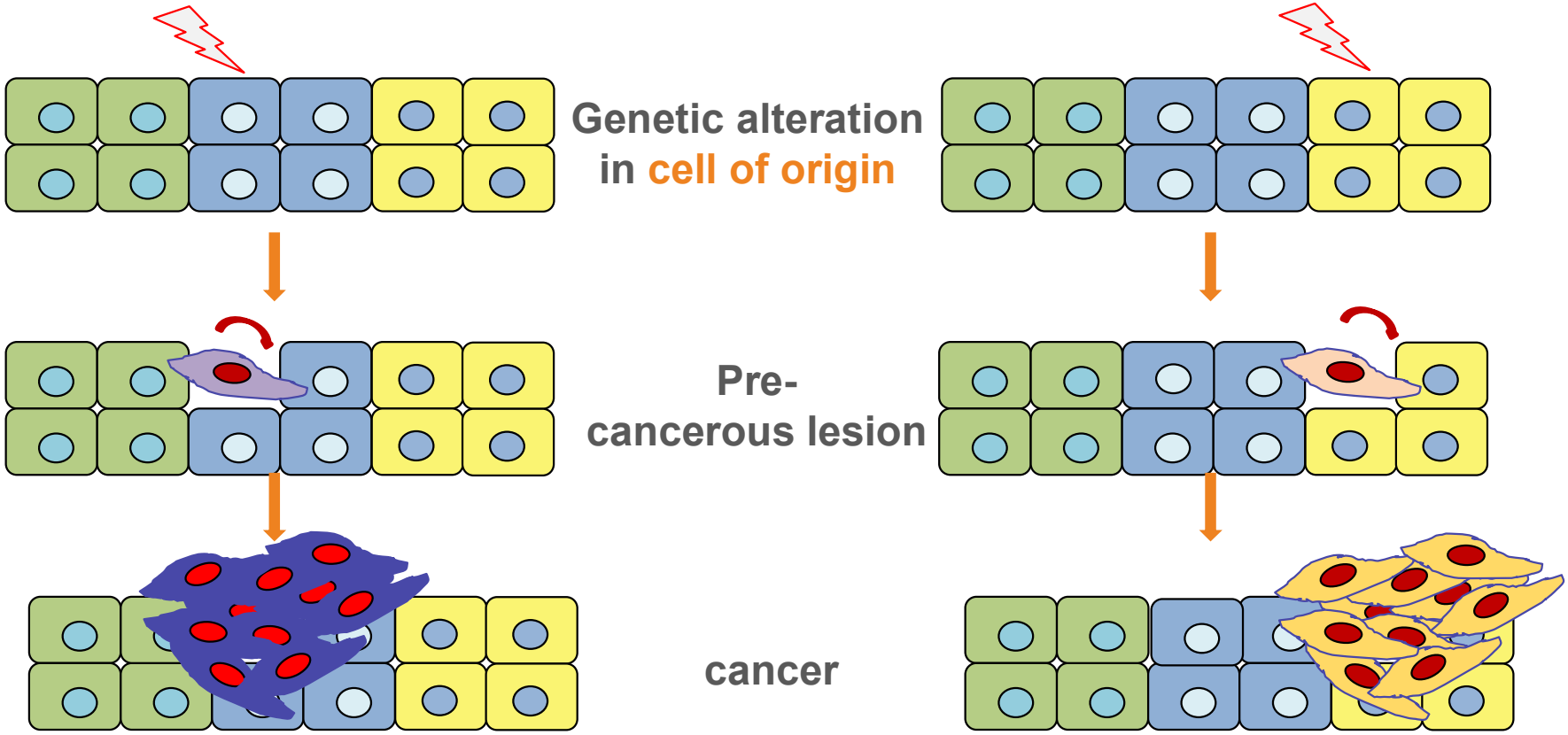
Cancers of a given location cluster together at their transcriptomic level
(express similar genes that are representative/specific of the tissue where the tumour develops

→ Specific lineage markers/cell identity define a tumor type

Cell of origin

Tumor progression

Genetic alterations : mutations…

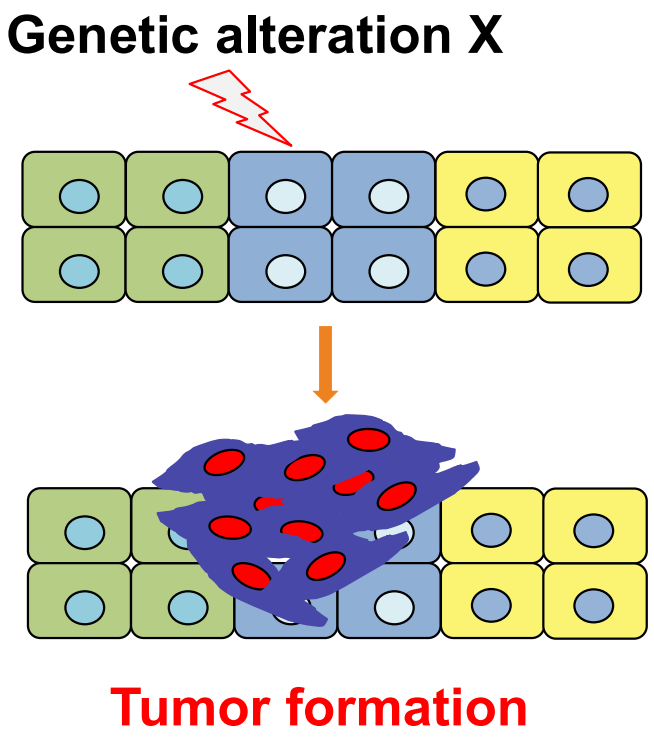


Cell of origin: Identify in which cell the first genetic lesion arises and promotes an pre-cancerous lesion.

-cell identity and lineage-

CONCEPT OF LINEAGE ADDICTION

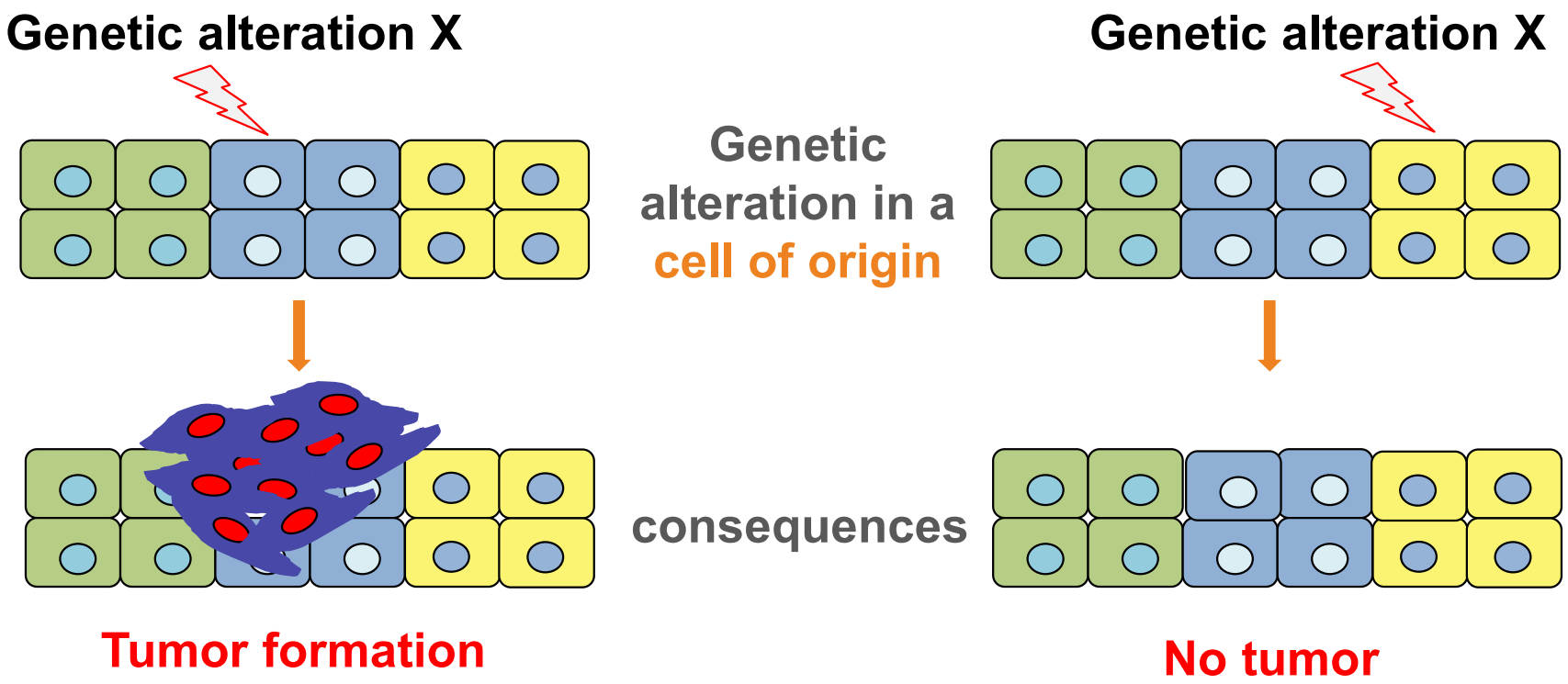
Cell of origin



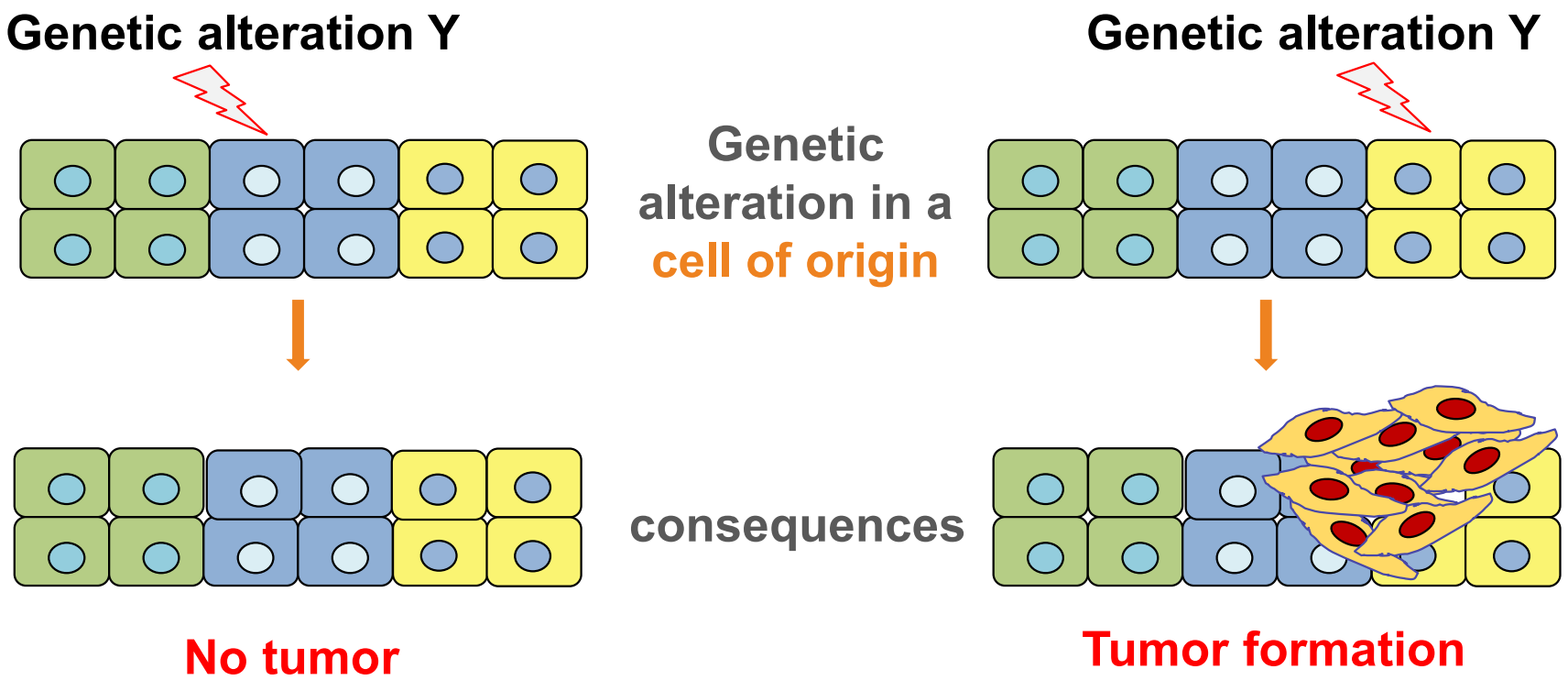
Genetic alteration in a **cell of origin**

consequences

Cell of origin



Cell of origin



Pediatric Cancers

Pediatric cancer:

Child **vs** **Adult**

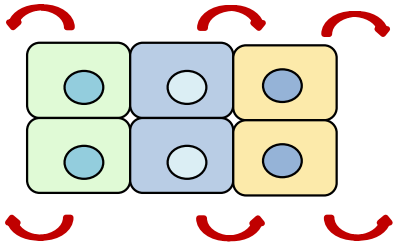
Pediatric Cancers

Pediatric cancers arise in a developing tissue

Pediatric cancer:

Genetic alteration during development

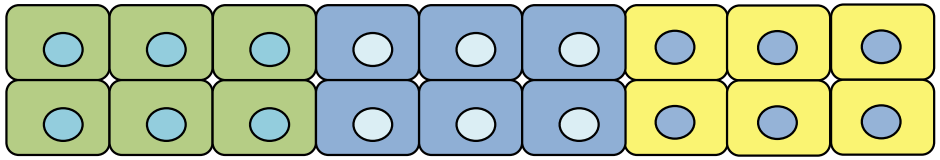
Child



DEVELOPMENT

Maturation
differentiation

Adult



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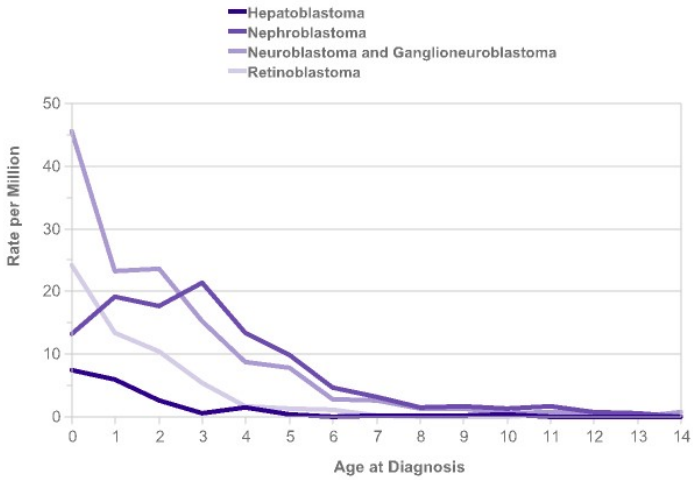

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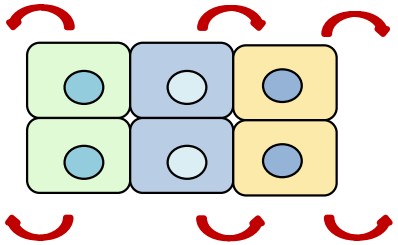

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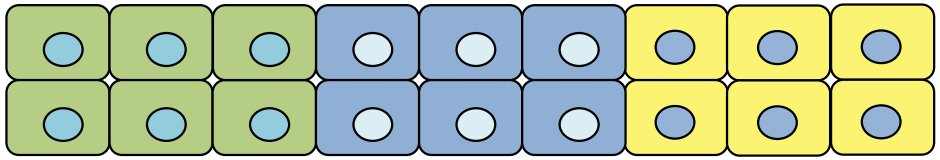

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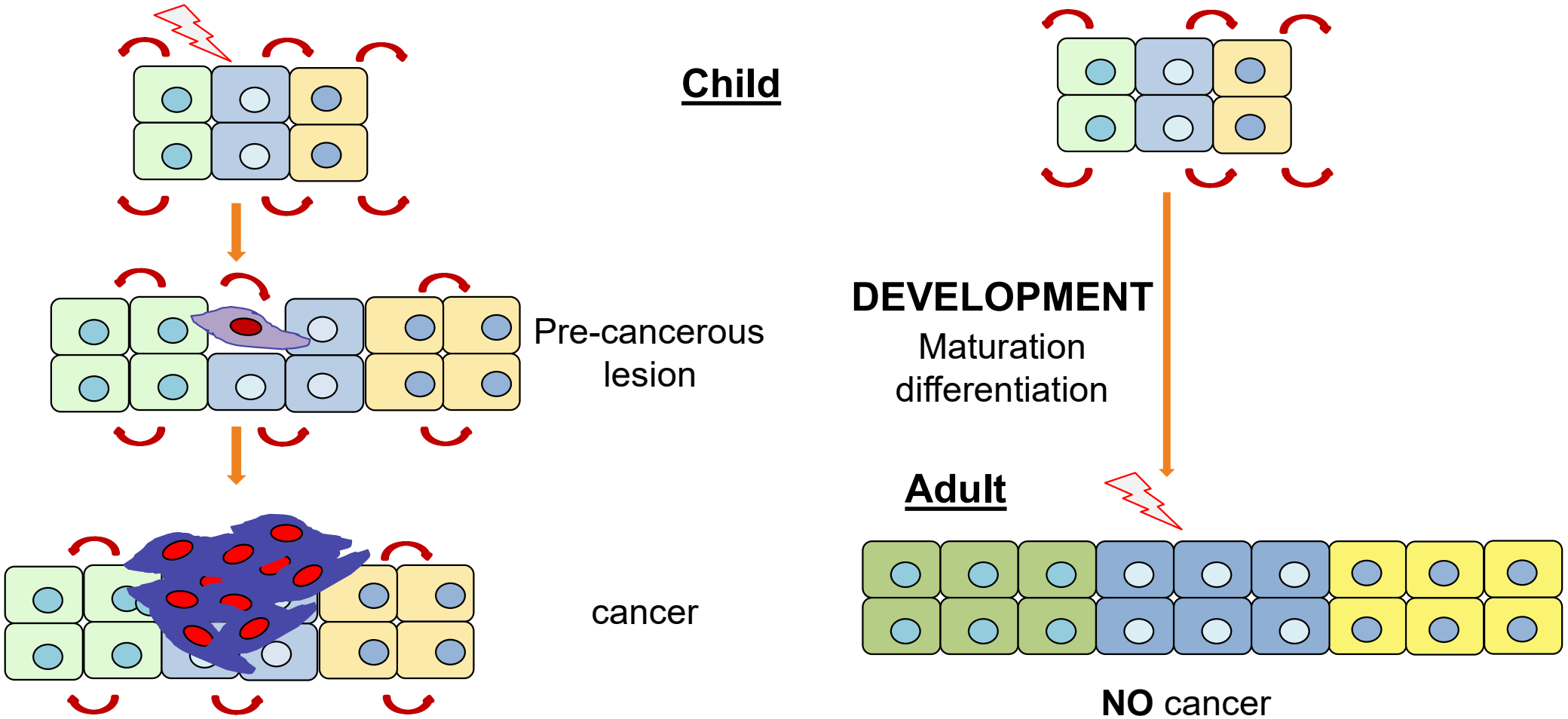

Cell of origin can explain some specificities of pediatric cancers, present in a defined window of time – not present in adult (spectrum)

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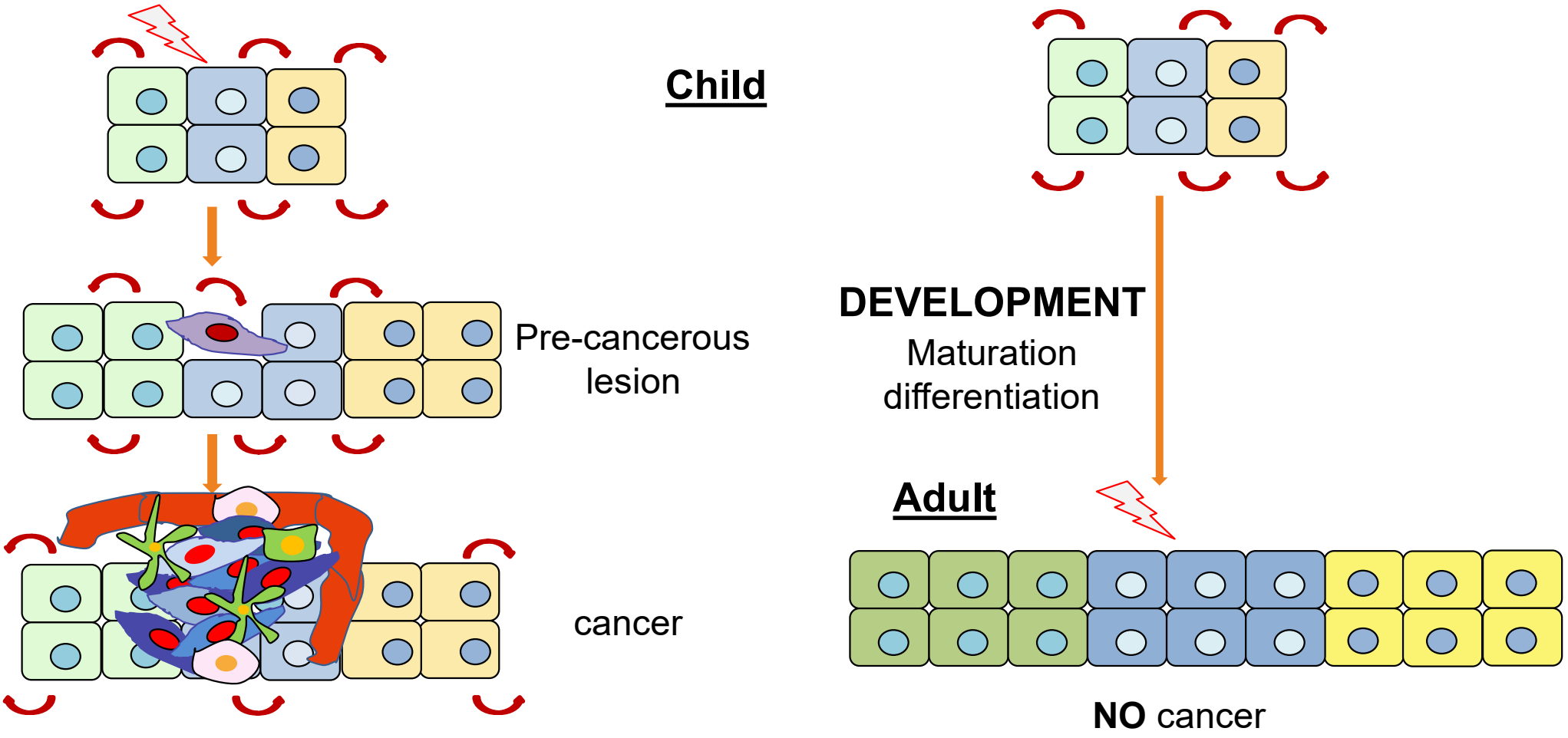

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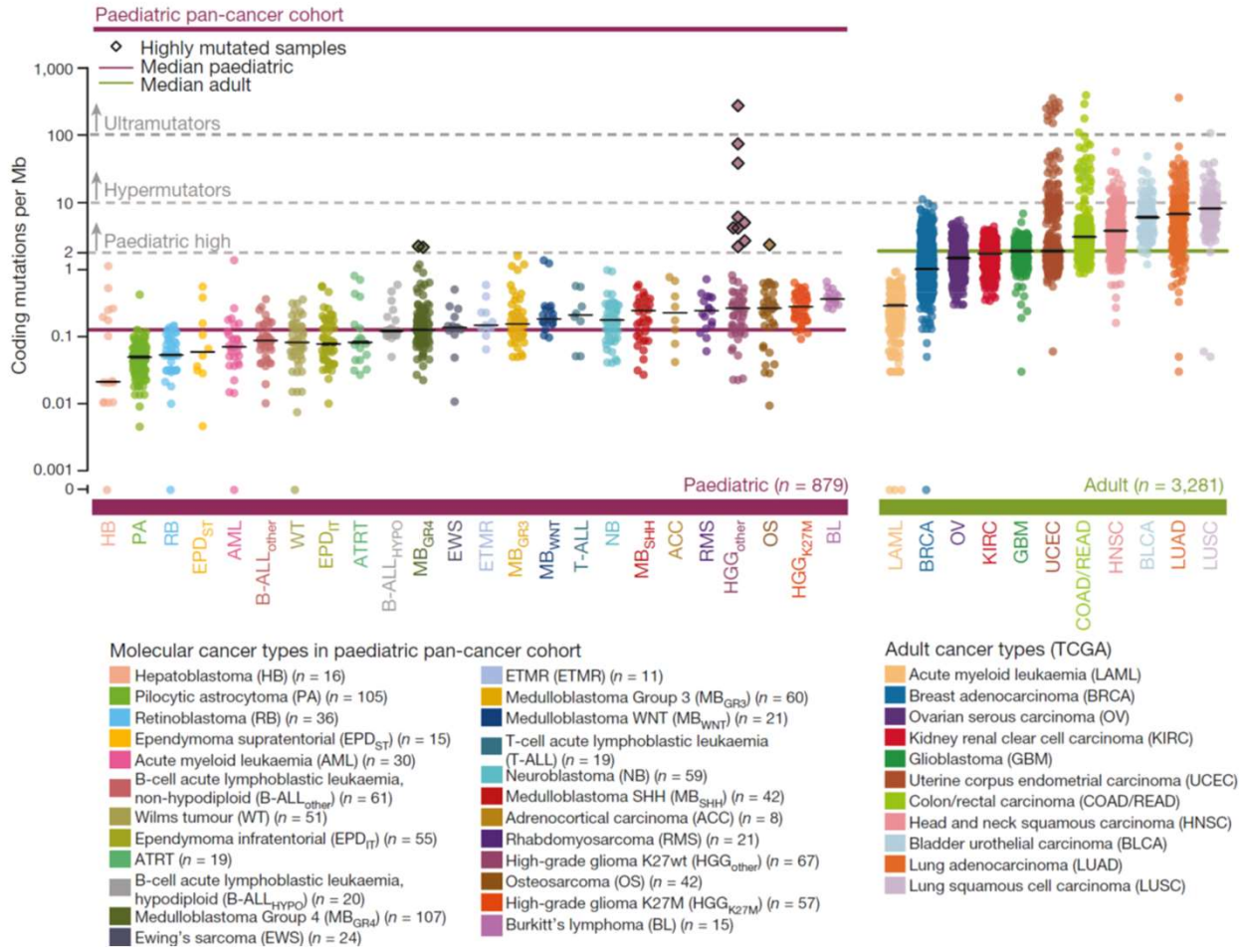

