Význam bakalářské práce

Vítězslav BRYJA

OFIŽ
Ústav experimentální biologie, PřF MU

Co jste možná nevěděli

Možnost zapsat si:

- Bakalářská práce nyní může být i praktická

- Časopisový klub Imunologie I a II
- Časopisový klub Fyziologie živočichů I a II
- Časopisový klub Vývojová biologie I a II

KONTAKT

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Mgr. Jakub Harnoš, Ph.D.

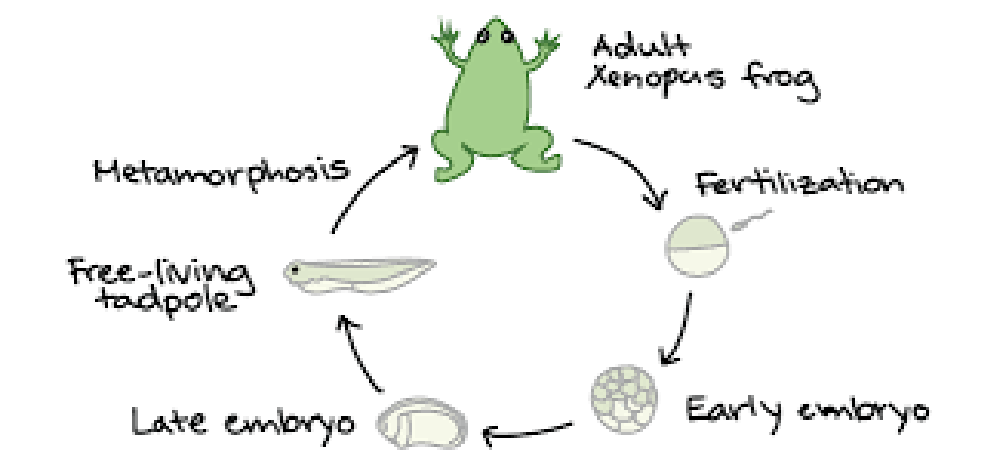
Odd. fyziologie a immunologie živočichů

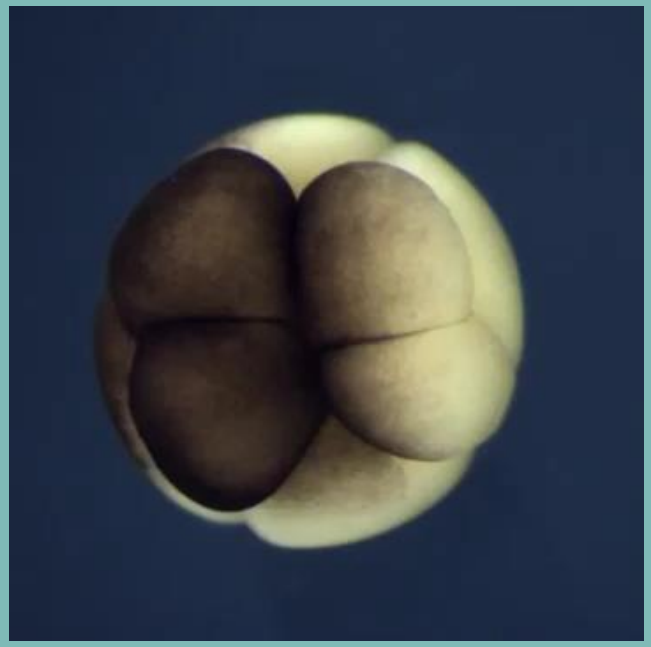
budova D36, místnost 1S16

HARNOS lab

EST 2021

MODEL *XENOPUS*