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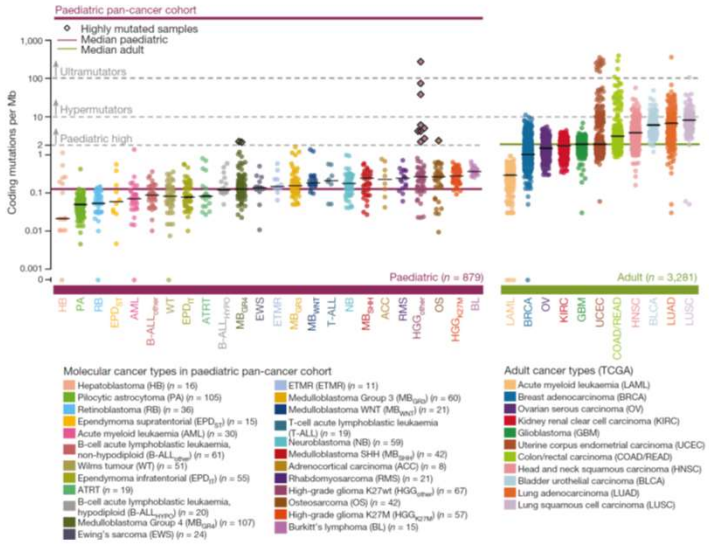
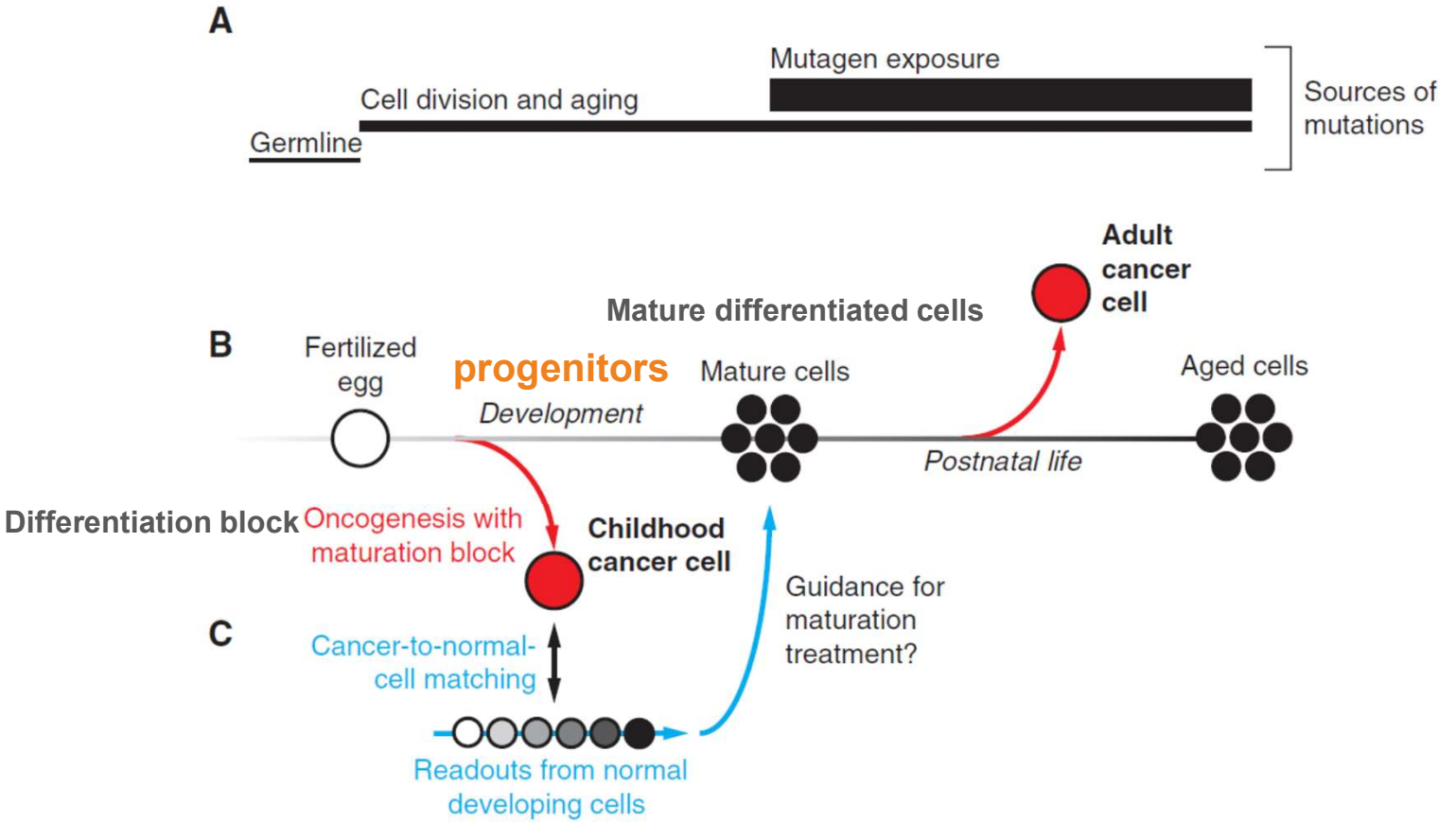
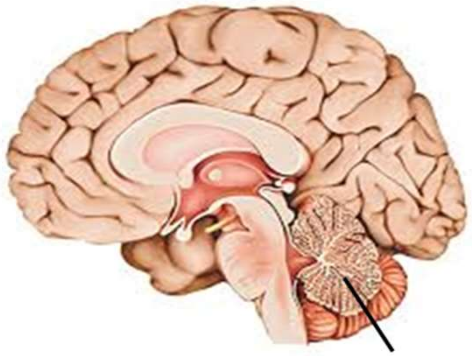




Medulloblastoma

Pediatric tumor

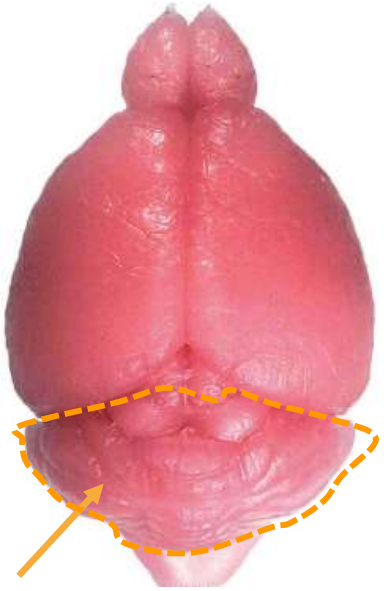
Medulloblastoma (MB)



cerebellum

- Pediatric tumor of the cerebellum (Median age 7 yrs)

The Cerebellum



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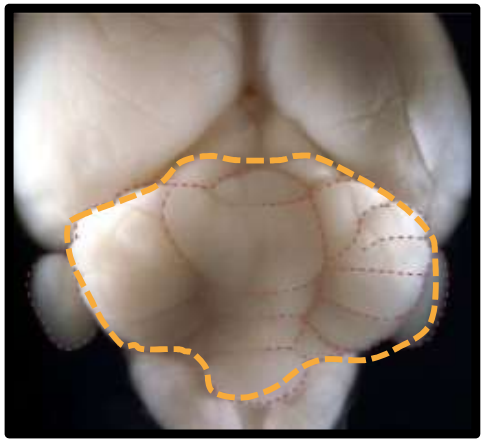
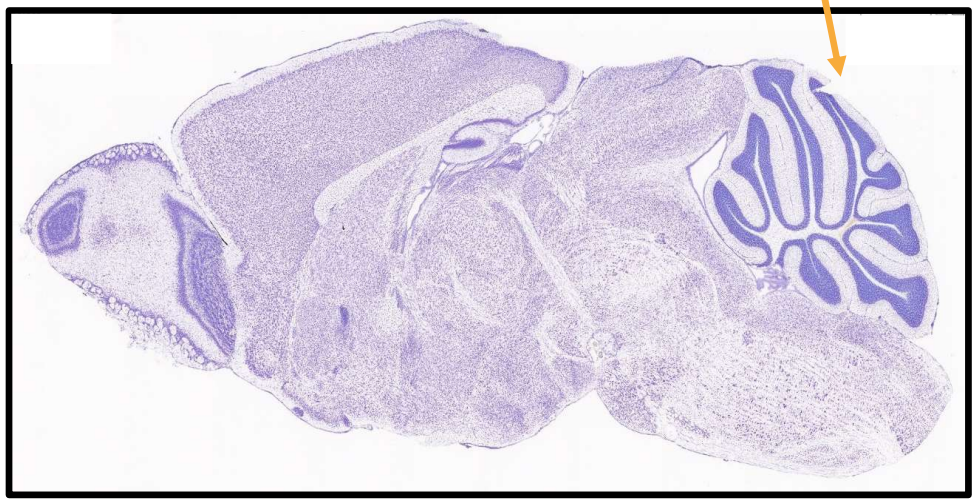


hemisphere vermis hemisphere

Cerebellum

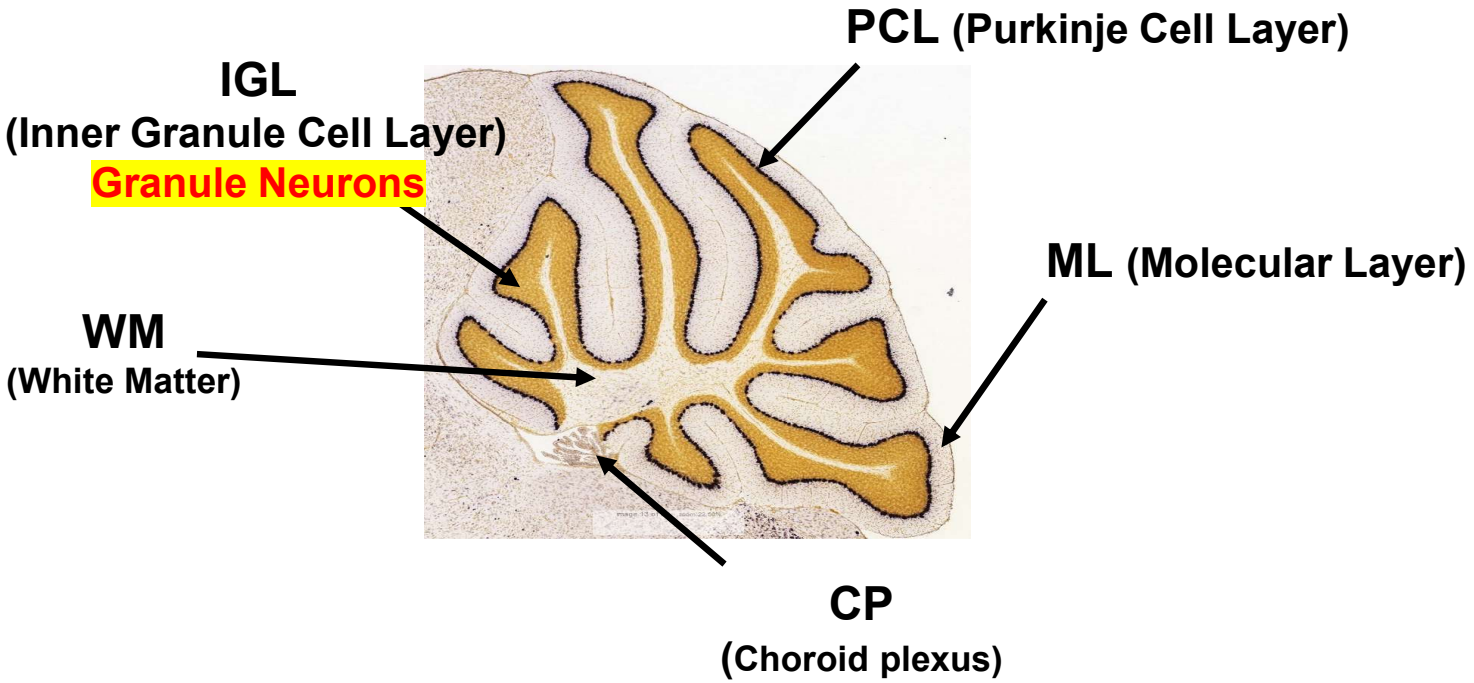


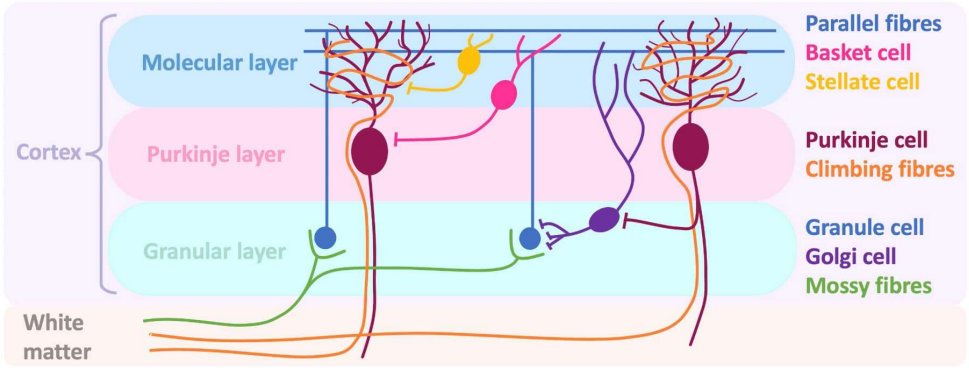
Controls:

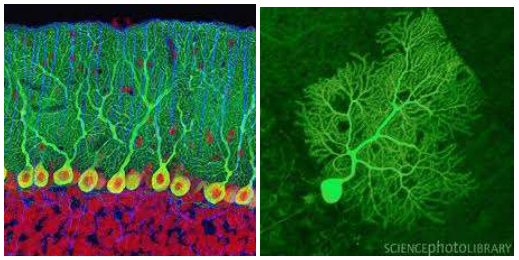
Balance, posture
Mouvement coordination
Some learning functions

The Cerebellum

Foliated and Stratified structure:
Composed of lobules and different layers





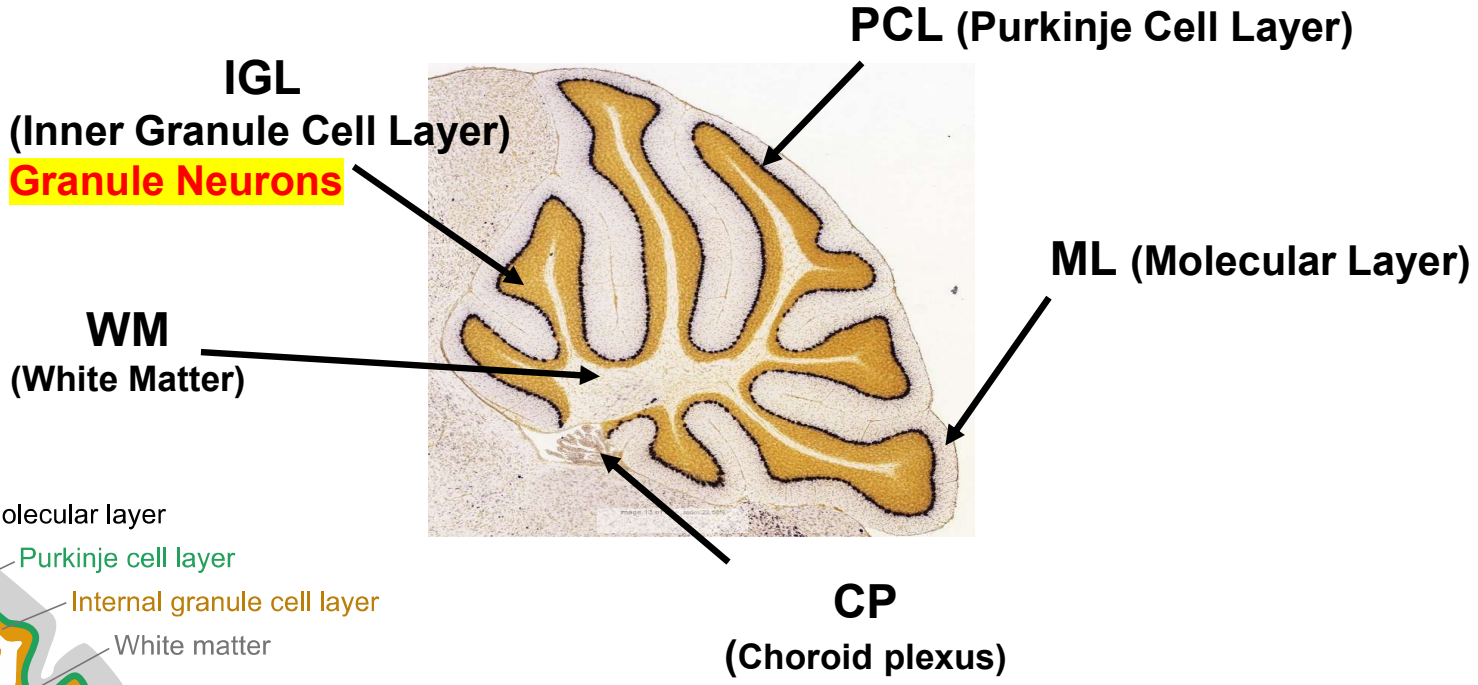


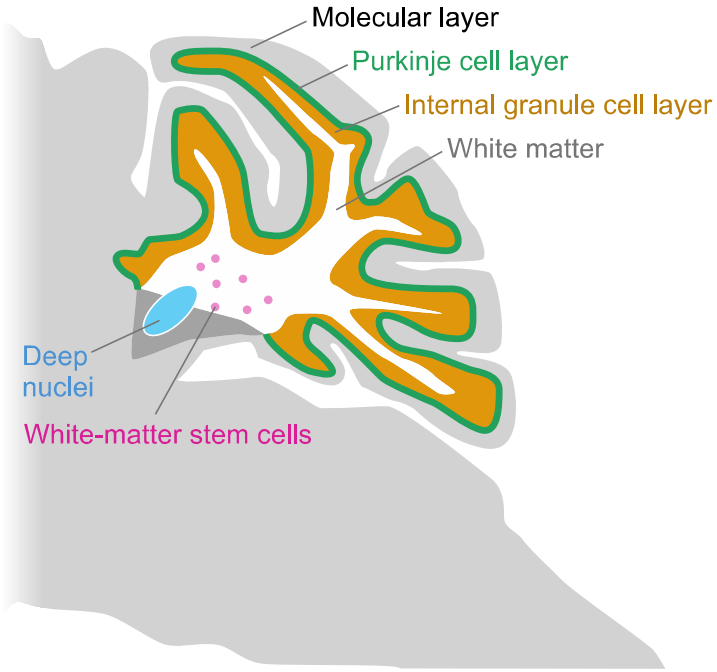
Purkinje cells

→ **Contains 80% of all brain neurons (human)/ 60% in mice**

The Cerebellum

Foliated and Stratified structure:
Composed of lobules and different layers

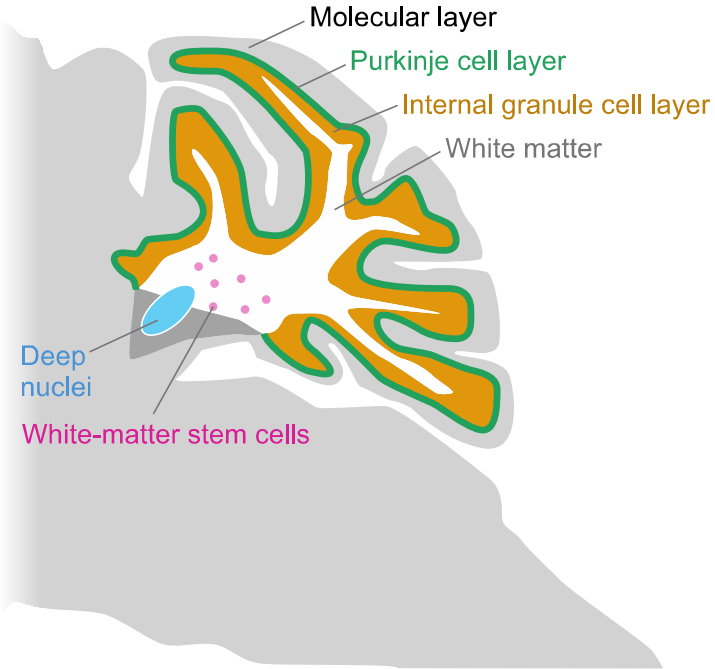




The Cerebellum

Mature cerebellum

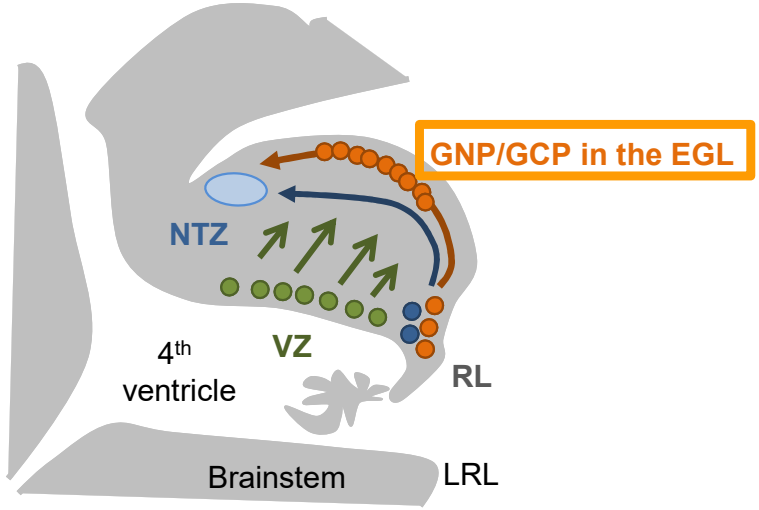
- **Cell types**
  - Granule cells (GC)
  - Purkinje cells (PC)
  - 6 Interneurons



Adapted from Gilbertson and Ellison, 2008

Developping cerebellum

- **Germinal zones**
  - Ventricular zone (VZ)
    - GABAergic neurons ➔ PCs
  - Rhombic Lip (RL)
    - Glutamatergic neurons ➔ GCs

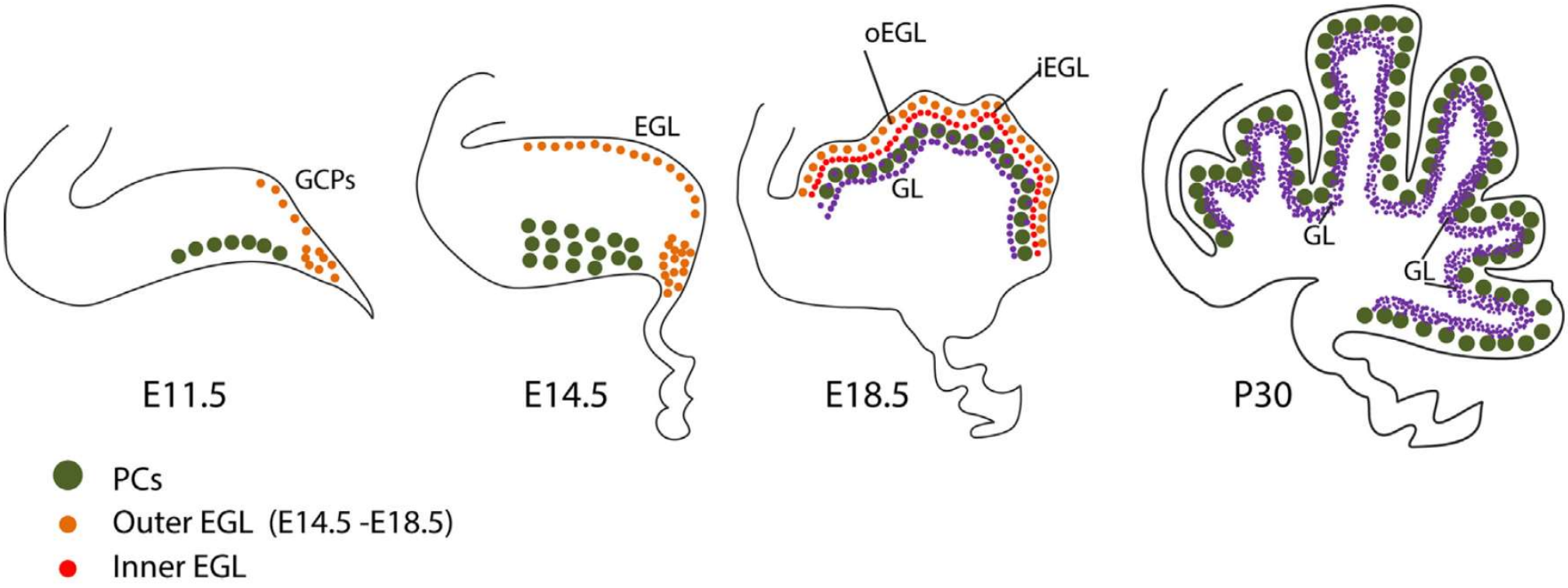


- GABAergic precursors (Purkinje cells and interneurons)
- Precursors of the deep nuclei (glutamatergic)
- Granule cell precursors (GCP or GNP)