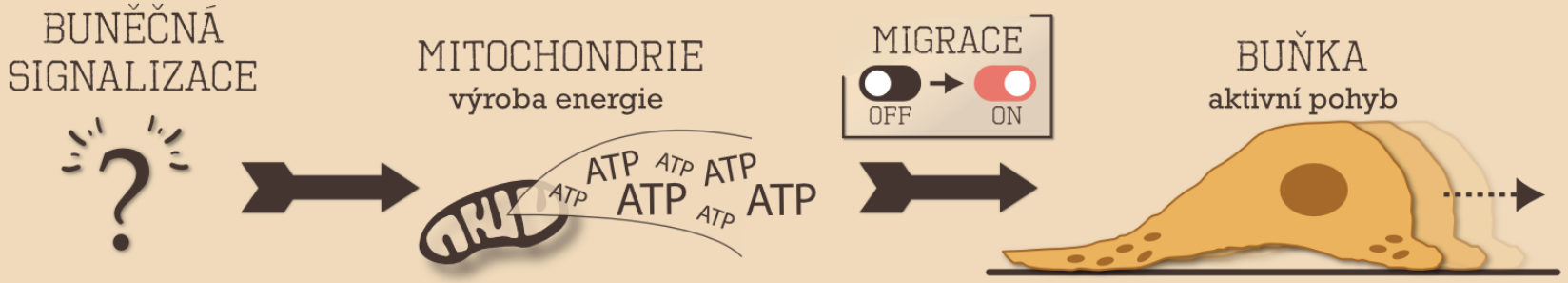




Nancy Papalopulu

TÉMA NAŠEHO VÝZKUMU:

Buněčná migrace hraje zásadní roli jak při vývoji organismu tak při patogenních procesech, např. metastáze. Navzdory její důležitosti však dosud neznáme, jak buňka získává **energii** pro tyto procesy. Konkrétně nás zajímá, jak buňka přesně instruuje mitochondrie pro výrobu energie.



CO HLEDÁME?

Hledáme motivované studenty pro studium procesů, jakými buňky získávají energii pro svoji migraci.

Pro studium bioenergetických procesů budeme využívat unikátní model obratlovců:
ranné stádia embryí žáby *Xenopus*.

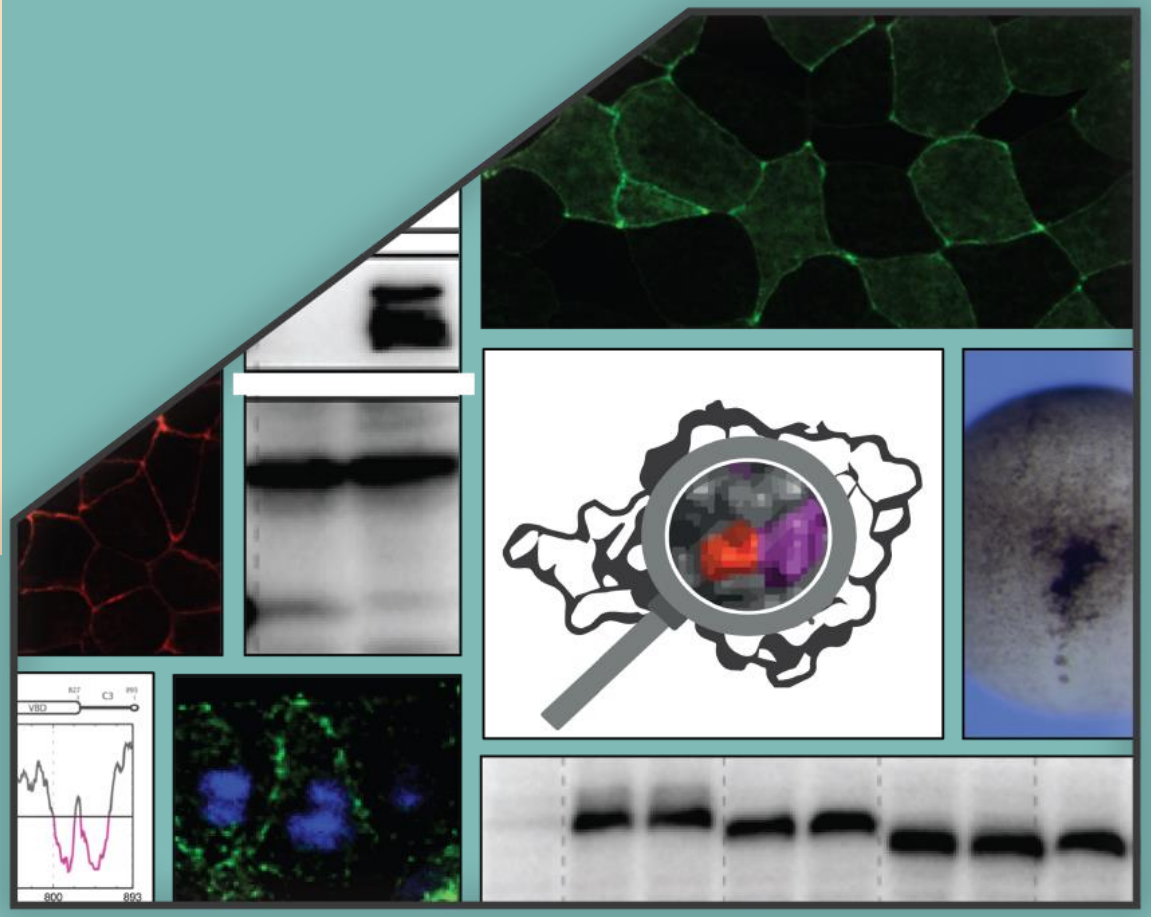
CO NABÍZÍME

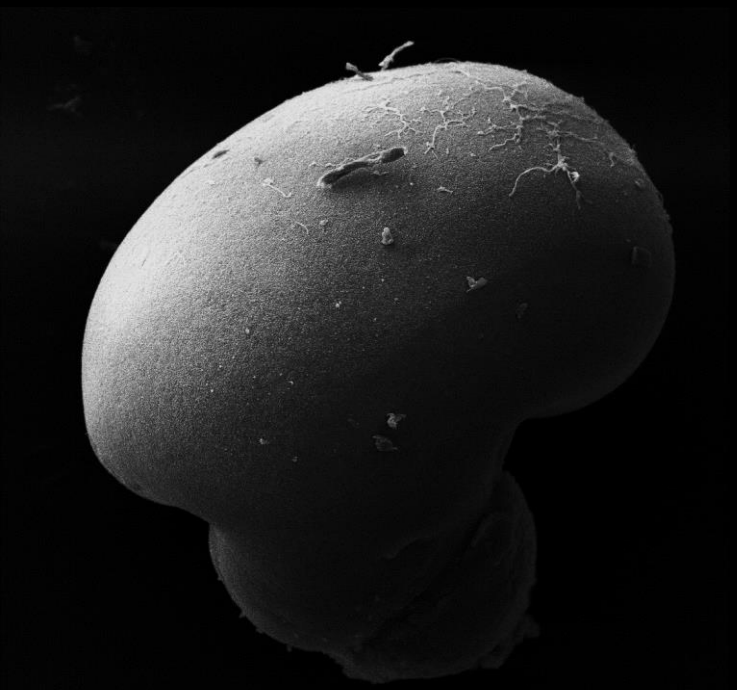
- Atraktivní a ambiciózní téma
- Cenné znalosti a zkušenosti s laborartorními technikami
- Osvojení si práce a zacházení s živými zvířaty - žábami
- Možnost krátkodobých stáží na spolupracujícím pracovišti v New Yorku
- Zajímavé finanční ohodnocení

METODY

Zahrnuty základní i pokročilé metodiky:

- “live imaging” a konfokální mikroskopie,
- optogenetika,
- biofyzika and NMR spectroskopie,
- genetické manipulace,
- molekularní & buněčná biologie metabolismus