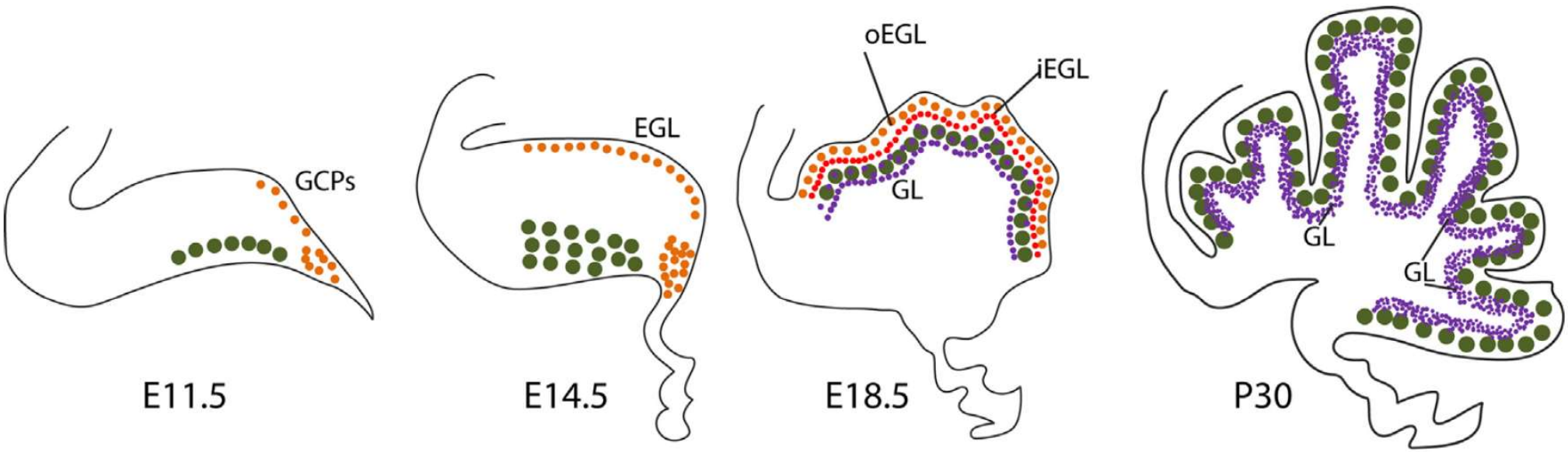
NTZ: nuclear transitory zone

Cerebellar development

Focus on Granule neurons lineages

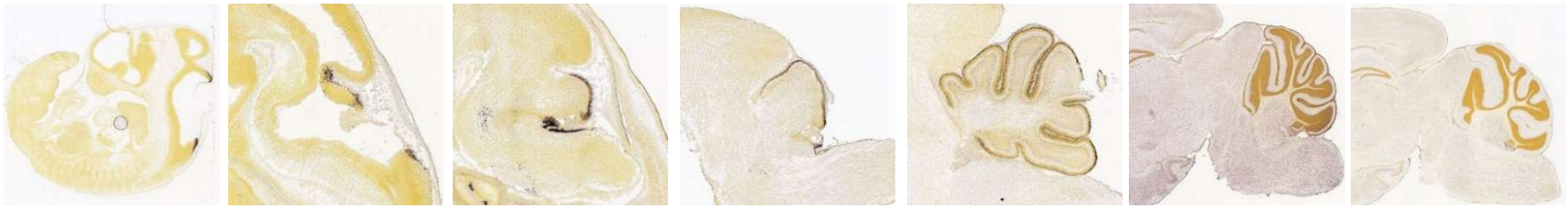


Cerebellar development



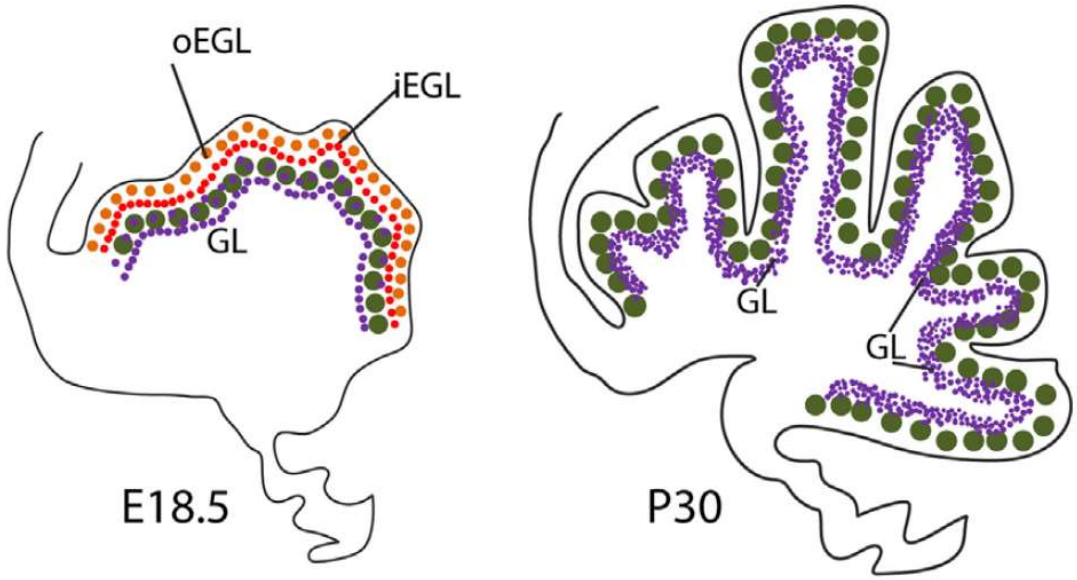
Atoh1 is a master regulator of the GCP

Atoh1



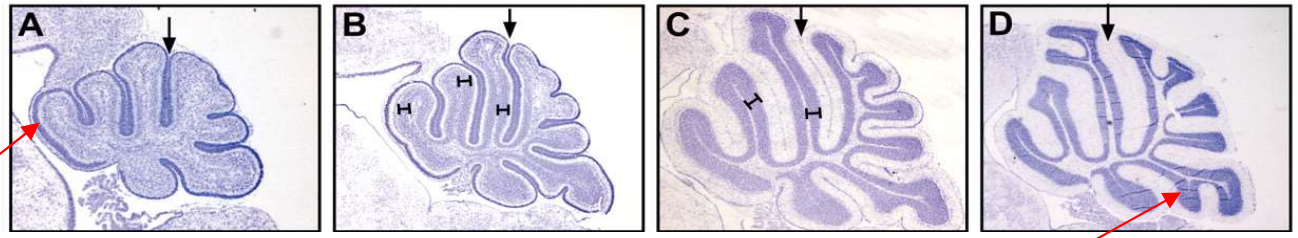
E11.5 **E13.5** **E15.5** **E18.5** **P4** **P14** **P28**

Late post-natal Cerebellar development



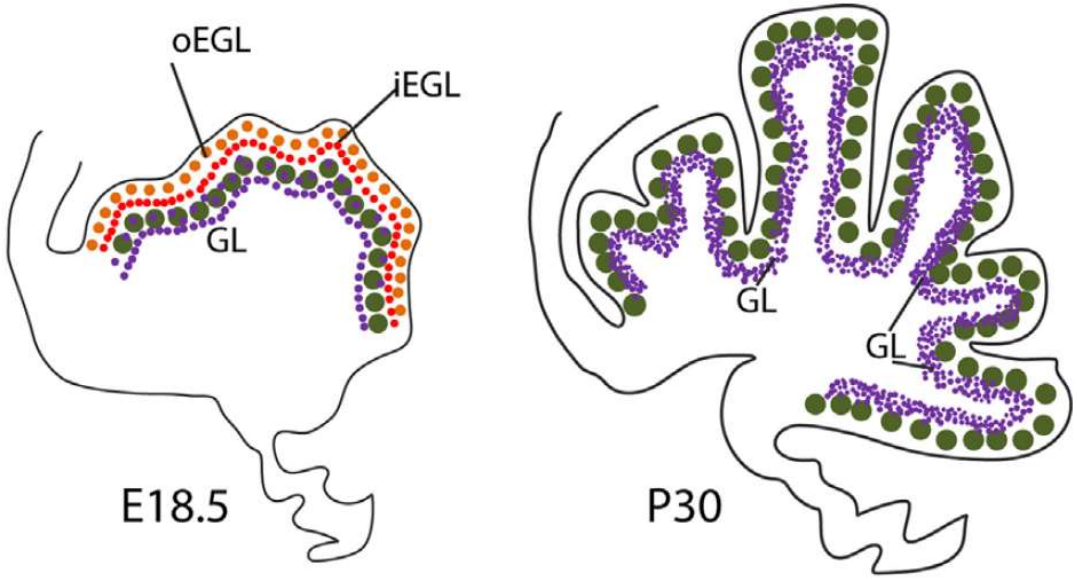
Postnatal cerebellar development

New Born **Adult**

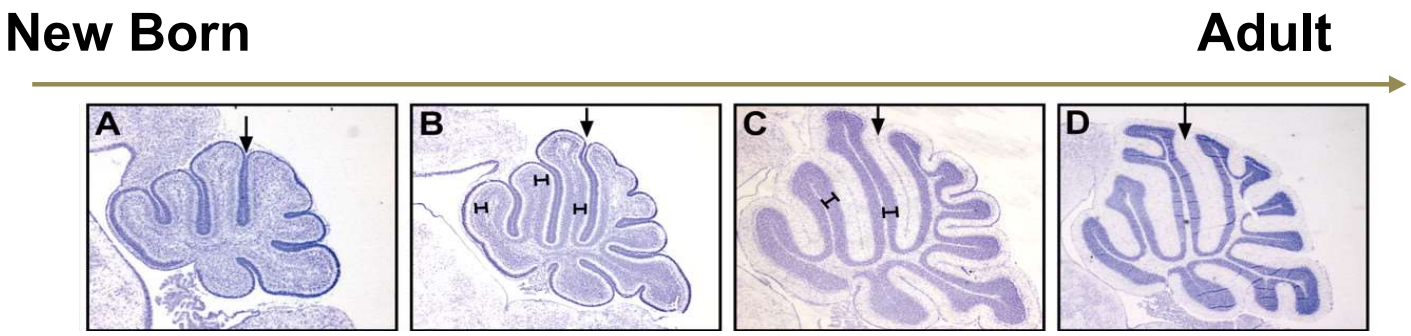


EGL (external granular layer)
Granule Neuron progenitors (GNP)/
Granule Cell progenitors (GCP)

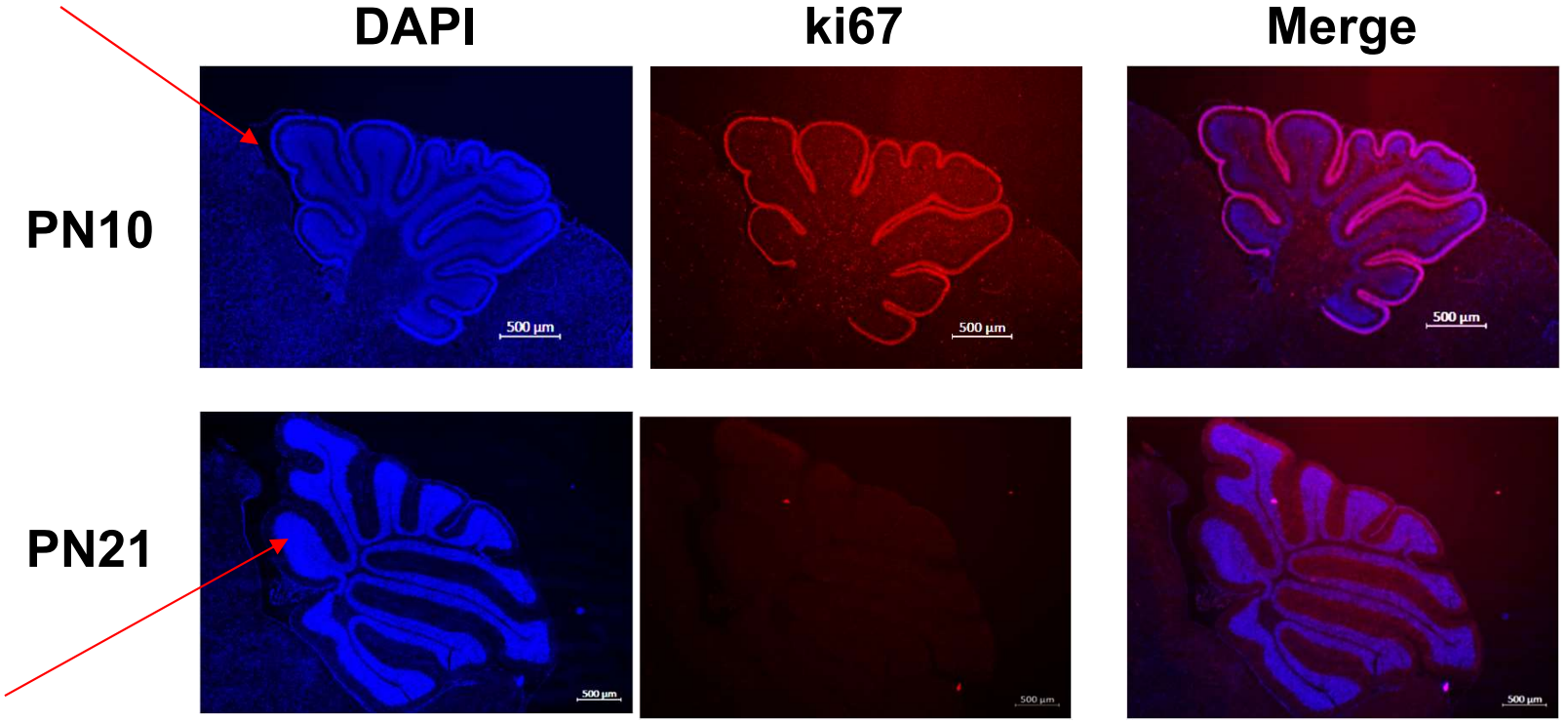
IGL (inner granular layer)
Differentiated Granule neurons



Postnatal cerebellar development



EGL (external granular layer)
Granule Neuron progenitors/GCP



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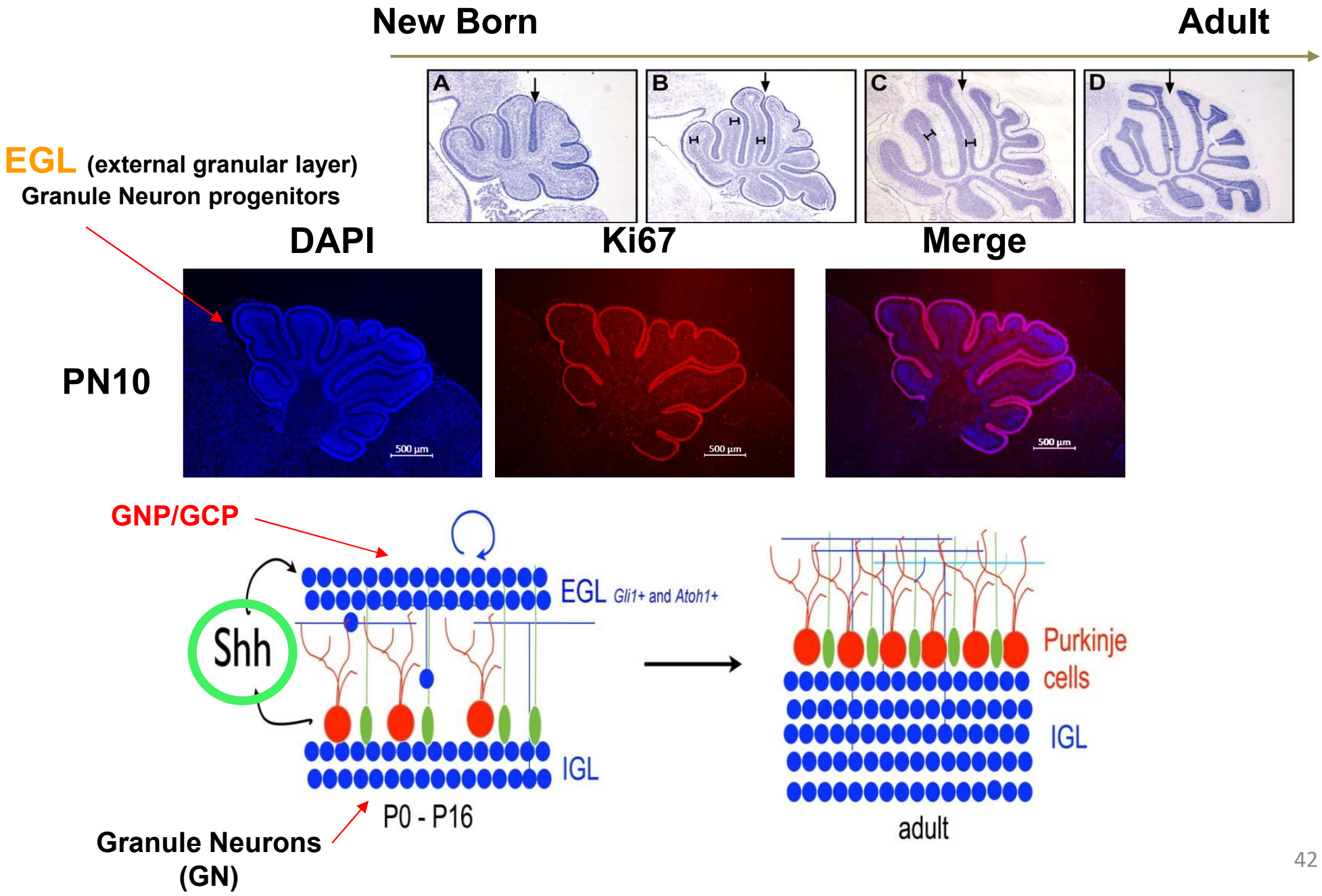
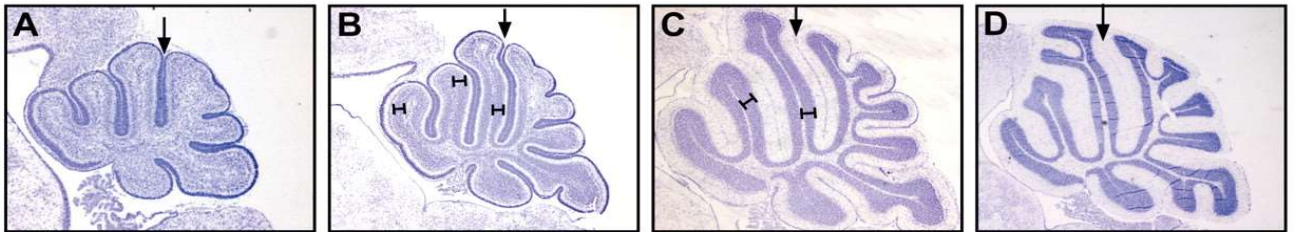


Postnatal cerebellar development

New Born **Adult**



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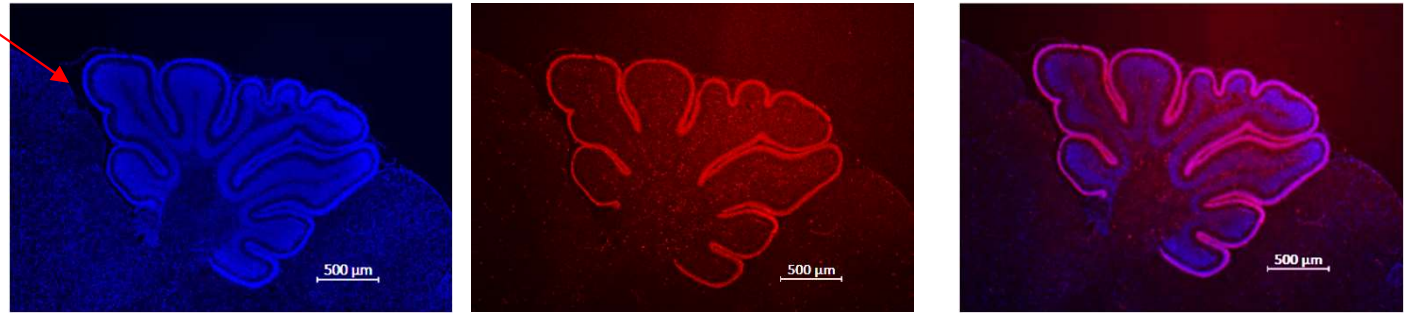
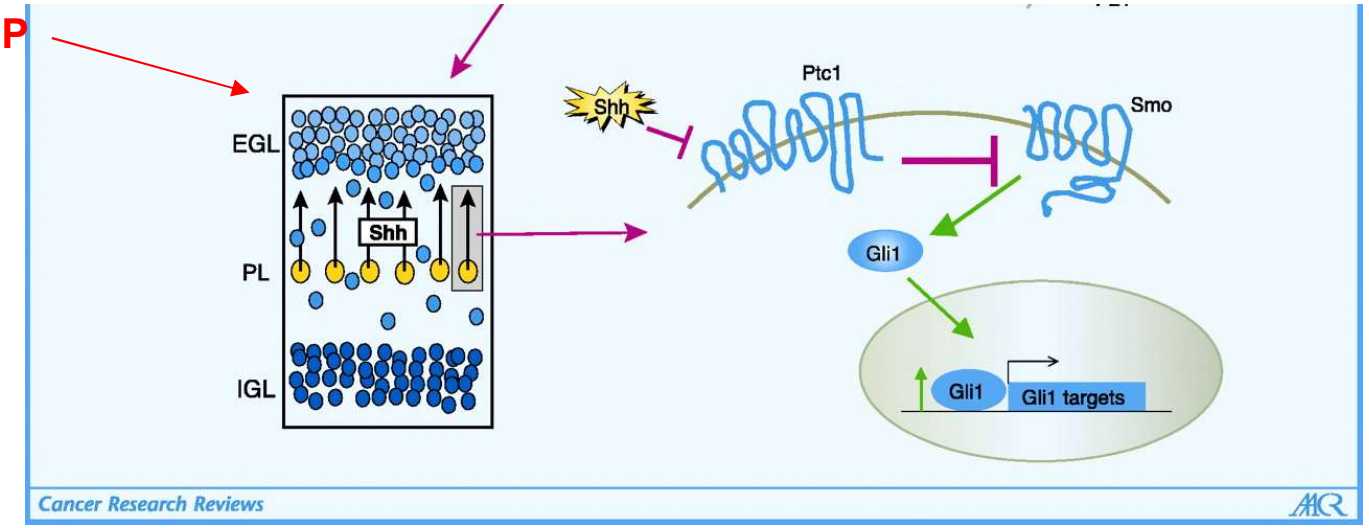
Granule Neuron progenitors

DAPI **Ki67** **Merge**

PN10

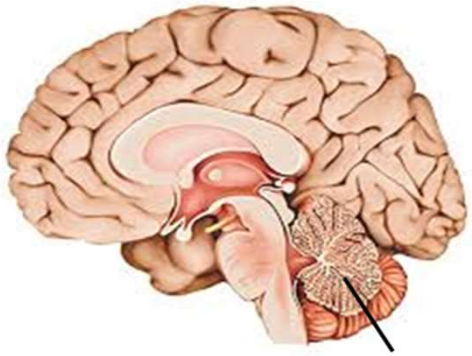


GNP/GCP



SHH signaling sustains GNP/GCP proliferation

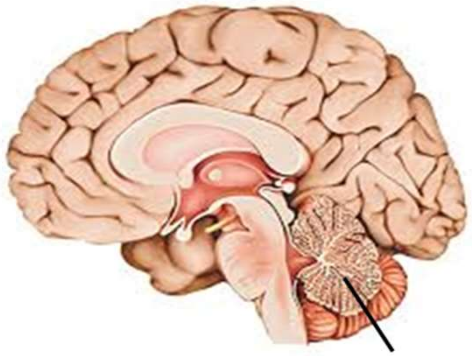
Medulloblastoma (MB)



cerebellum

- Pediatric tumor of the cerebellum (Median age 7 yrs)
- Most frequent Malignant brain tumor of childhood (~150 cases/year in France)

Medulloblastoma (MB)



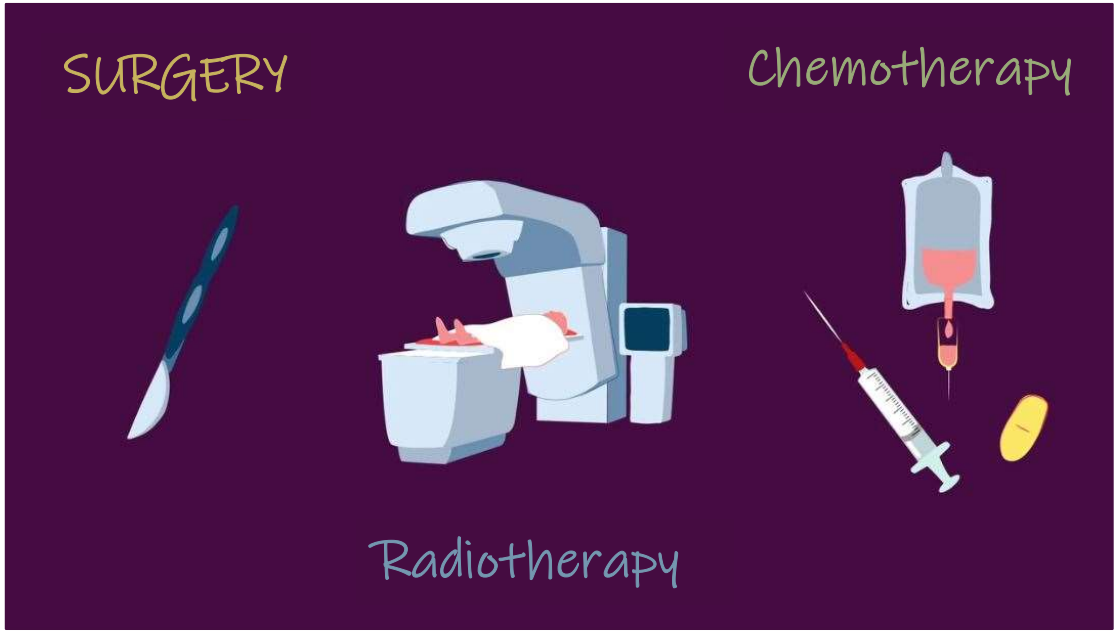
cerebellum

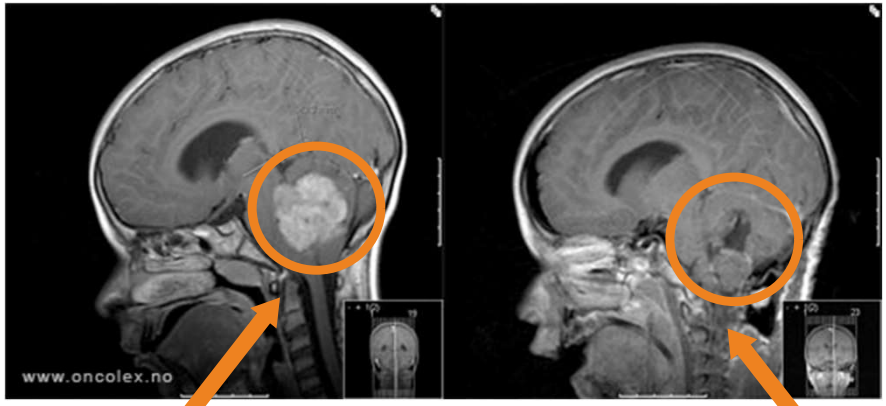
- Pediatric tumor of the cerebellum (Median age 7 yrs)
- Most frequent Malignant brain tumor of childhood (~150 cases/year in France)
- Treatment : surgery, chemotherapy, radiotherapy