Mgr. Tomáš Bárta, PhD

Department of Histology and Embryology

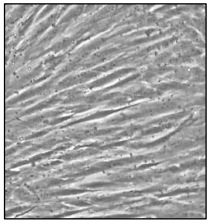
&

DEPARTMENT
OF EXPERIMENTAL BIOLOGY
MUNI SCI

SECTION
OF ANIMAL PHYSIOLOGY
AND IMMUNOLOGY

Čím se zabýváme

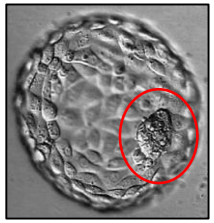
Lidské pluripotentní kmenové buňky

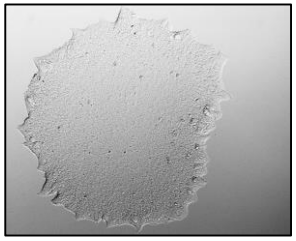


Sir John B. Gurdon

Shinya Yamanaka

Oct4, Klf4, Sox2, c-myc





Lidské embryonální kmenové buňky

Lidské indukované pluripotentní kmenové buňky

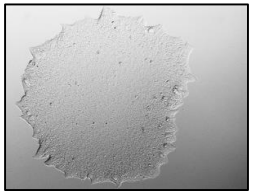
Endoderm

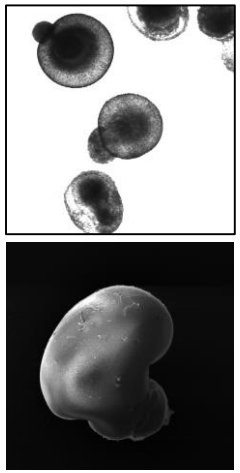
Mesoderm

Ectoderm

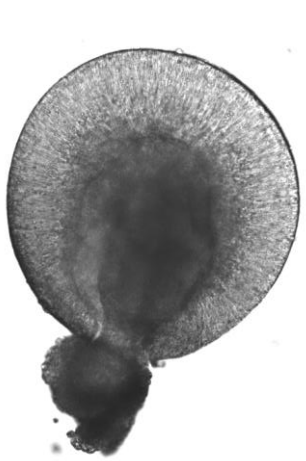
Čím se zabýváme

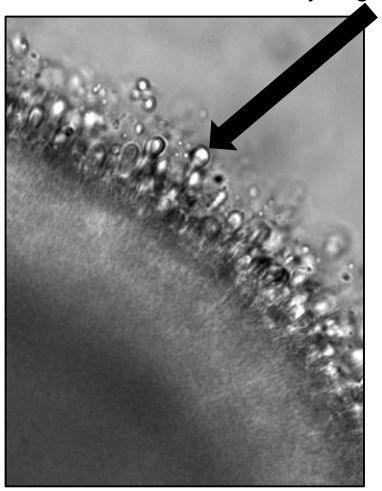
Retinální organoidy

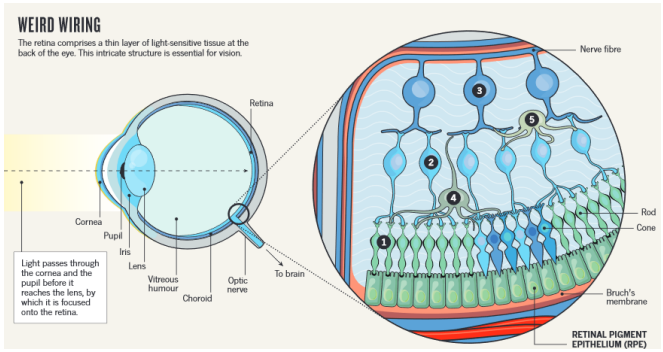




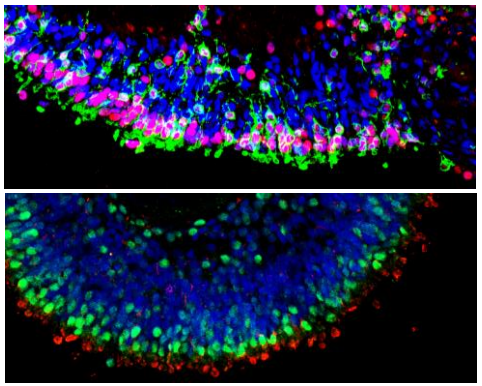
Vnější segmenty fotoreceptorů







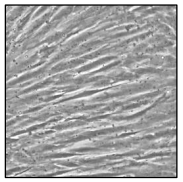
Průřez retinou



Průřezy retinálním organoidem – obarveny fotoreceptory a Opsiny

Co studujeme