➔70-80% overall survival at 5 years
➔Important secondary effects

Medulloblastoma Treatment

- Current treatment: surgery, radiotherapy and chemotherapy





TREATMENT TOXICITY

- **~80 % survivors** after treatment

<u>But</u>

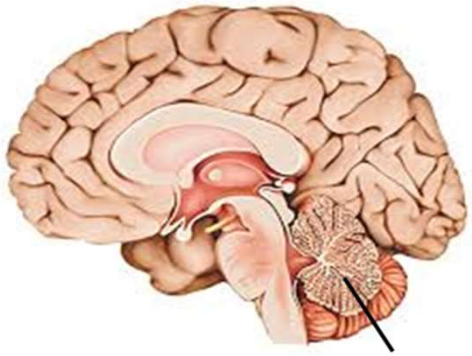
Very strong sequeala: TREATMENT TOXICITY

- Mutism
- Hearing deficit, Deafness
- Learning deficit (attention, memory deficit, mental retardation)
- Growth deficit, puberty
- Risk of secondary cancers

→ **Strong decrease in quality of life in the adult life**
(social dependency)

Decrease the secondary effects of therapy

Medulloblastoma (MB)



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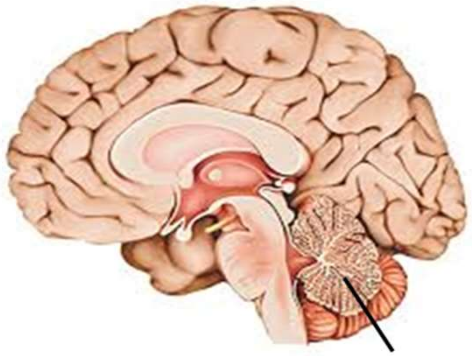
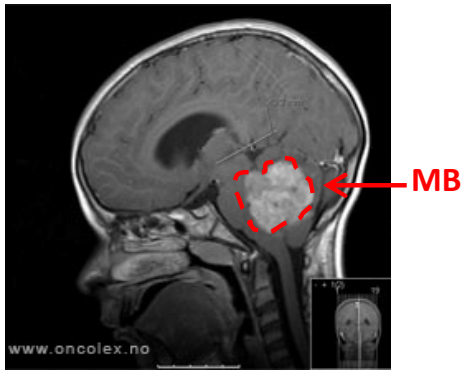


cerebellum



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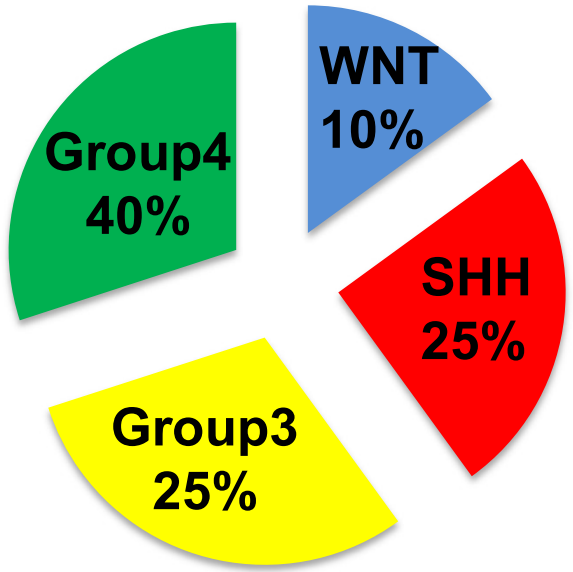
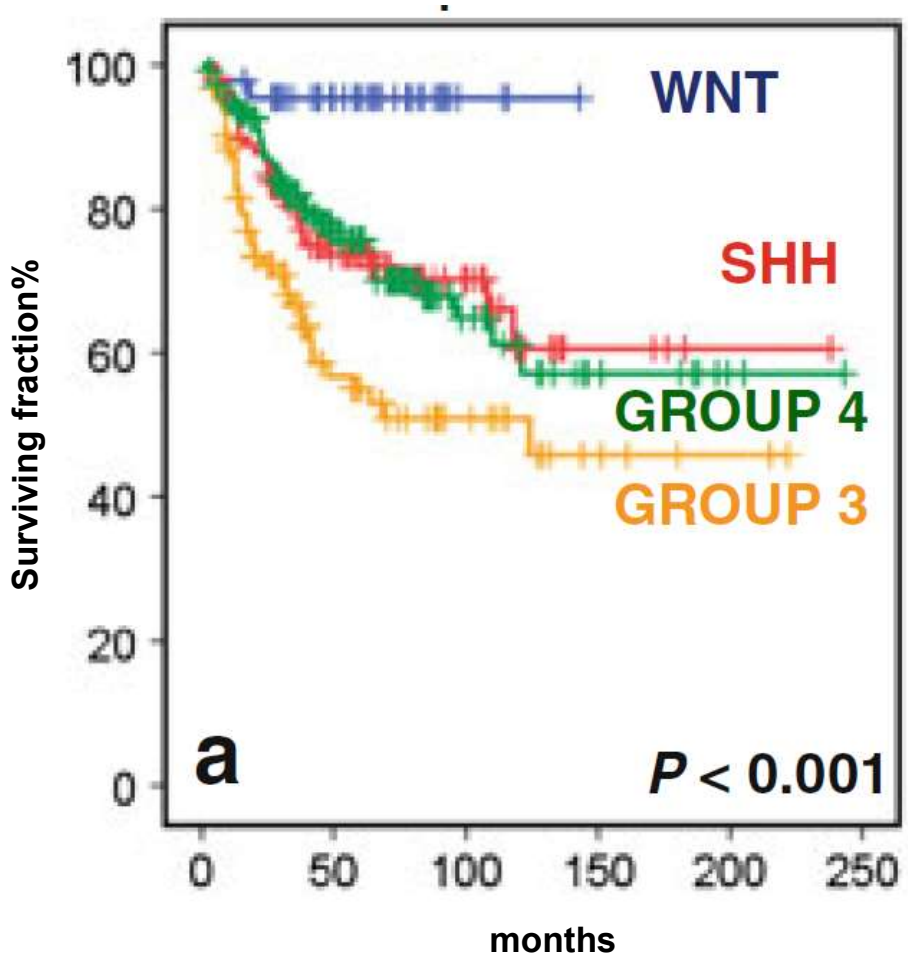


The different groups of MB
(prognostic)



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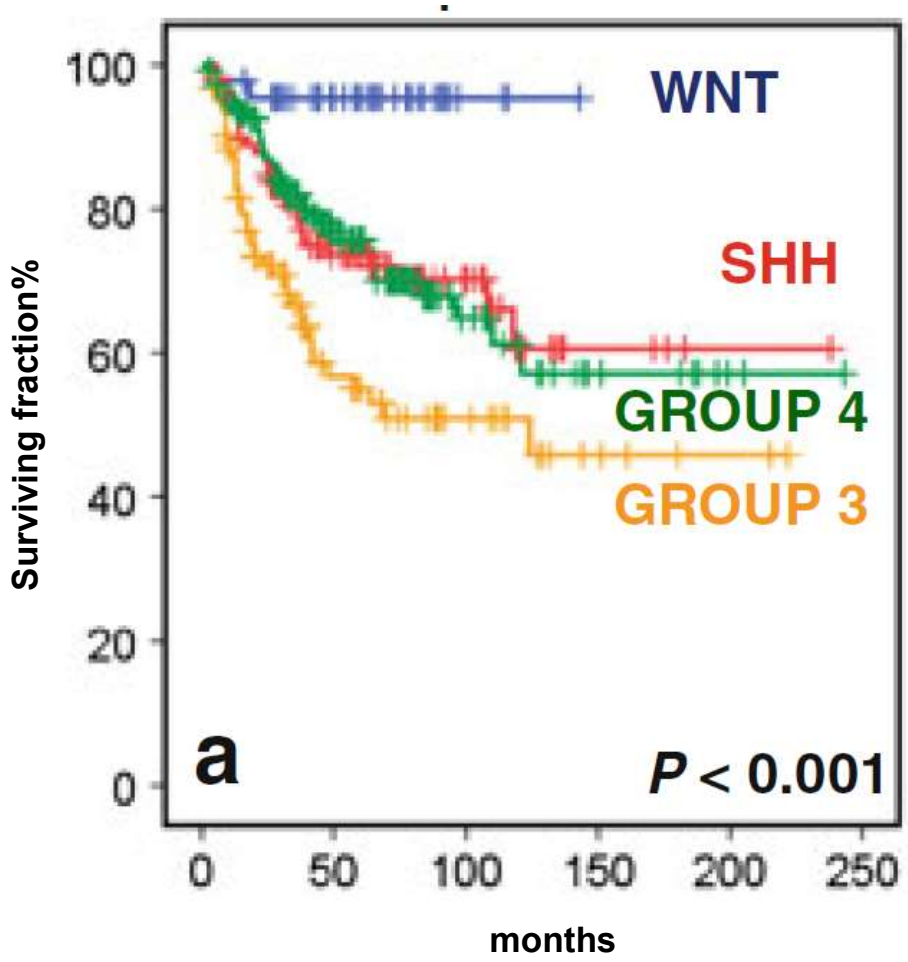
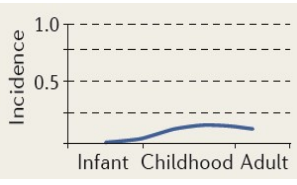
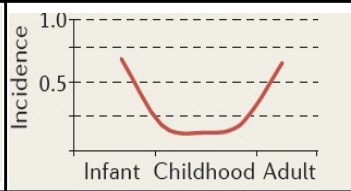
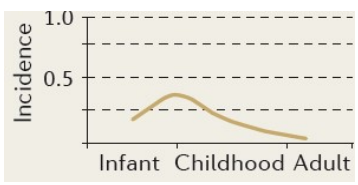
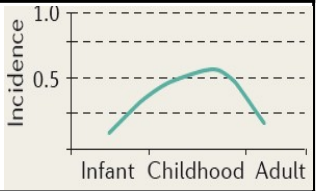


G3 has the worse prognosis

Medulloblastoma (MB)

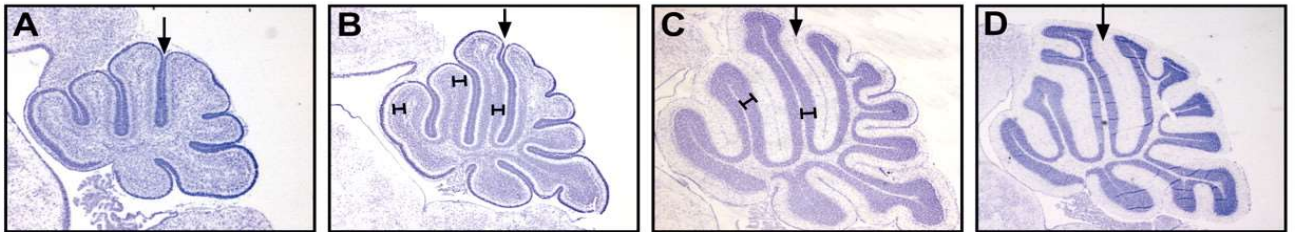
➢ Based on gene expression profile : 4 different molecular groups

MB subtype		WNT	SHH	3	4
Clinical features	Age distribution				
	incidence	10%	25%	25%	40%
	histology	Classic (very rare LCA)	Classic > desmoplastic/ nodular> LCA	Classic>LCA	Classic; rarely LCA
	Metastasis at diagnosis	rare 5-10%	intermediate 15-20%	frequent 40-45%	frequent 35-40%
	Overall survival (5years)	Very good 95%	Intermediate 75%	Bad 50%	intermediate 75%
	Cell of origin	Lower rhombic lip precursor	?	Unknown	
Genomic features	Driver pathway/genes	WNT/βCAT CTNNB1 mutation	SHH PTCH1 mutation	MYC amplification OTX2 amplification	MYCN & CDK6 amplification
	Expression signature	WNT signaling	SHH signaling	MYC & GABAergic & photoreceptor signature	Neuronal & glutamatergic signature

Adapted from Northcott & al., 2012

Cell of origin SHH medulloblastoma

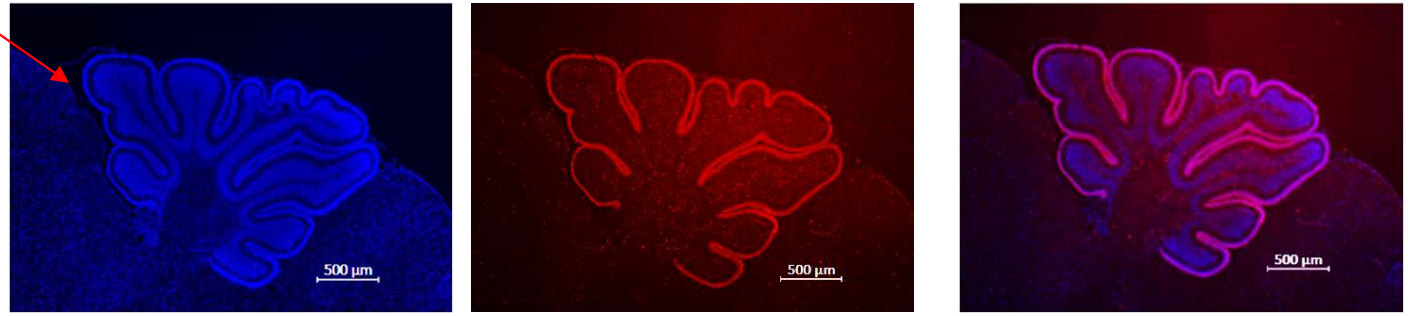
New Born **Adult**



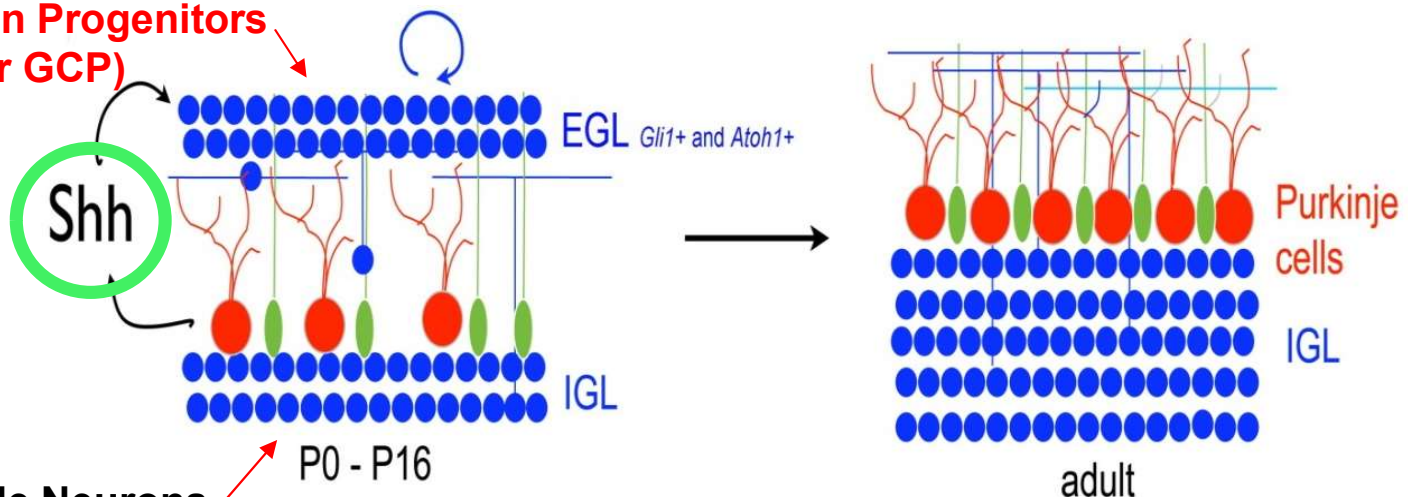
EGL (external granular layer)
Granule Neuron progenitors

DAPI **Ki67** **Merge**

PN10

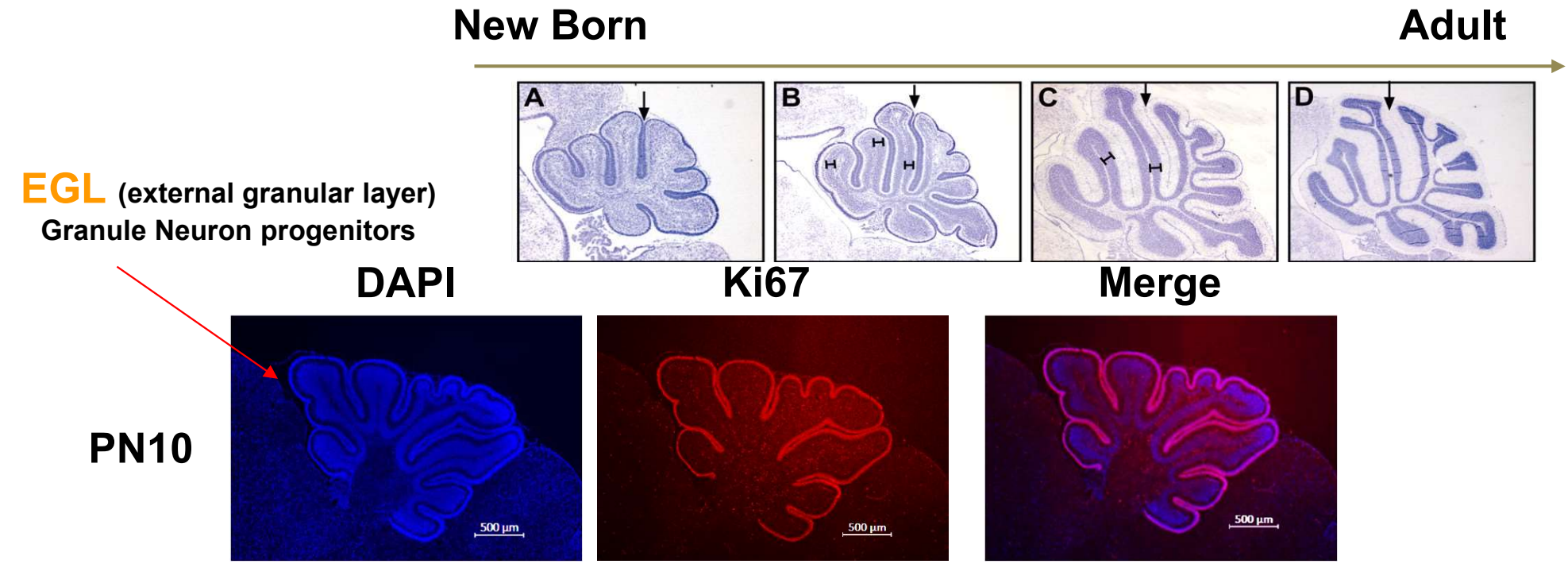


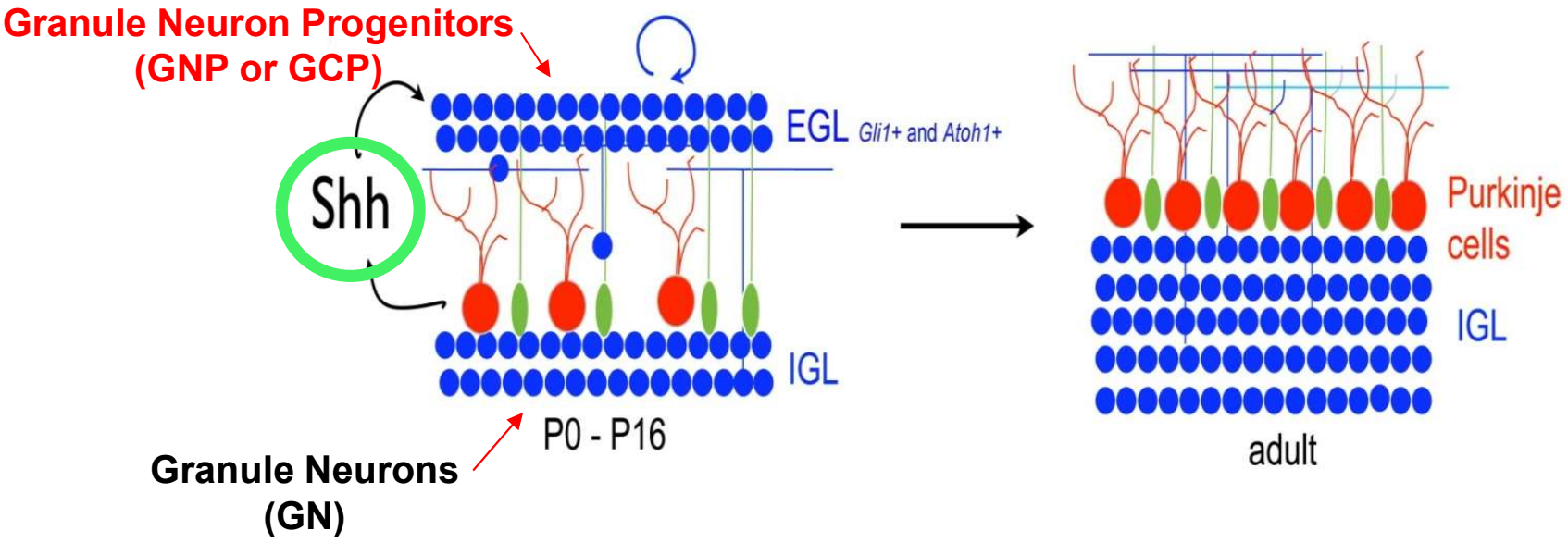
Granule Neuron Progenitors (GNP or GCP)



Granule Neurons (GN)

Are the GCP the cell of origin SHH medulloblastoma?