Co se děje při přeprogramování buněčného osudu?



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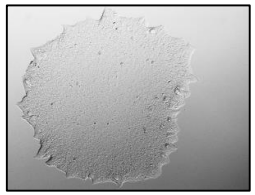
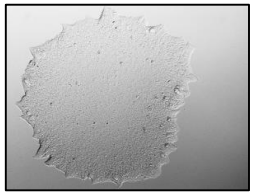


iPSCs

Jak vzniká a funguje lidská retina?



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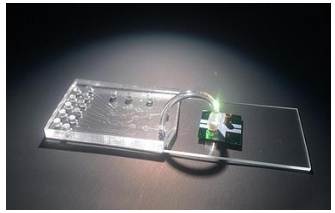
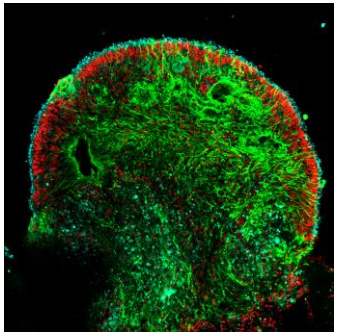
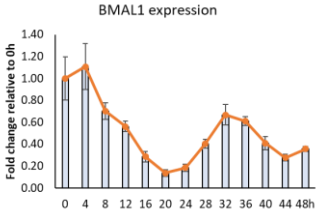
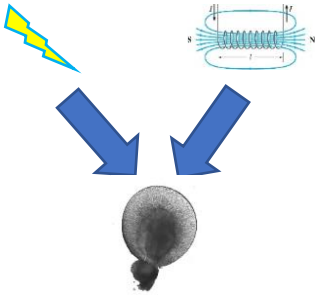
Jak vytvořit lepší organoidy?



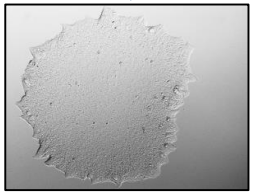


Jak fungují cirkadiánní rytmy u lidí?





Modelování retinálních onemocnění



S kým spolupracujeme

UPPSALA UNIVERSITET

Michael Andäng

Karolinska Institutet

Mikael Altun
Johan Boström

Evelyn Sernagor

MASARYKOVA UNIVERZITA

Martin Vácha

Pavel Krejčí

Markéta Bebarová

Michaela Bosáková

Marcela Buchtová

Jiří Pacherník

Newcastle University

Majlinda Lako

Lyle Armstrong

Valeria Chichagova

IMG
Ústav molekulární genetiky AV ČR, v. v. i.