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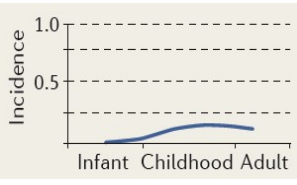
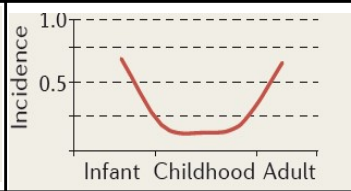
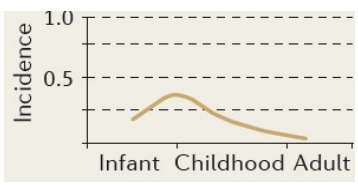
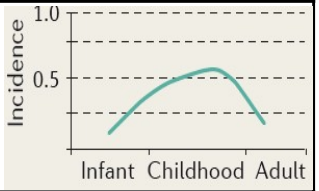
Clinical features	Age distribution				
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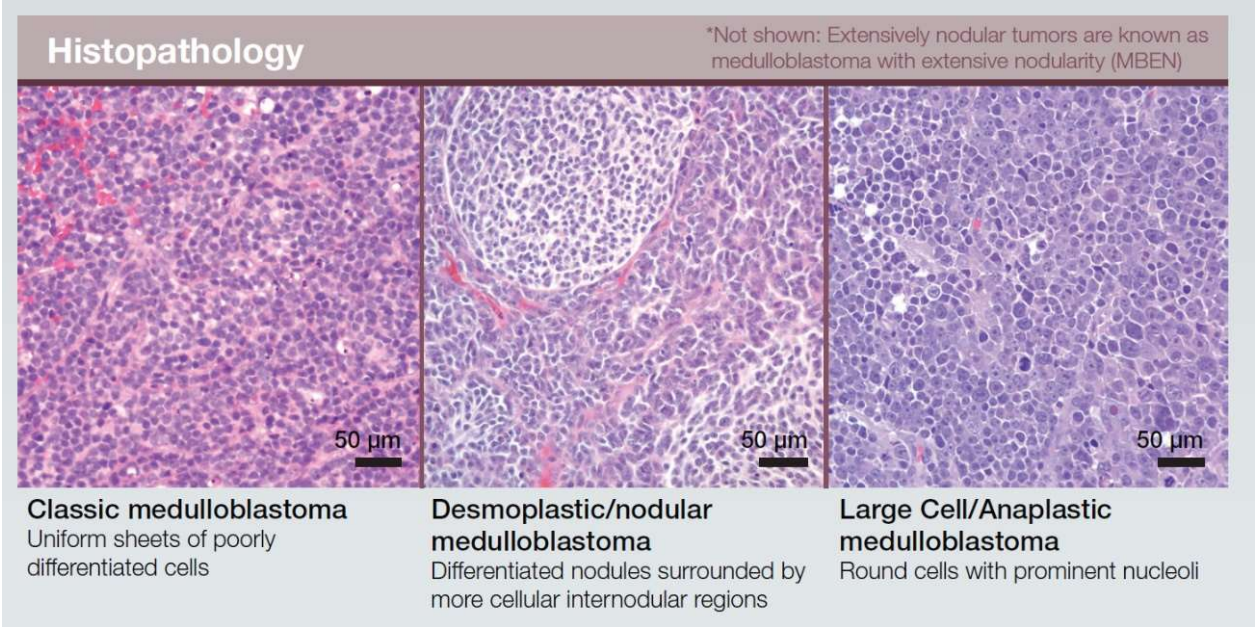
Adapted from Northcott & al., 2012

Medulloblastoma (MB)

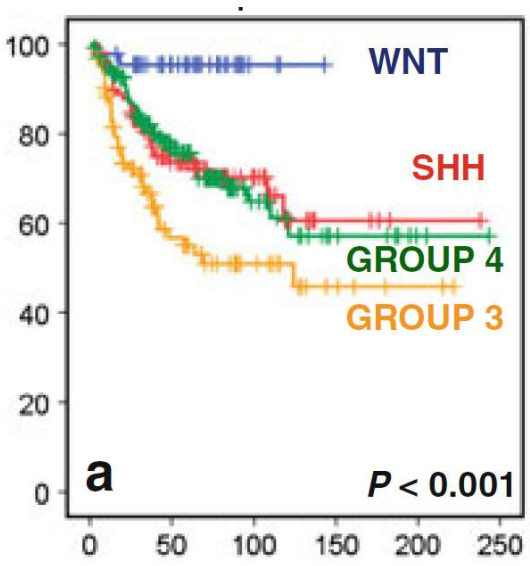
Histological classification

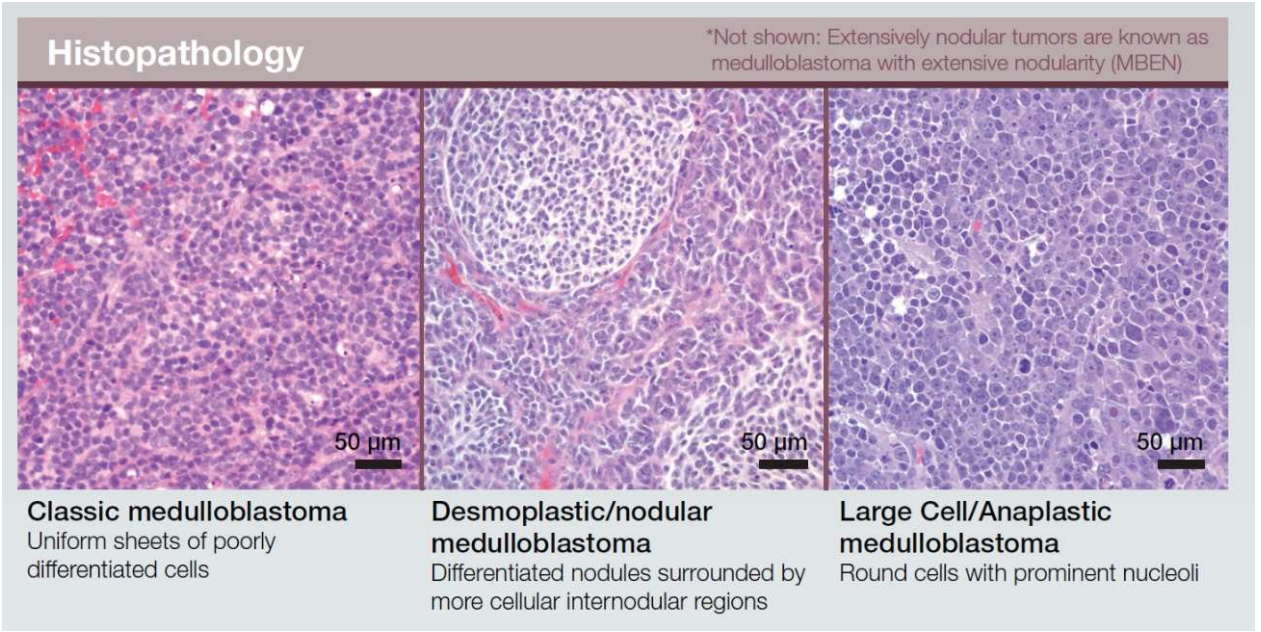


(intermediate prognosis) **(good prognosis)** **(bad prognosis)**

Medulloblastoma (MB)

Histological classification





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Medulloblastoma (MB)

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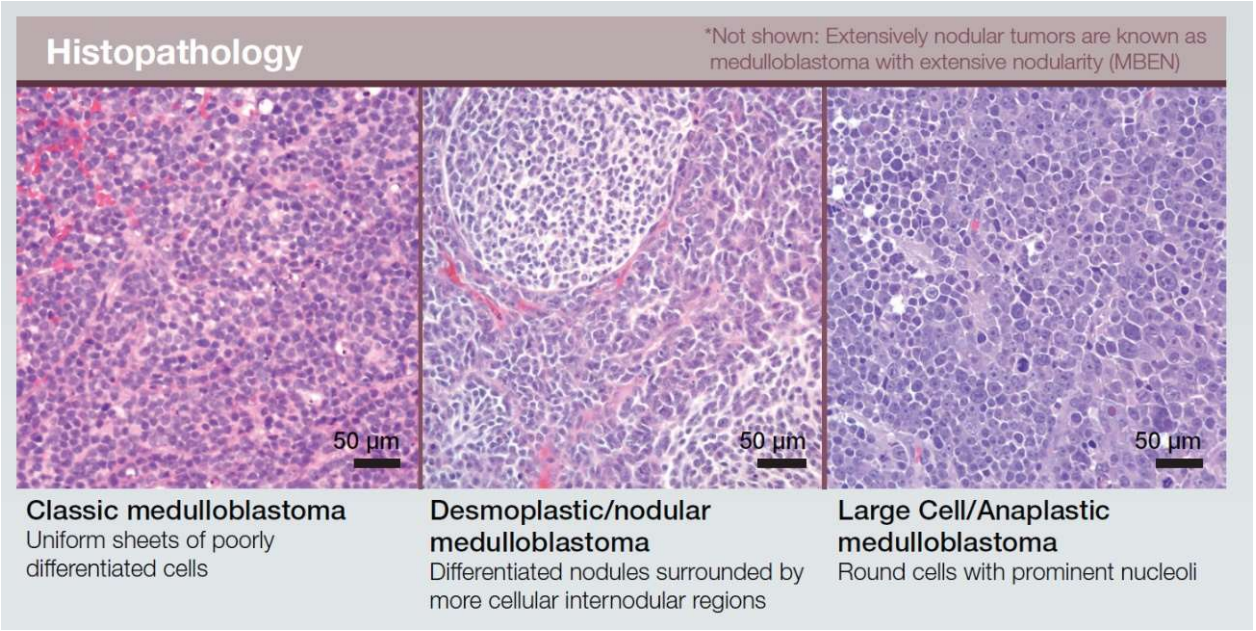
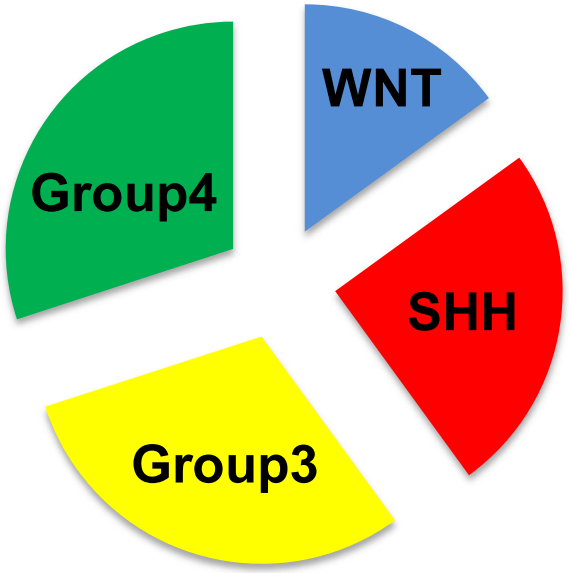


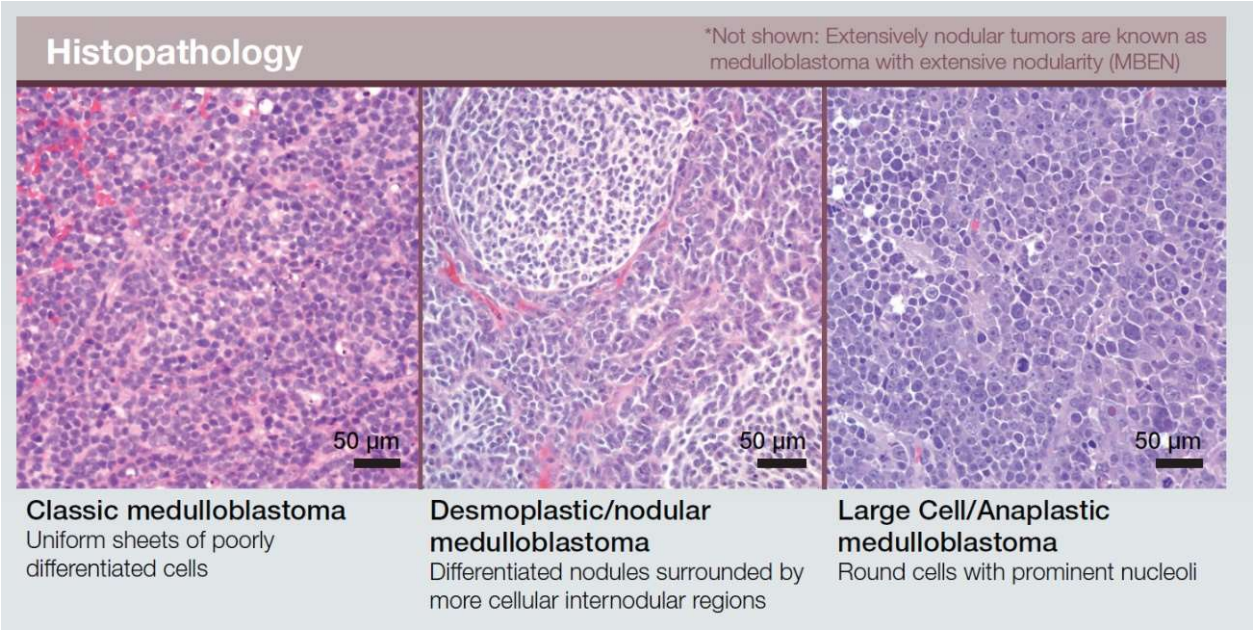


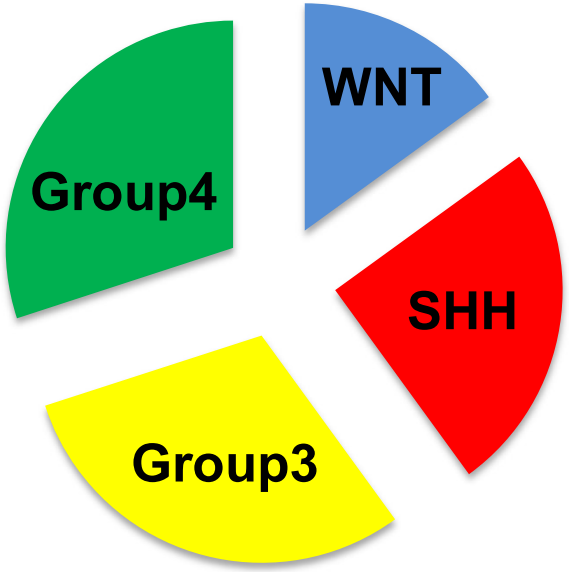
Transcriptomic classification

(intermediate prognosis)	(good prognosis)	(bad prognosis)
WNT	SHH	Group3
Group3		SHH
Group4		Group4
SHH		

Medulloblastoma (MB)

Histological classification



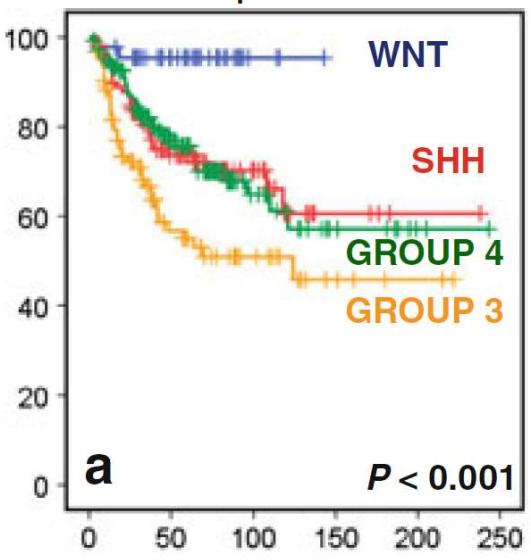


Transcriptomic classification

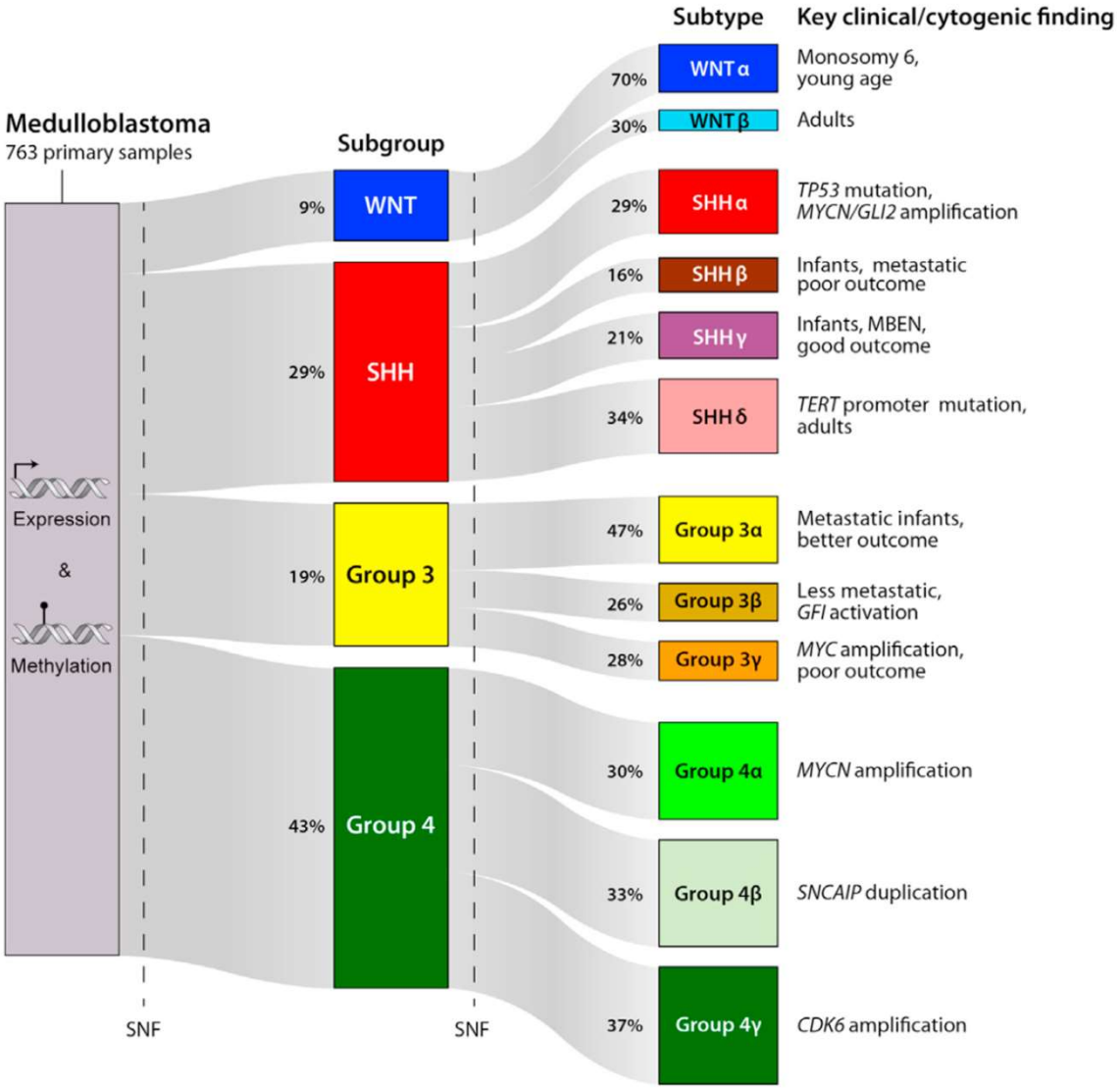
(intermediate prognosis) **(good prognosis)** **(bad prognosis)**

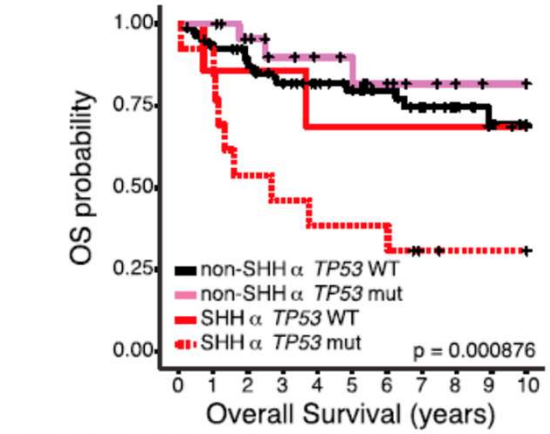
WNT **Group3** **Group4** **SHH**

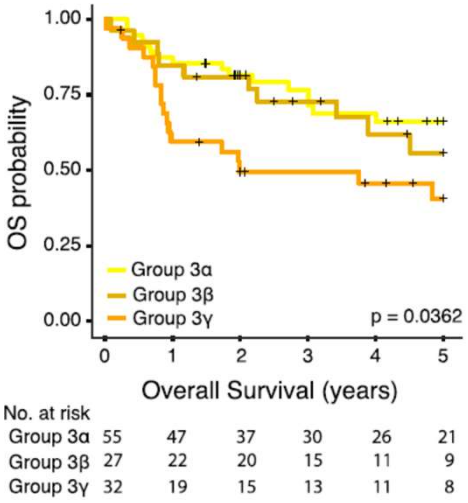
SHH **Group3** **SHH** **Group4**



MB groups can be stratified in subtypes







Are the GCP the cell of origin SHH medulloblastoma?

How to test that the GCP/GNP are the cell of origin of SHH medulloblastoma?

Are the GCP the cell of origin SHH medulloblastoma?

How to test that the GCP are the cell of origin of SHH medulloblastoma?

-match the transcriptome of SHH medulloblastoma to that of different normal cerebellar progenitors during development

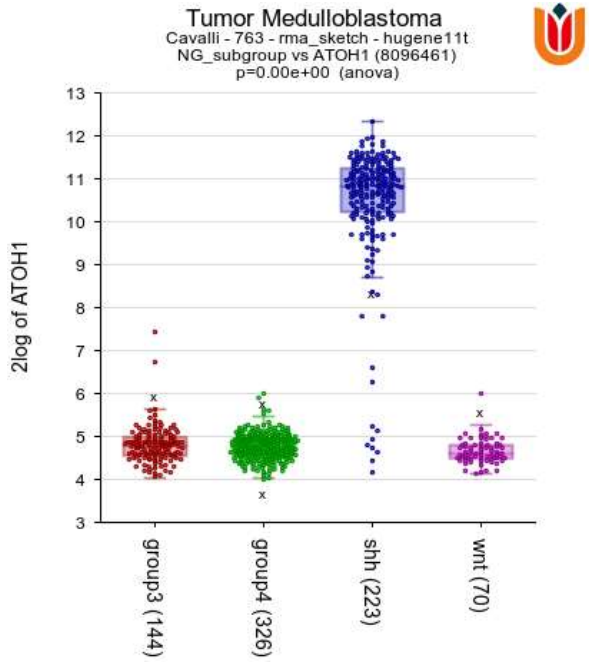
-use mouse genetics

Cell of origin of SHH-MB

GCP/GNP is the cell of origin of SHH-MB

Atoh1

Atoh1 expression across MB groups

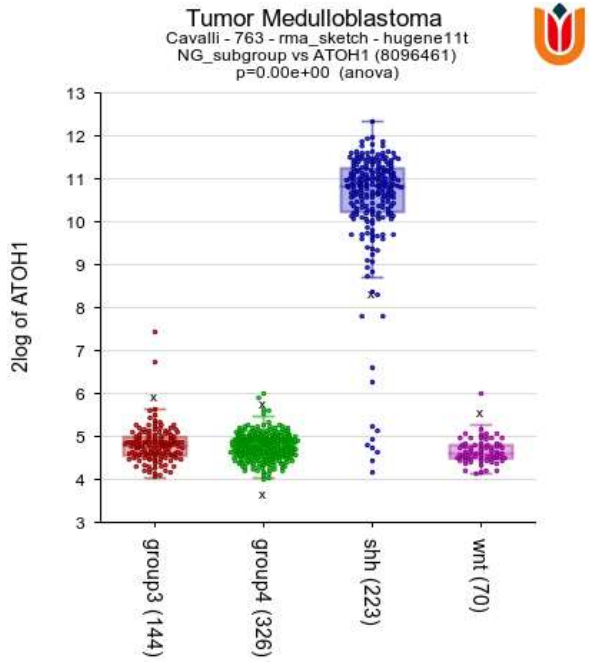


Cell of origin of SHH-MB

GCP/GNP is the cell of origin of SHH-MB

Atoh1

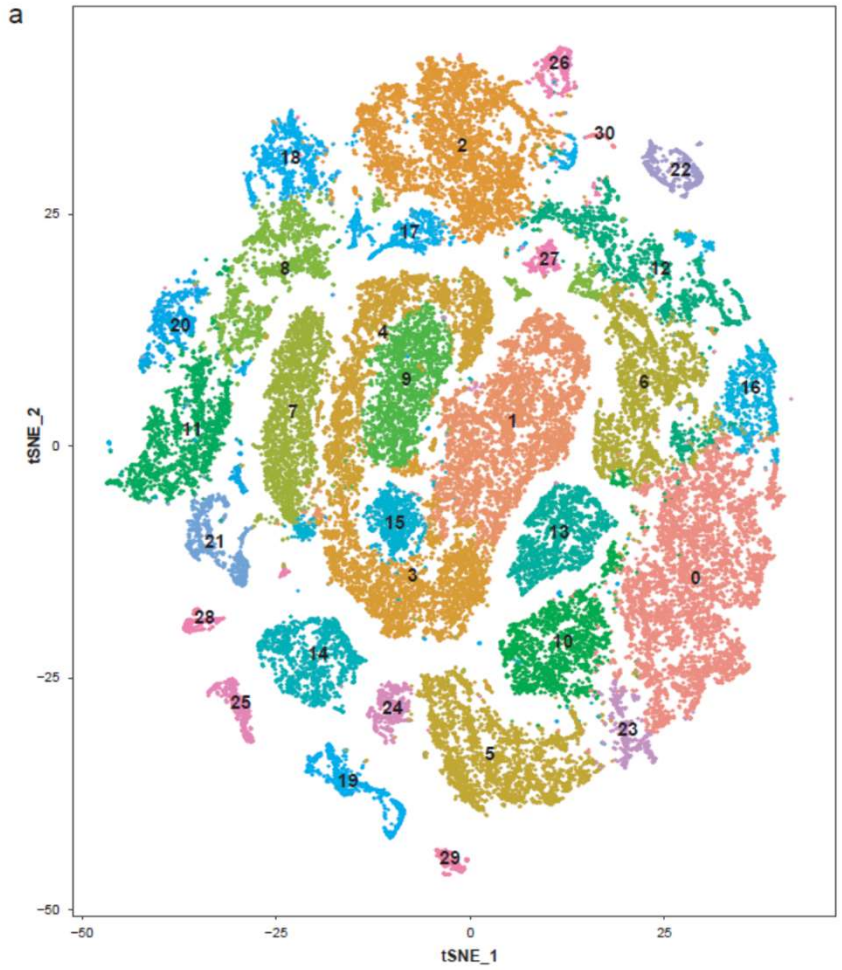
Atoh1 expression across MB groups



ATOH1 expression is enriched in SHH- group

Cell of origin SHH group

matching the transcriptome:

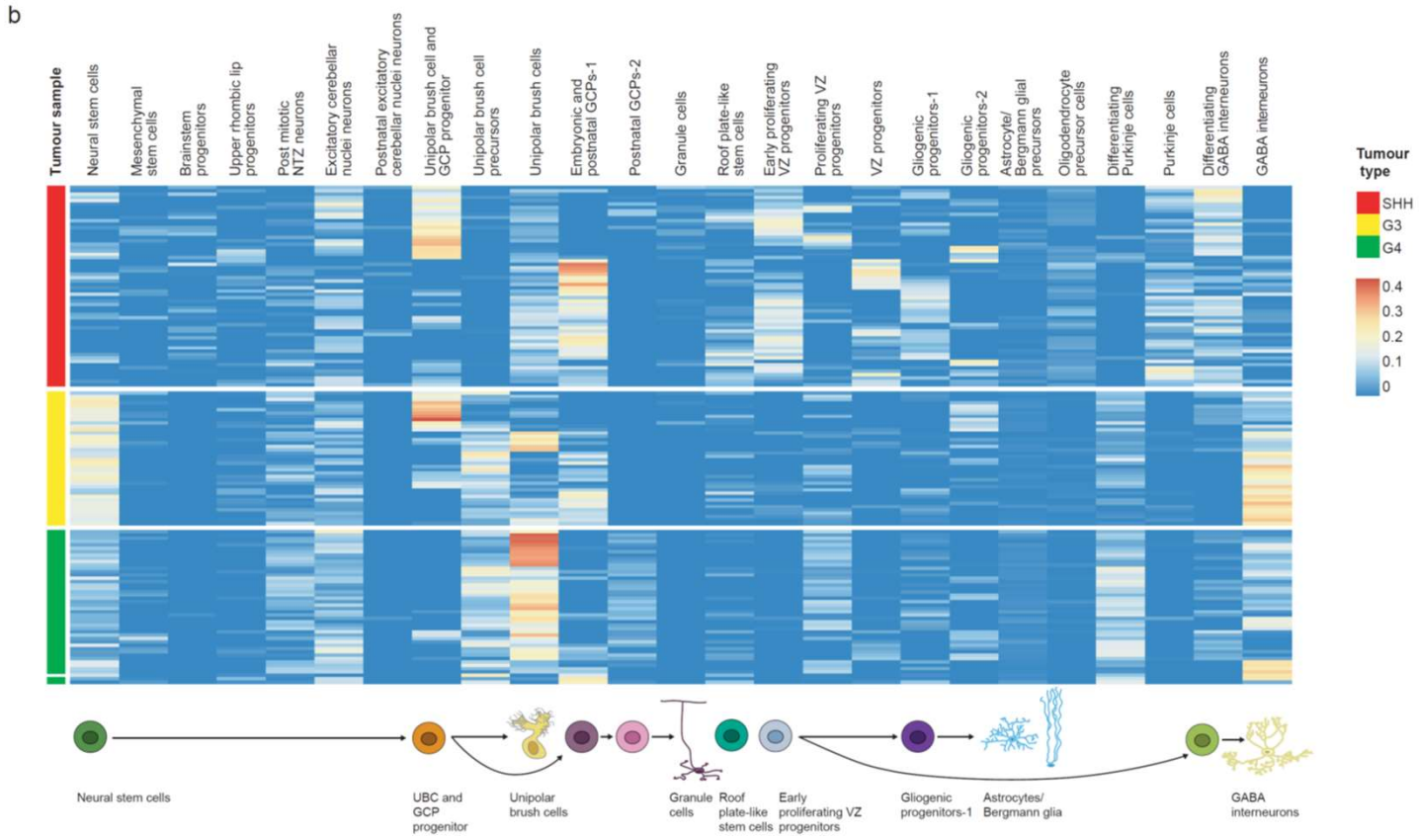


0- Excitatory cerebellar nuclei neurons
1- Embryonic and postnatal GCPs-1
2- Neural stem cells
3- Unipolar brush cell and GCP progenitor
4- Unipolar brush cells
5- GABA interneurons
6- Brainstem progenitors
7- Granule cells
8- VZ progenitors
9- Unipolar brush cell precursors
10- Differentiating Purkinje cells
11- Gliogenic progenitors-1
12- Upper rhombic lip progenitors
13- Mesenchymal stem cells-1
14- Purkinje cells
15- Postnatal GCPs-2
16- Post mitotic NTZ neurons
17- Roof plate-like stem cells
18- Proliferating VZ progenitors
19- Oligodendrocyte progenitor cells
20- Gliogenic progenitors-2
21- Astrocyte/Bergmann glia precursors
22- Endothelial cells
23-Postnatal excitatory cerebellar nuclei neurons
24- GABA interneuron precursors
25- Pericytes
26- Early proliferating VZ progenitors
27- Mesencgymal stem cells-2
28- Microglia
29- Meninges
30- Red blood cells

scRNAseq of cerebellar cells

Cell of origin SHH group

matching the transcriptome:

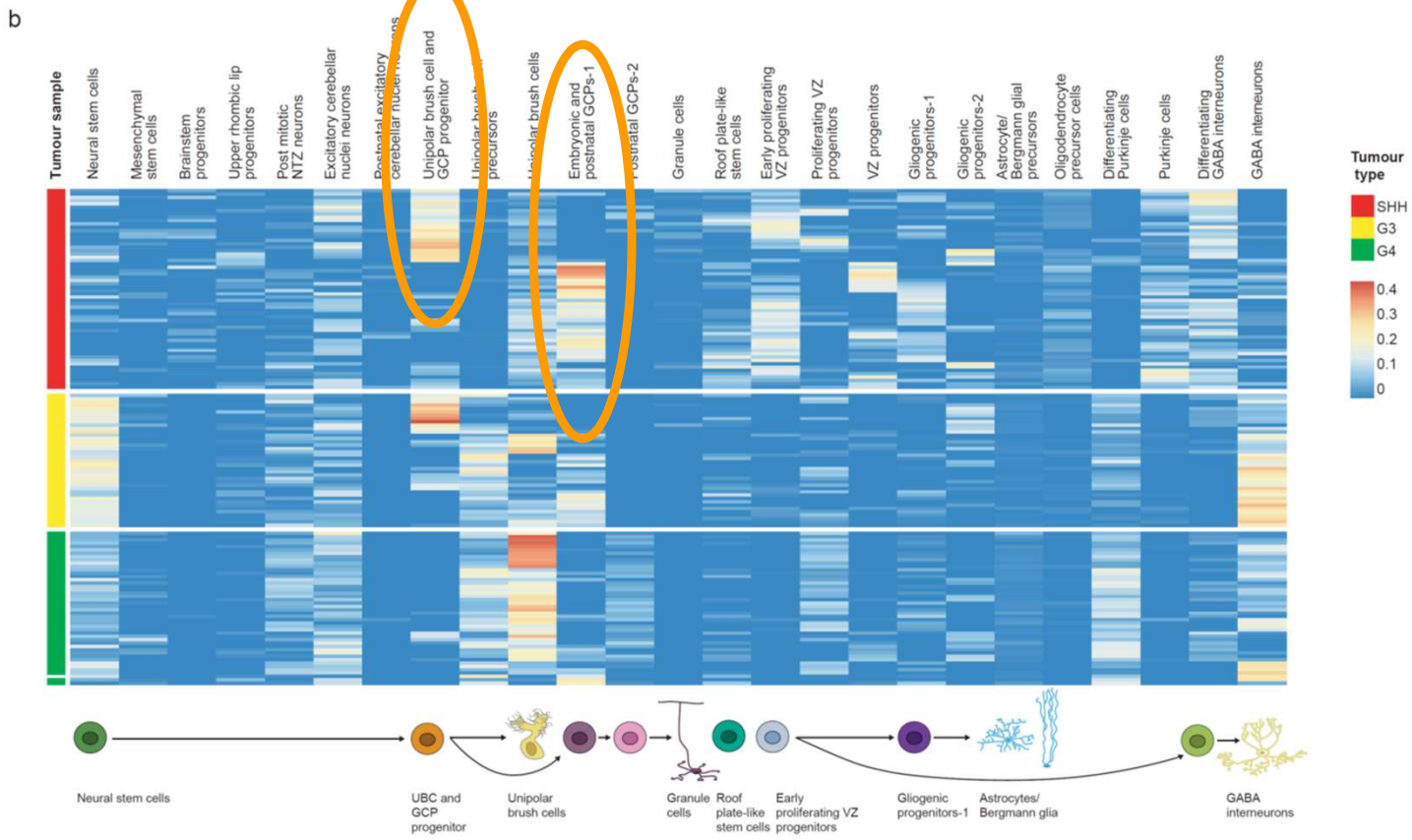


Matching the MB to cerebellar cell transcriptomes

Cell of origin SHH group

matching the transcriptome:

Atoh1



SHH-MB has a transcriptome closed to that of GCP (Granule cell progenitors)

Cell of origin SHH group

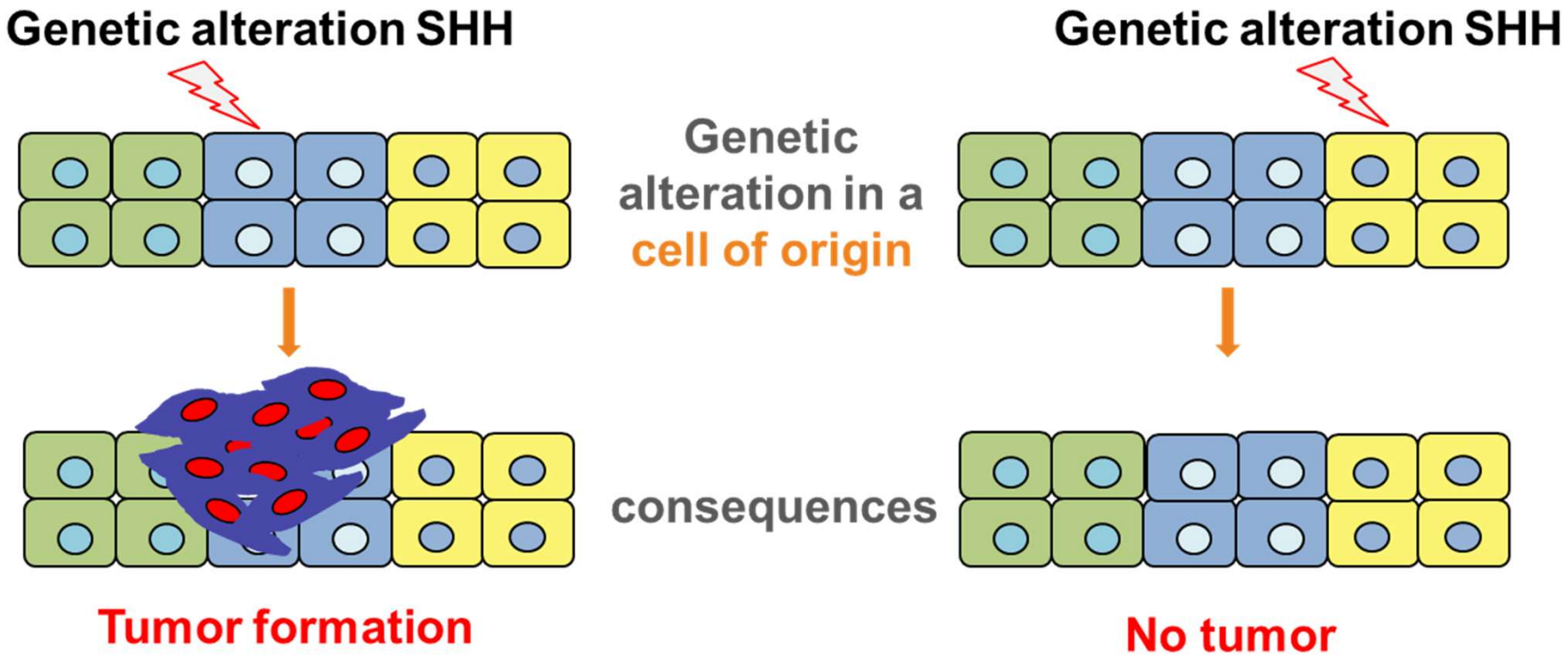
Mouse genetics:

Atoh1

Cell of origin SHH group

Mouse genetics:

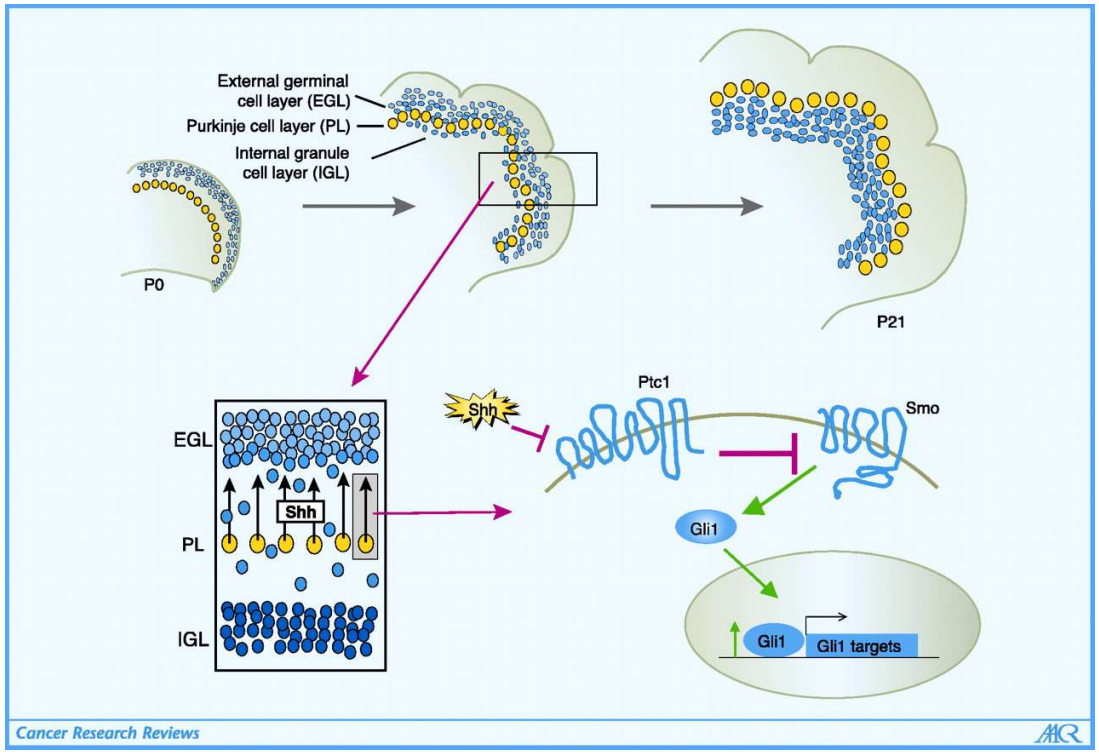
Atoh1



Cell of origin SHH group

Mouse genetics:

Atoh1



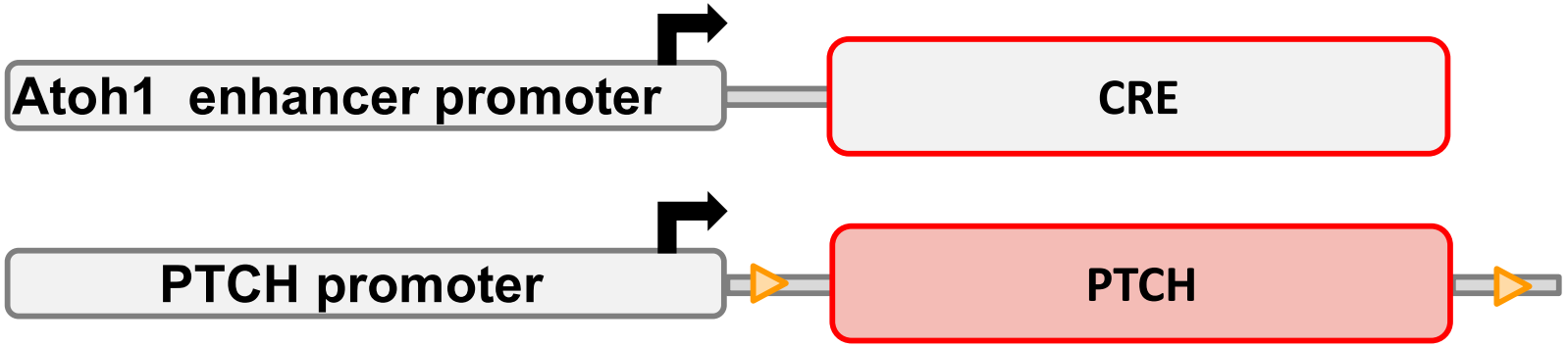
Cell of origin SHH group

Mouse genetics:

Atoh1

Mouse

Atoh1-cre::PTCH$^{F/F}$ to generate the PTCH$^{GCP/GCP}$ mice



Transgenic/KI

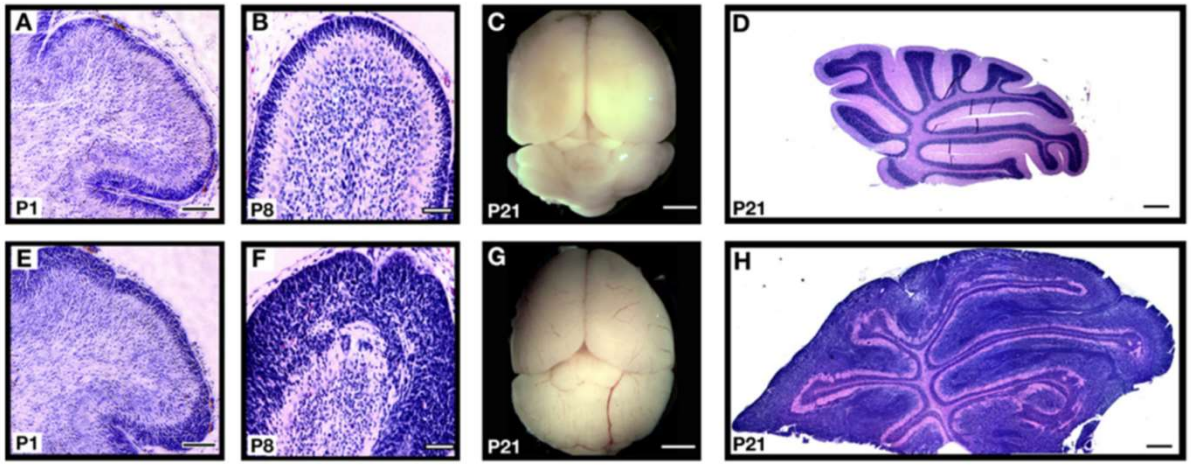
PTCH$^{F/F}$ (PTCH locus)

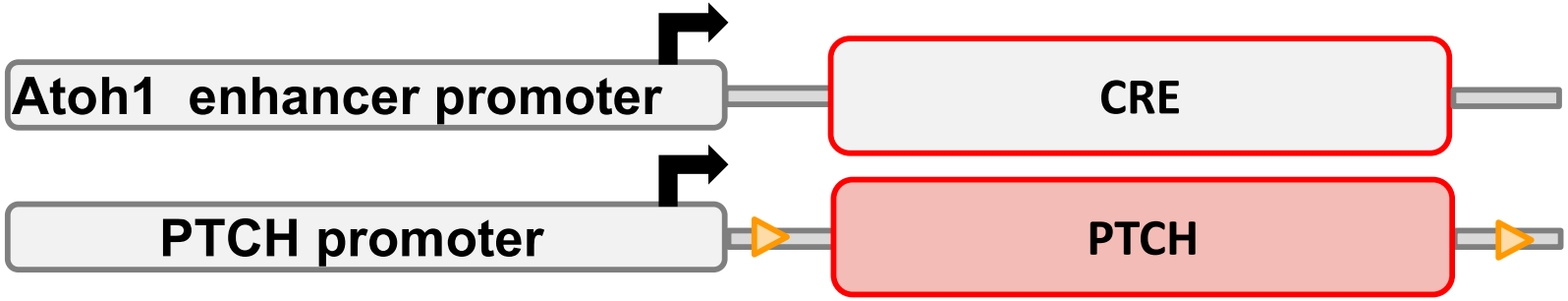
Cell of origin SHH group

Atoh1Cre::PTCH$^{F/F}$:

WT

PTCH$^{GCP/GCP}$





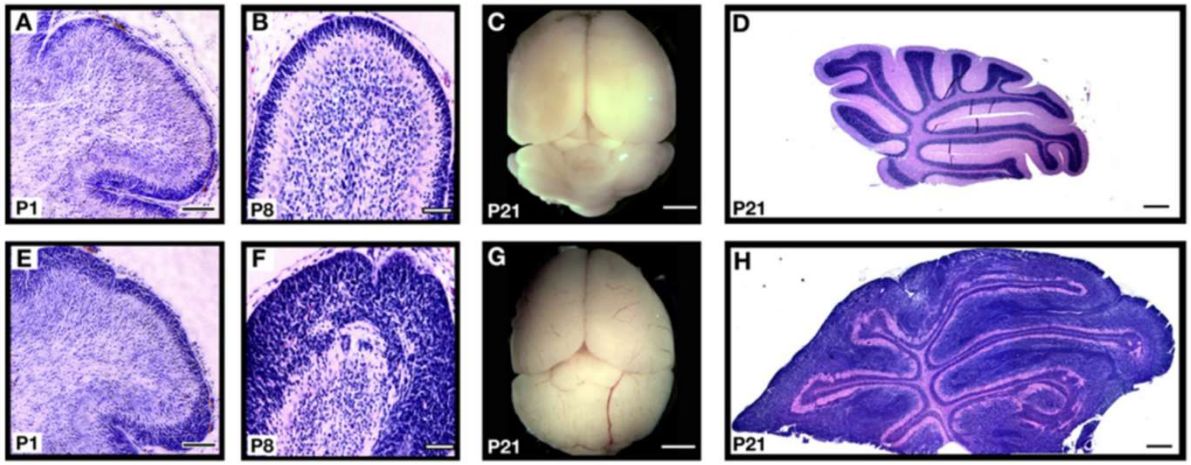
PTCH$^{GCP/GCP}$

Cell of origin SHH group

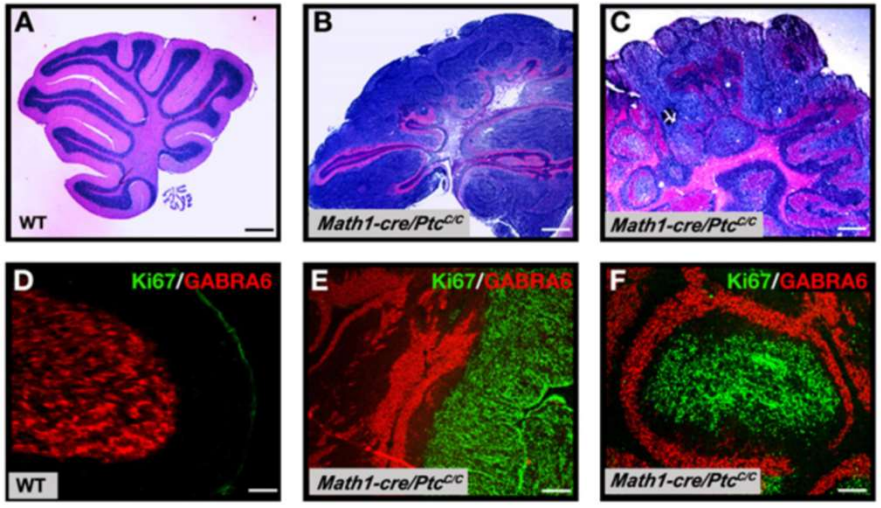
Atoh1Cre::PTCH$^{F/F}$:

WT

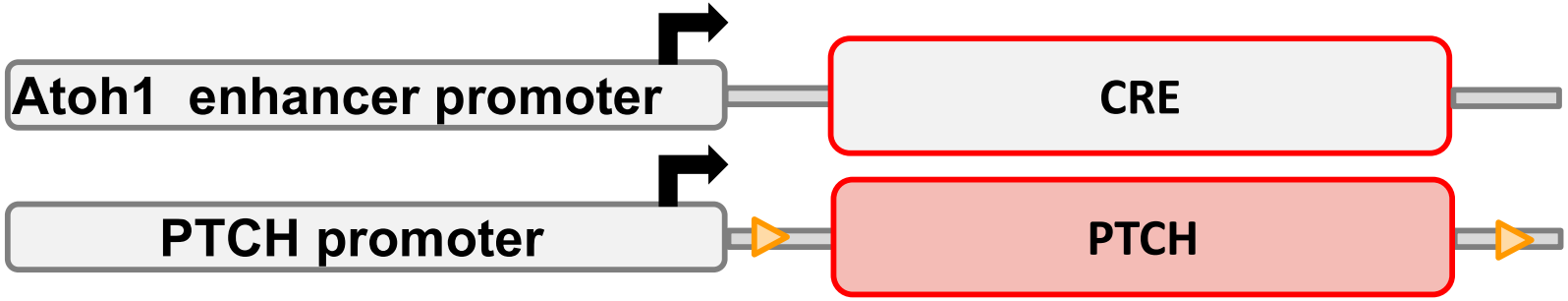
PTCH$^{GCP/GCP}$



6 weeks old animals



GABRA6 differentiated GN marker



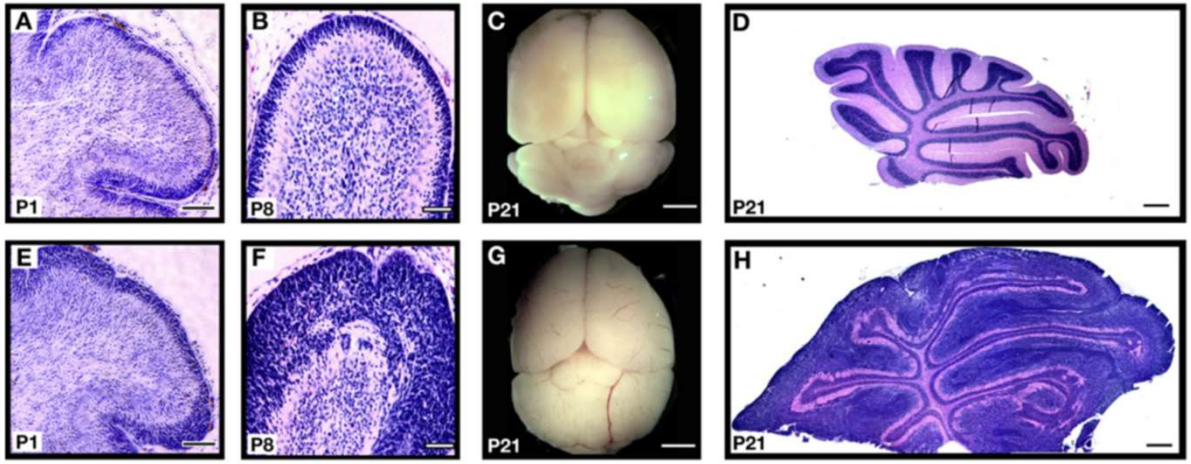
PTCH$^{GCP/GCP}$

Cell of origin SHH group

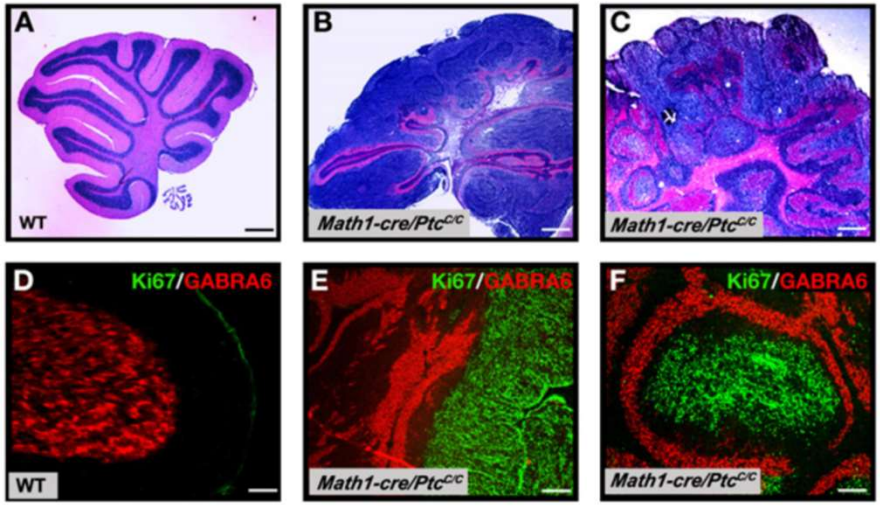
Atoh1Cre::PTCH$^{F/F}$:

WT

PTCH$^{GCP/GCP}$



6 weeks old animals



PTCH inactivation (activation of SHH pathway) in GCP leads to MB formation

GCP/GNP is the cell of origin of SHH-MB

Lineage addiction in cancer

Lineage addiction in cancer

GCP/GNP is the cell of origin of SHH-MB

Atoh1

Atoh1 is expresssed in granule cell progenitors (GCP)

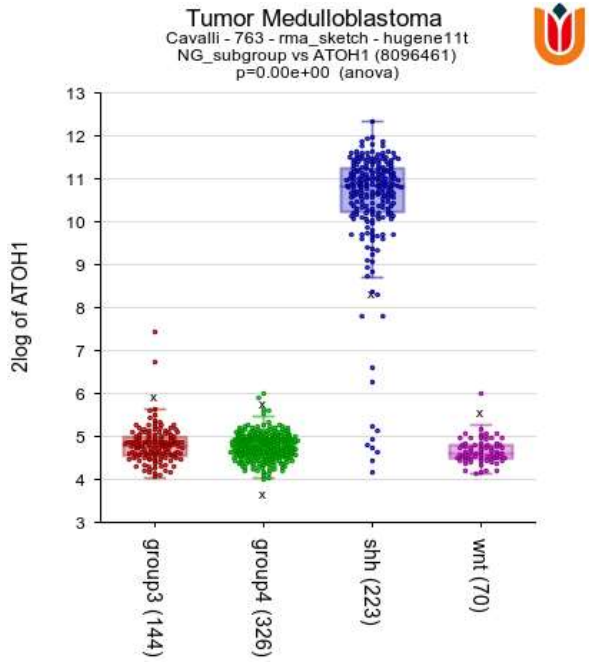
Atoh1 is a specific marker of the GCP lineage

Lineage addiction in cancer

GCP/GNP is the cell of origin of SHH-MB

Atoh1

Atoh1 expression across MB groups



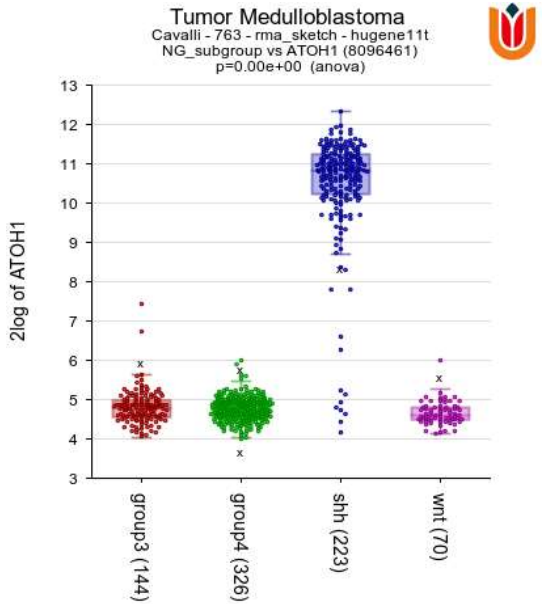
ATOH1 expression is enriched in SHH- group

Lineage addiction in cancer

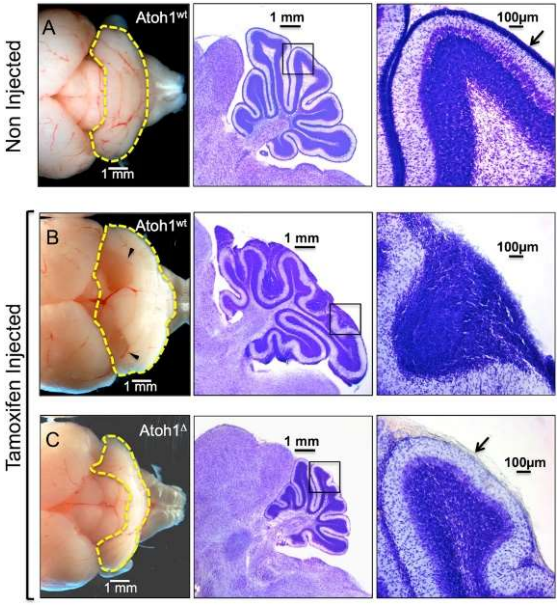
GCP/GNP is the cell of origin of SHH-MB

Atoh1

Atoh1 expression across MB groups



Atoh1 and SHH-MB formation (PTCH-/-)

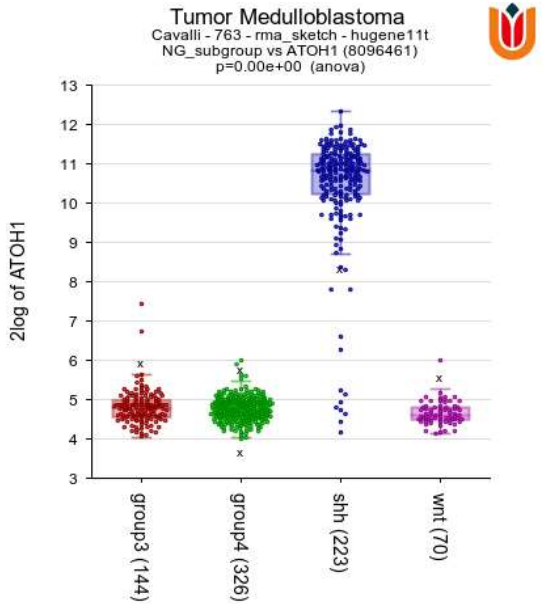


Lineage addiction in cancer

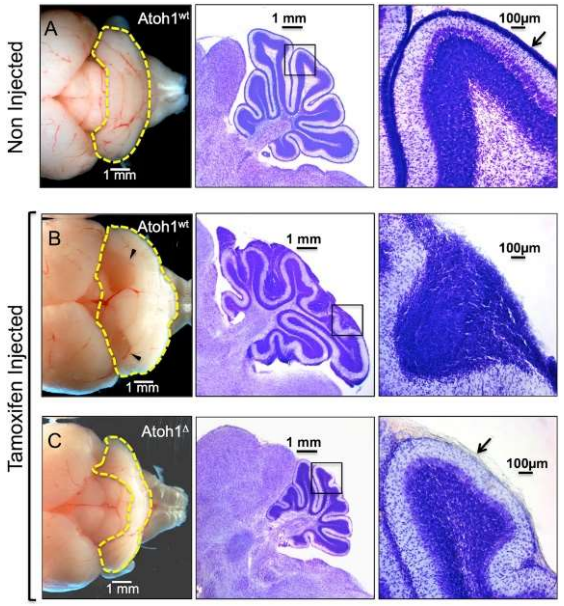
GCP/GNP is the cell of origin of SHH-MB

Atoh1

Atoh1 expression across MB groups



Atoh1 and MB formation



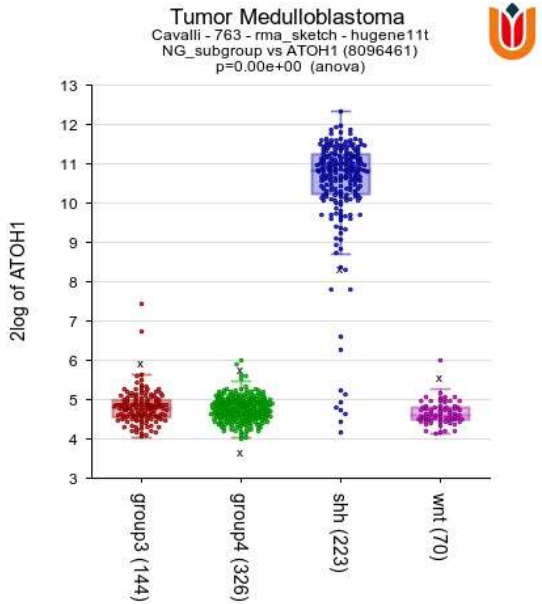
Deletion of Atoh1 Prevents MB formation

Lineage addiction in cancer

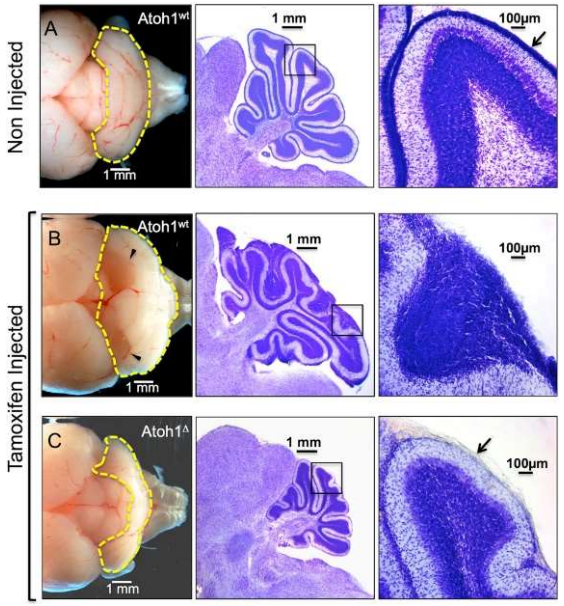
GCP/GNP is the cell of origin of SHH-MB

Atoh1

Atoh1 is expressed in SHH-MB

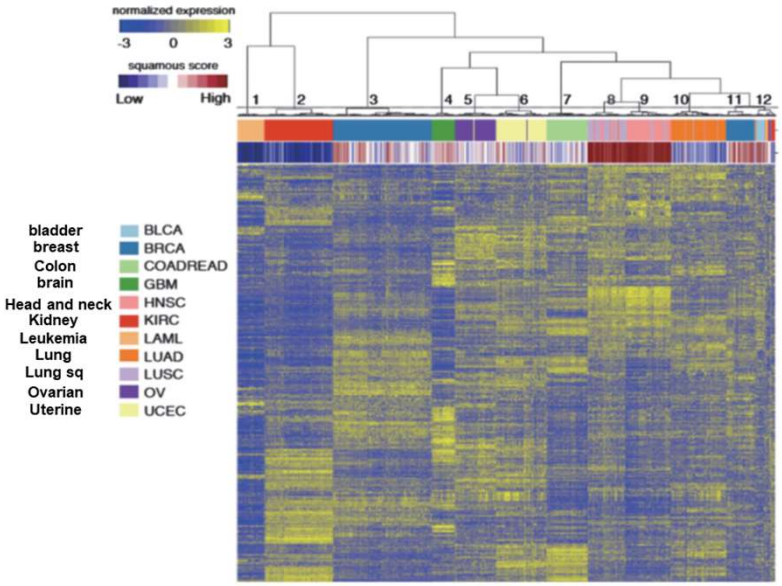


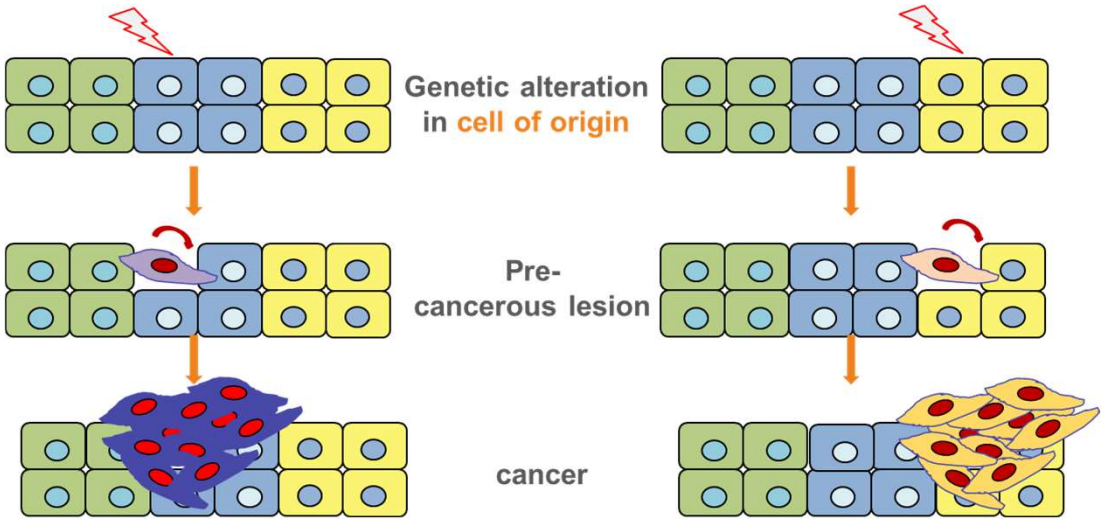
Atoh1 is required for SHH-MB formation



CONCEPT OF LINEAGE ADDICTION

Lineage addiction in cancer





•**LINEAGE ADDICTION IN CANCER:**

➔ **Cancer are classified by their localization (organ specificity)**

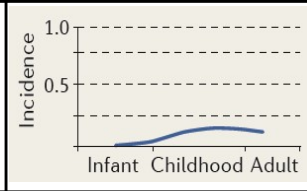
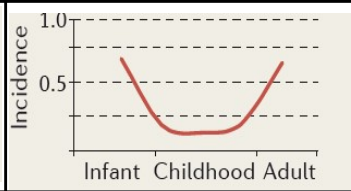
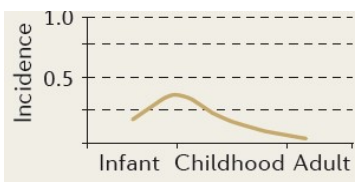
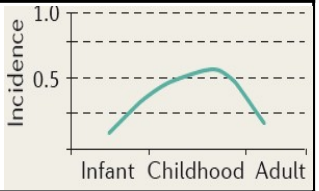
➔ **Close association between cell lineage and cancer phenotype**
(Cancer cells expressed specific lineage markers representative of cell of origin)

➔ **Lineage markers are key for tumour progression**
(Lineage dependency (ex: Atoh1in SHH-MB))

➔ **CONCEPT OF LINEAGE ADDICTION**

Medulloblastoma (MB)

➢ Based on gene expression profile : 4 different molecular groups

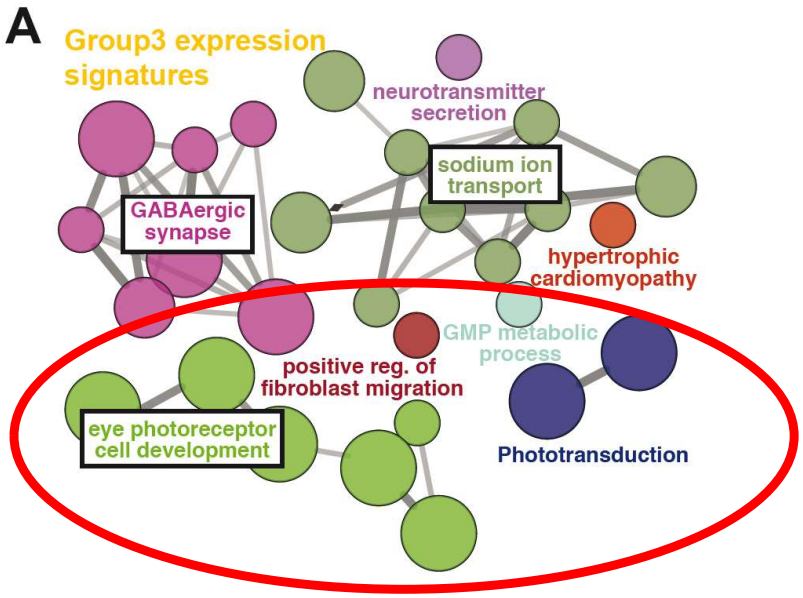
MB subtype		WNT	SHH	3	4
Clinical features	Age distribution				
	incidence	10%	25%	25%	40%
	histology	Classic (very rare LCA)	Classic > desmoplastic/ nodular> LCA	Classic>LCA	Classic; rarely LCA
	Metastasis at diagnosis	rare 5-10%	intermediate 15-20%	frequent 40-45%	frequent 35-40%
	Overall survival (5years)	Very good 95%	Intermediate 75%	Bad 50%	intermediate 75%
	Cell of origin	Lower rhombic lip precursor	Granular cell progenitor	Unknown	
Genomic features	Driver pathway/genes	WNT/βCAT CTNNB1 mutation	SHH PTCH1 mutation	MYC amplification OTX2 amplification	MYCN & CDK6 amplification
	Expression signature	WNT signaling	SHH signaling	MYC & GABAergic & photoreceptor signature	Neuronal & glutamatergic signature

Adapted from Northcott & al., 2012

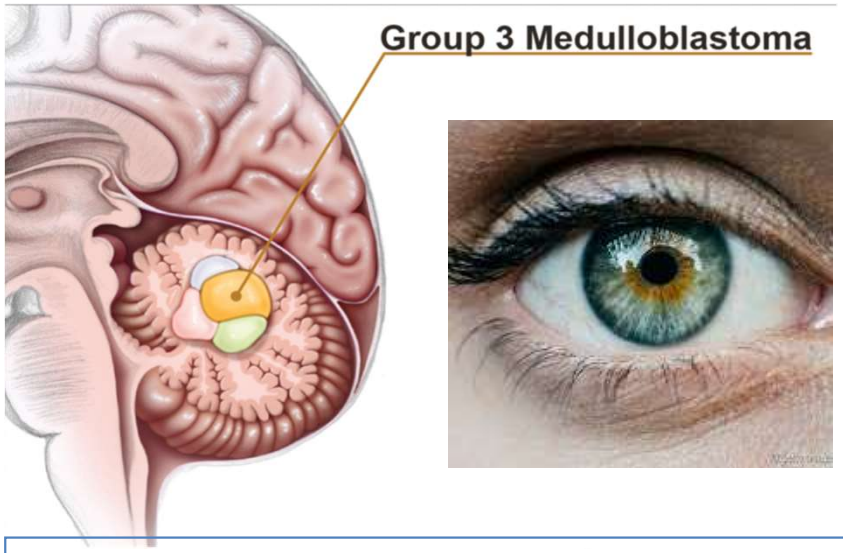
Medulloblastoma (MB)

➢ Based on gene expression profil : 4 different molecular groups

MB subtype	WNT	HH	3	4
incidence	10%	25%	25%	40%
Overall survival	Very good	Intermediate	Bad (meta+++)	Intermediate (meta++)
Driver pathway	WNT	HH	MYC amplification OTX2 amplification	MYCN & CDK6 amplification
Expression signature	WNT signaling	SHH signaling	Photoreceptor markers	Neuronal & glutamatergic signature



Group 3 Medulloblastoma (MB)



Group 3 MB displays an abnormal identity (photoreceptors of the retina)

• Identity unrelated to the tissue of origin : retina fate in a cerebellar tumor

•LINEAGE ADDICTION IN CANCER:

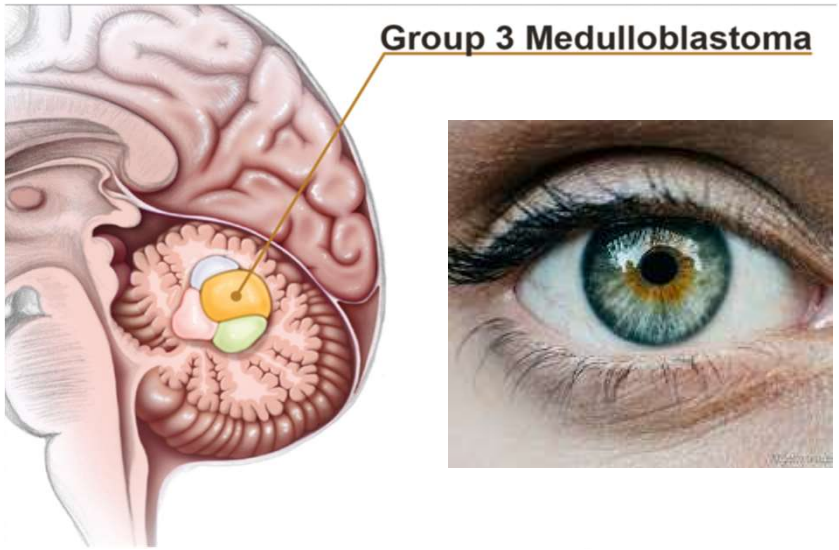
➔ Cancer are classified by their localization (organ specificity)

➔ Close association between cell lineage and cancer phenotype
(Cancer cells expressed specific lineage markers representative of their localization)

➔ Lineage markers are key for tumour progression
(Lineage dependency can guide treatment (ex ER in breast Cancer))

•Challenge the concept of lineage addiction in cancer

Group 3 Medulloblastoma (MB)



Group 3 MB displays an abnormal identity (photoreceptors of the retina)

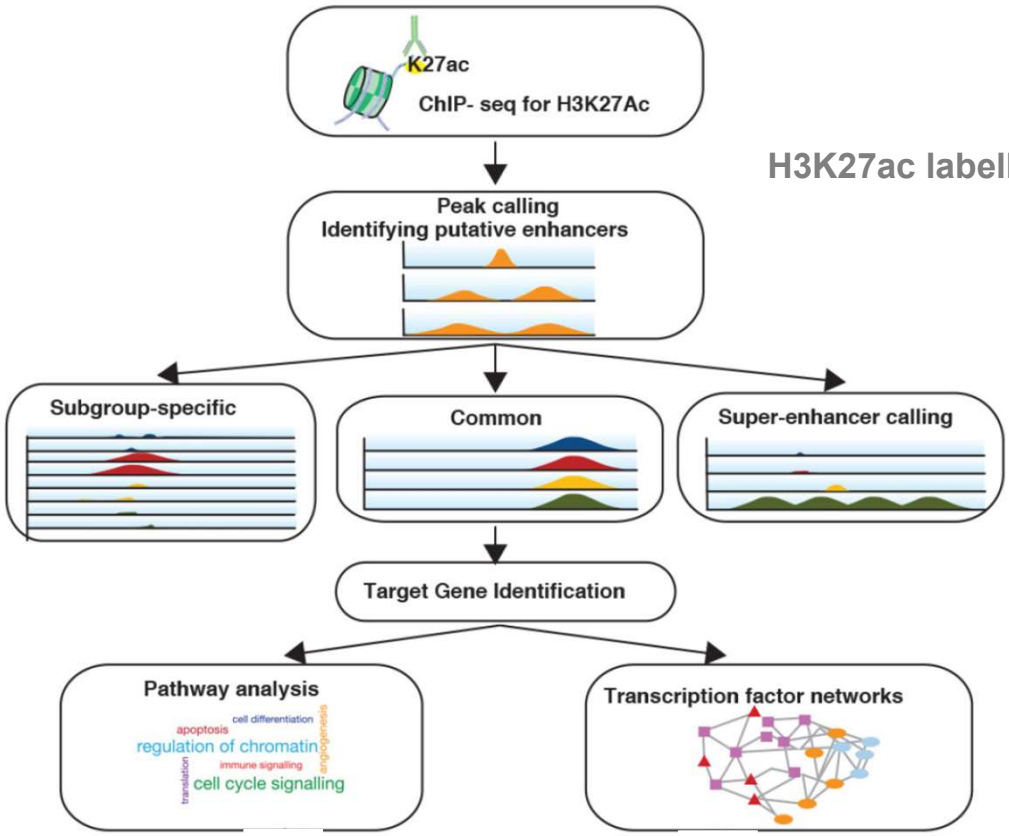
• Identity unrelated to the tissue of origin : retina fate in a cerebellar tumor

•Challenge the concept of lineage addiction in cancer

How this abnormal identity is established ?

Is there an addiction to this abnormal identity?

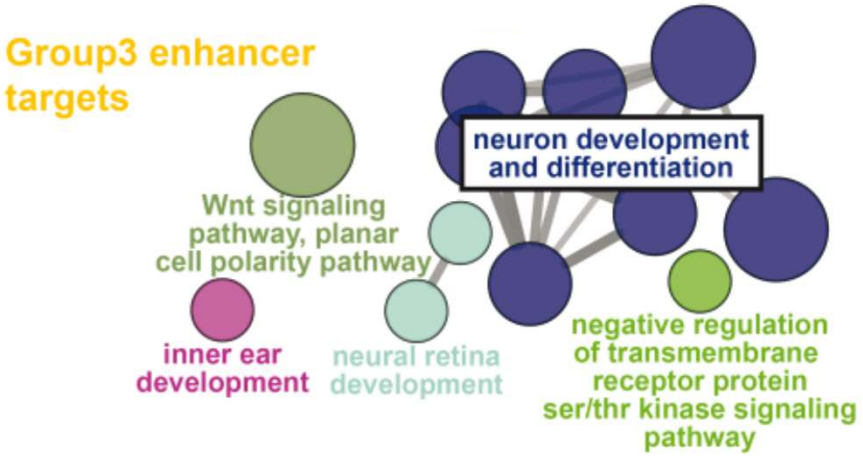
Identification of active enhancers in medulloblastoma



H3K27ac labelled active enhancers

In collaboration with Dr. P. Northcott (St Jude, USA) & C. Lin (Houston USA)

Group 3 are enriched in active enhancer/SE controling photoreceptor genes



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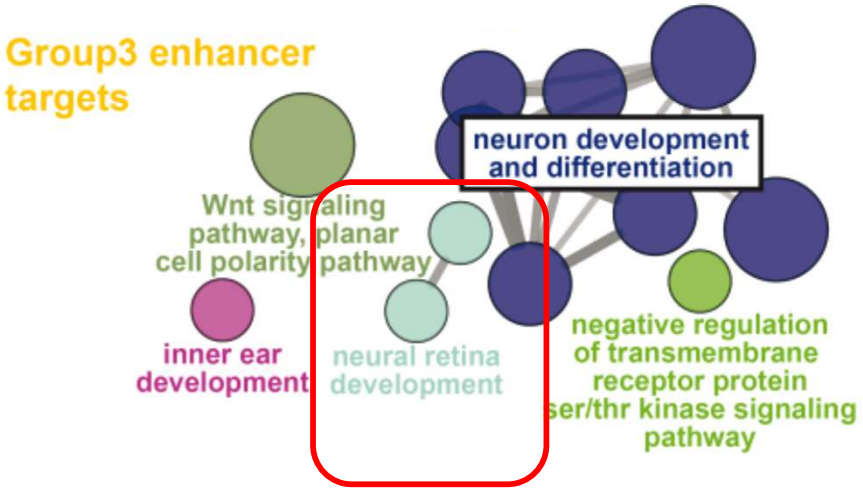

H3K27ac labelled active enhancers (Identification of G3 enhancers) Controlled genes involved in retina development

In collaboration with Dr. P. Northcott (St Jude, USA) & C. Lin (Houston USA)

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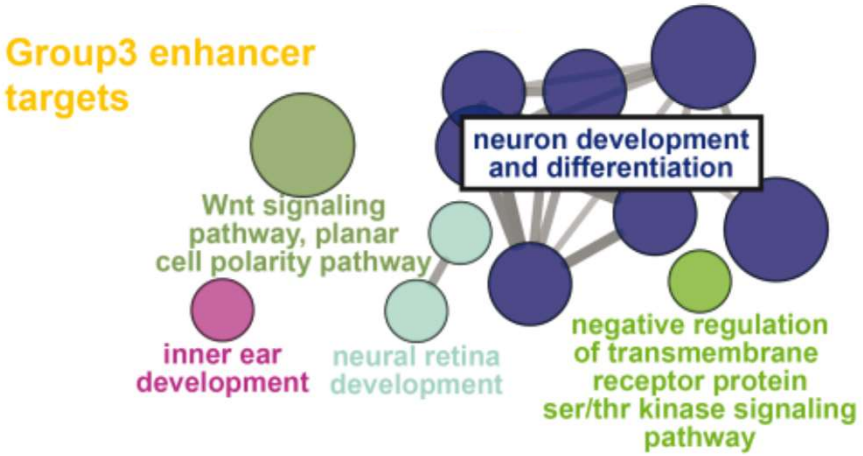
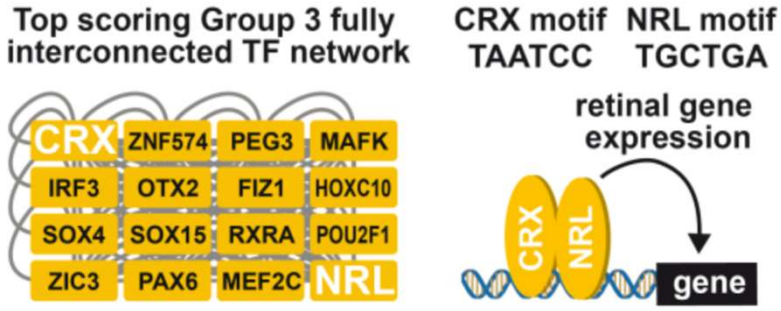


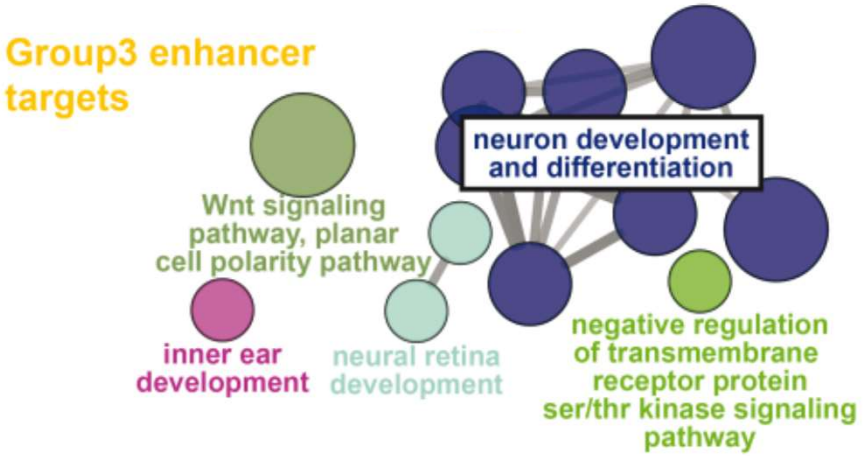


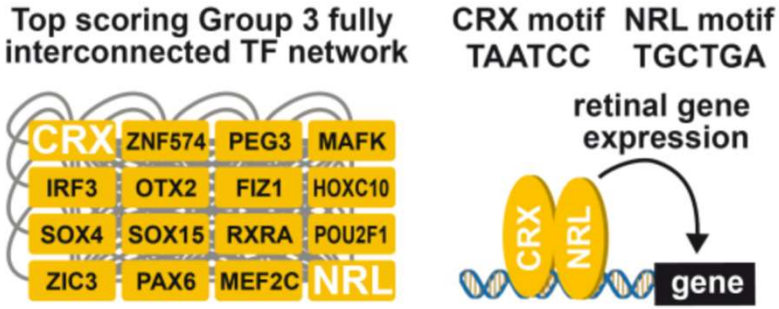
H3K27ac labelled active enhancers (Identification of G3 enhancers) Controlled genes involved in retina development

Look for enrichment of binding sites for TFs on these enhancers

In collaboration with Dr. P. Northcott (St Jude, USA) & C. Lin (Houston USA)

Group 3 are enriched in active enhancer/SE controling photoreceptor genes





H3K27ac labelled active enhancers (Identification of G3 enhancers) Controlled genes involved in retina development

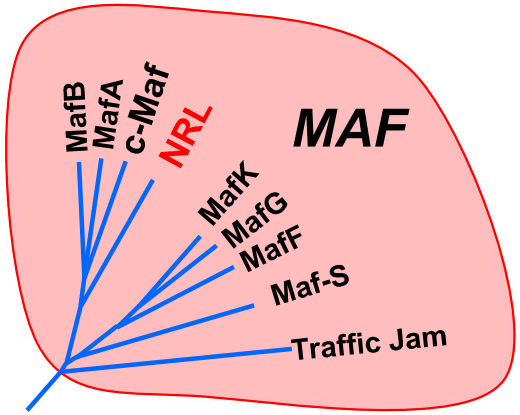
Look for enrichment of binding sites for TFs on these enhancers

NRL and CRX binding sites are enriched in Group 3 enhancers
NRL AND CRX are lineage restricted photoreceptor TFs

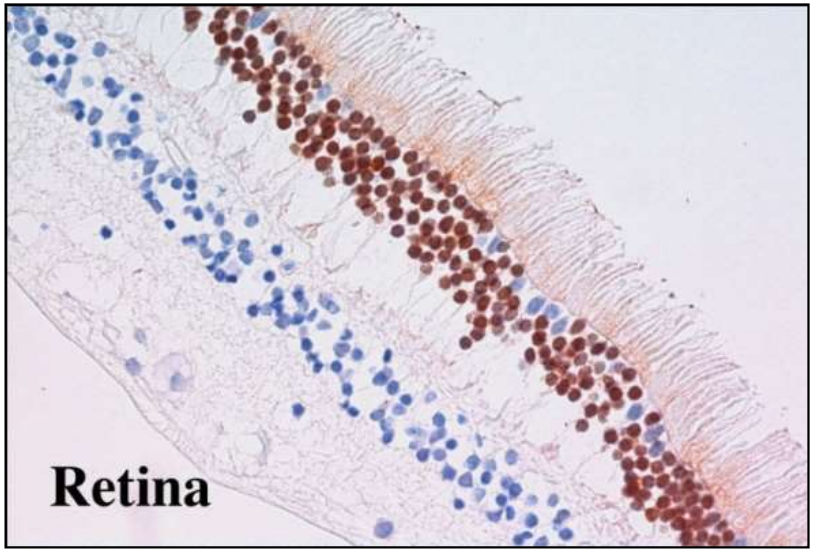
In collaboration with Dr. P. Northcott (St Jude, USA) & C. Lin (Houston USA)

NRL in Medulloblastoma

Role of NRL in medulloblastoma



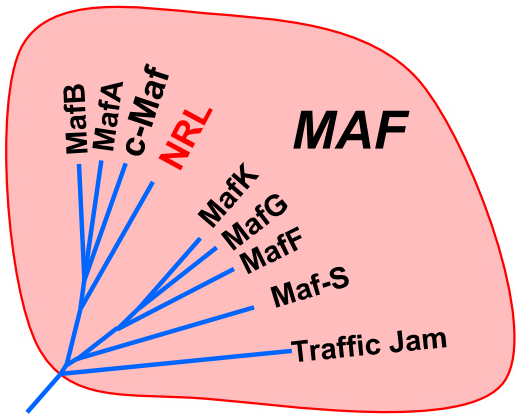
- NRL expression is restricted to the photoreceptors of the retina



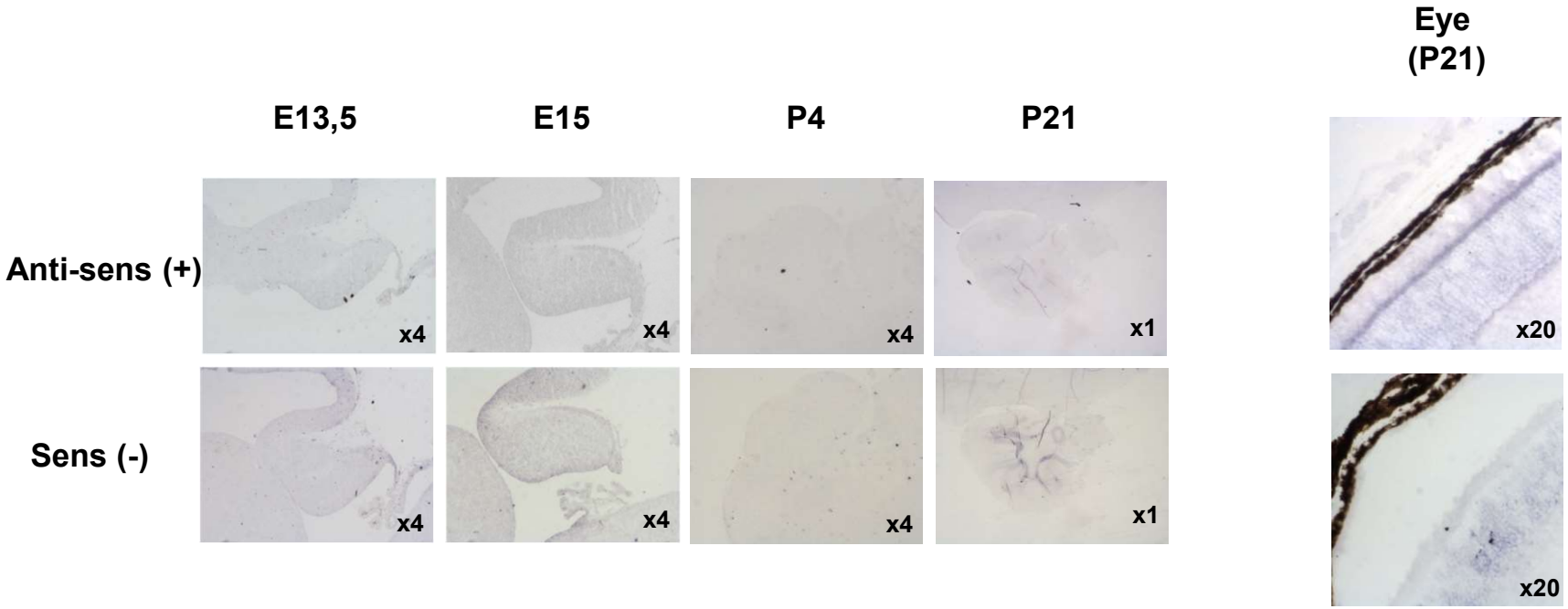
(immunohistochemistry (IHC))

NRL in Medulloblastoma

Role of NRL in medulloblastoma



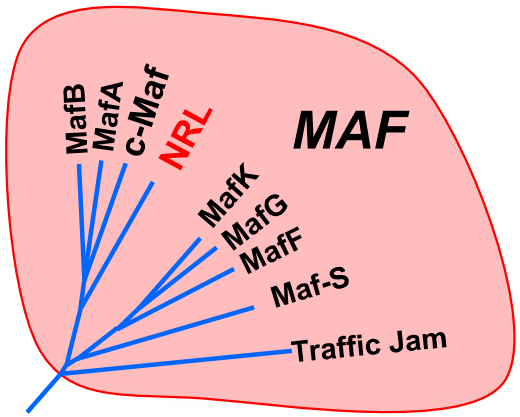
- **NRL expression is restricted to the photoreceptors of the retina**
  (not expressed in the cerebellum)



In situ hybridization (ISH)

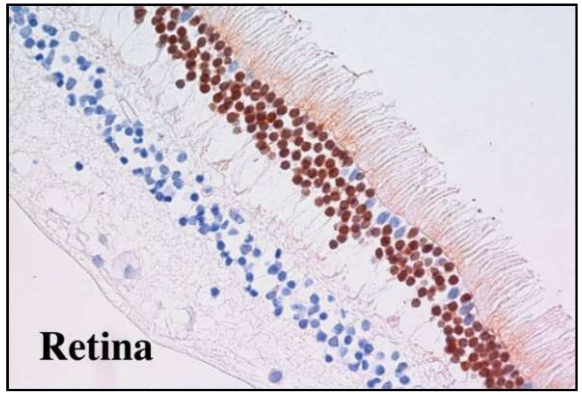
NRL in Medulloblastoma

➡ Role of NRL in medulloblastoma



- NRL expression is restricted to the photoreceptors of the retina
  (not expressed in the cerebellum)

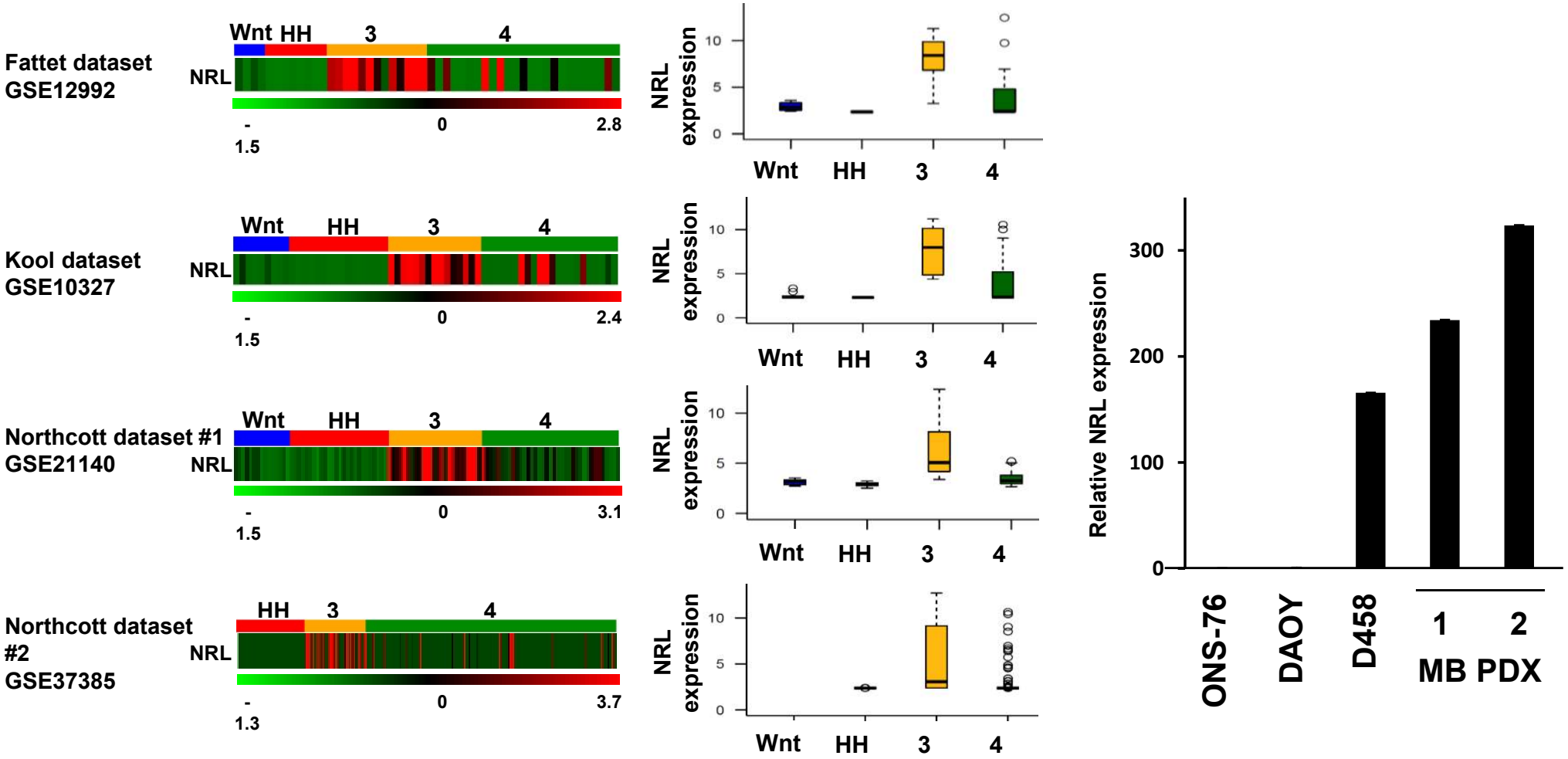
- Role in photoreceptor terminal differentiation
  (KO NRL: lack of a subset of photoreceptors)
  Mutations of NRL in human (retinitis pigmentosa)



➡ Role of NRL in medulloblastoma?

➔ NRL has not yet been demonstrated to be involved in oncogenesis

NRL overexpression in MB



NRL is overexpressed in the metastatic MB group 3

Collaboration with Dr. F.Bourdeault & Dr. O. Delattre (Institut Curie)

Group 3 MB PDXs

PDX : Patient-derived xenograft

Group 3 MB PDXs

PDX : Patient-derived xenograft

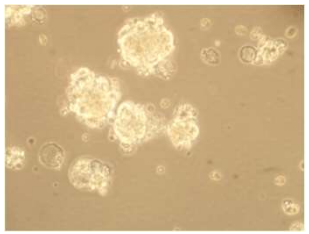
Medulloblastoma
Surgery

Necker Hospital

grafting

Tumour growth and serial passages

Culture

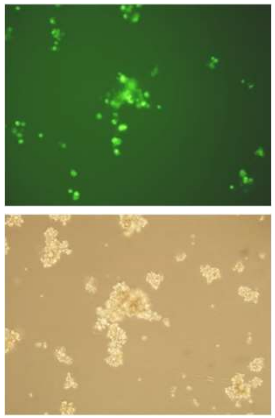


neurospheres

Genetic manipulations

LENTIVIRAL INFECTION
Gene extinction, overexpression

GFP



phase

Tumour
growth

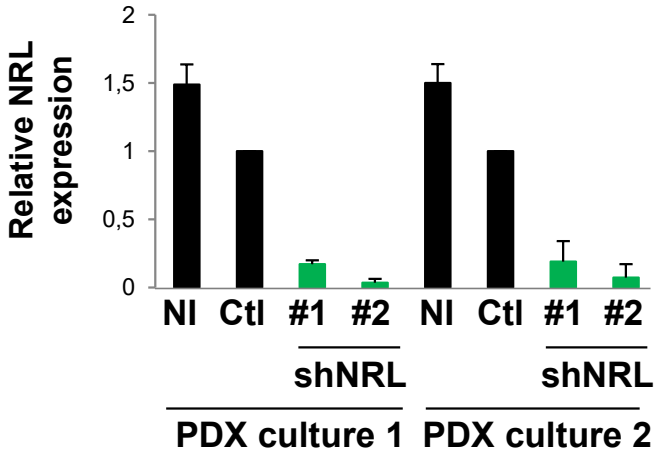
Tests in vitro
(proliferation…)

In collaboration with Dr. O. Ayrault (Institut Curie)

NRL is required for MB growth in vivo

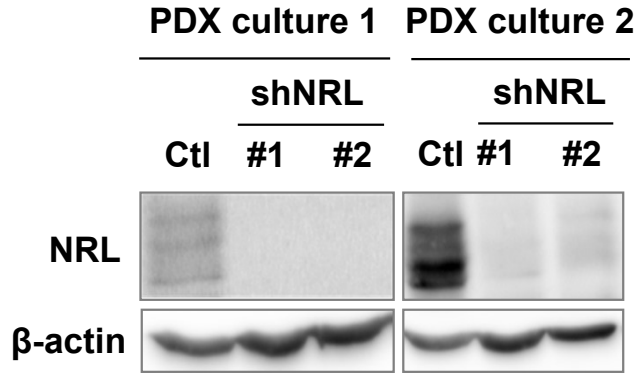
➢ **NRL knock-down in 2 PDX**

RT-qPCR



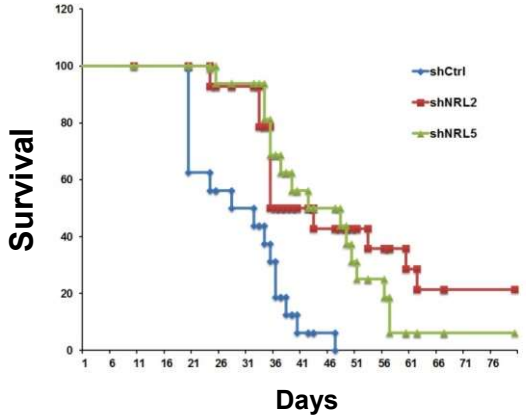
Western-blot

NI : non infected



Orthotopic Grafting

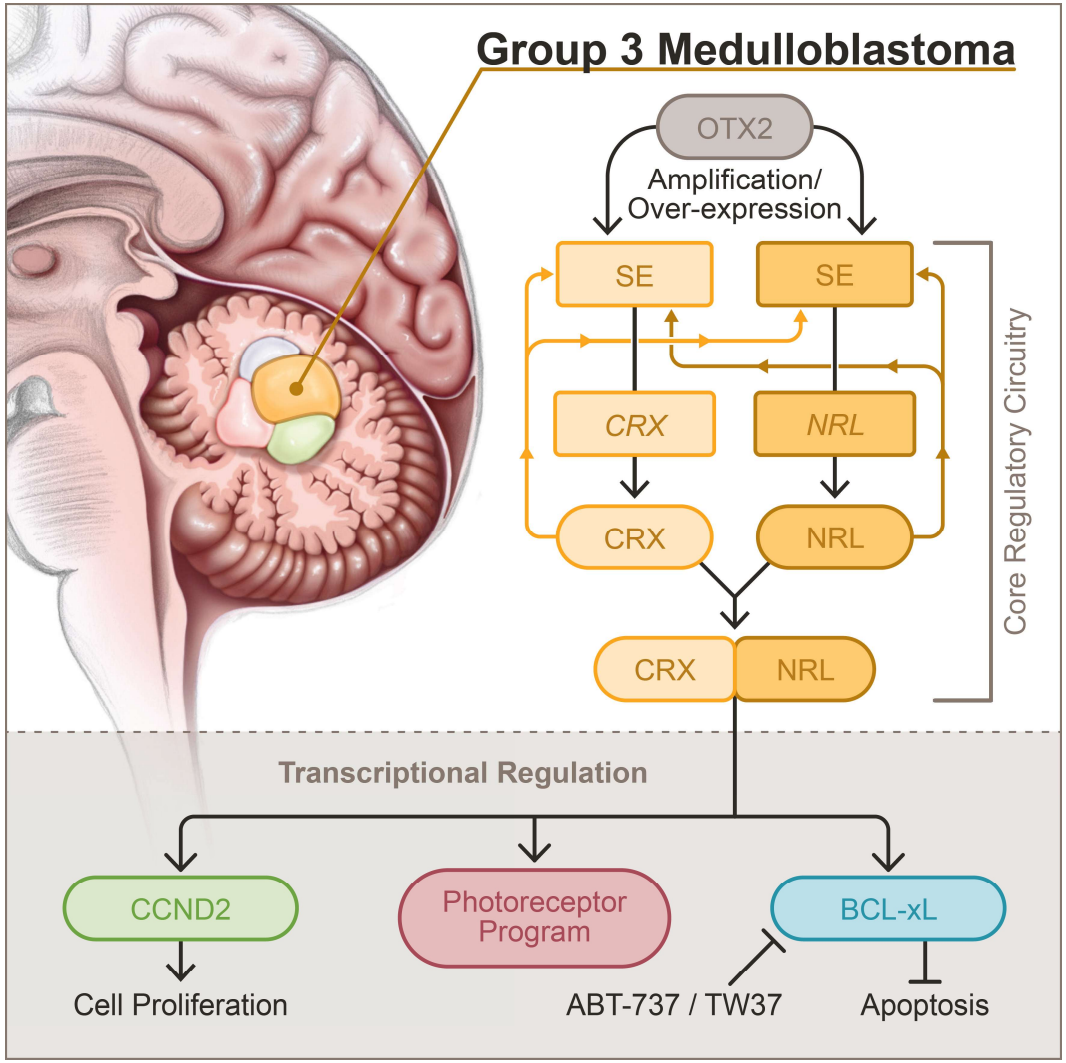
tumor growth



survival

➔ **NRL is required for growth in vivo both in cell lines and in PDXs**

Model



- **NRL and CRX are master regulators of the Group 3 MB photoreceptor program**
- **Photoreceptor transcriptional program is a dependency in Group 3 MB**
- **NRL control cell cycle by inducing *CCND2* expression**
- **NRL protects MB from apoptosis by inducing *BCL-XL* expression**
- **BCL-XL as a potential therapeutic target in Group 3 MB**

G33-MB: Addiction to abnormal lineage

Signaling and Cancer progression


Andy Warhol n'a qu'à bien se tenir
By Chloé Foray

Pediatric Cancers

In general rare cancers

- **75-80% Cured**
  - Better than for adult cancers.
  - Important side effects
  - **Much less mutations ????**