David Staněk

Petra Lišková

Kdo jsme

Tomáš Bárta

Kontakt: tbarta@med.muni.cz

Postdoci

Kamila Weissová

Tereza Váňová

Ph.D. studenti

Canan Çeliker

M.Sc. studenti

Kateřina Konečná

Jana Šebestíková

Absolventi

Lucie Pešková – M.Sc. a Ph.D. student
Vienna BioCenter, Vienna, Austria

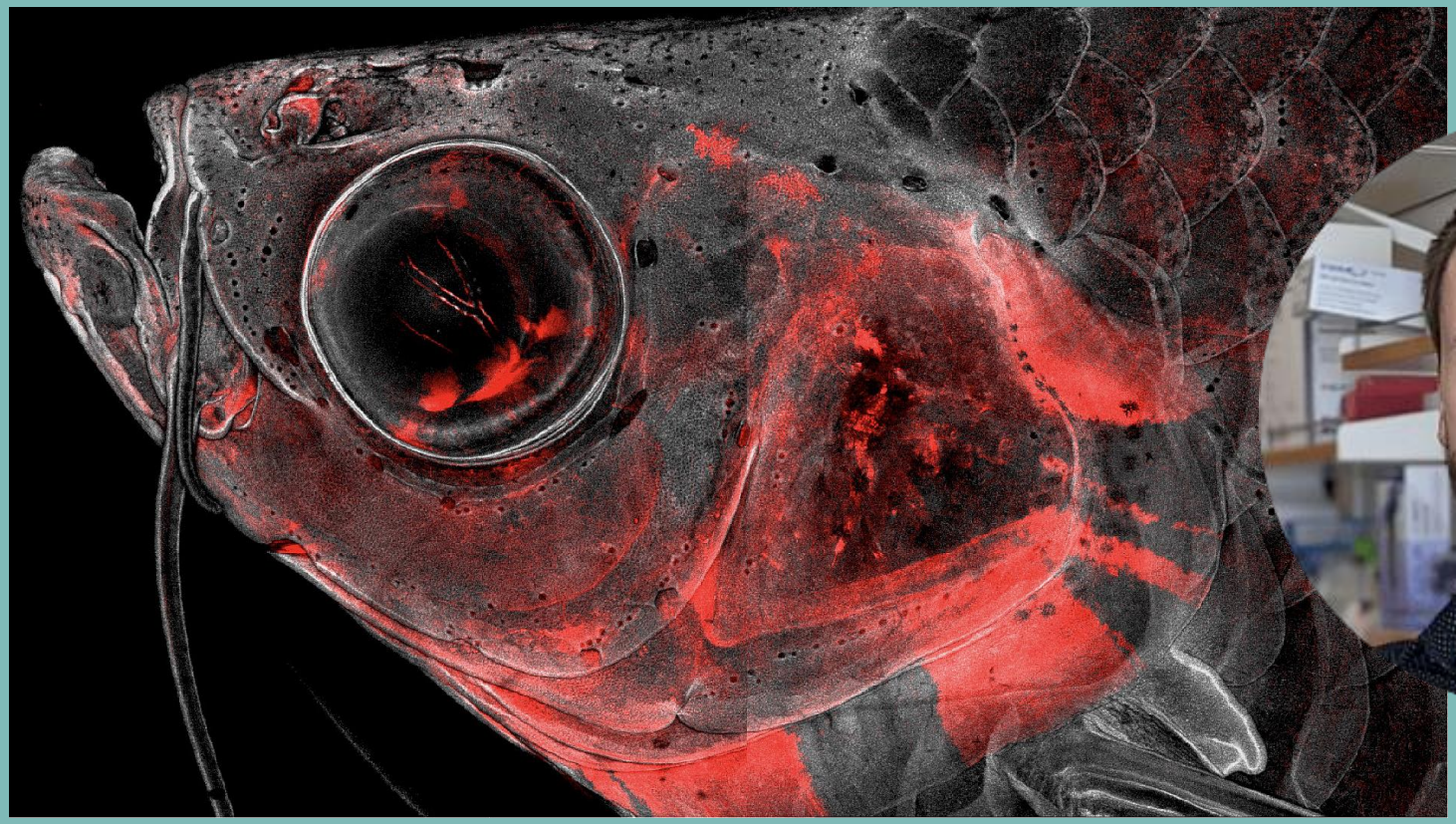
Denisa Jurčíková – B.Sc. a M.Sc. student
Roche, Switzerland

A další….

Laboratoř Petera Fabiana

Starting 1.9.2022

Kontakt: Peter.Fabian@med.usc.edu



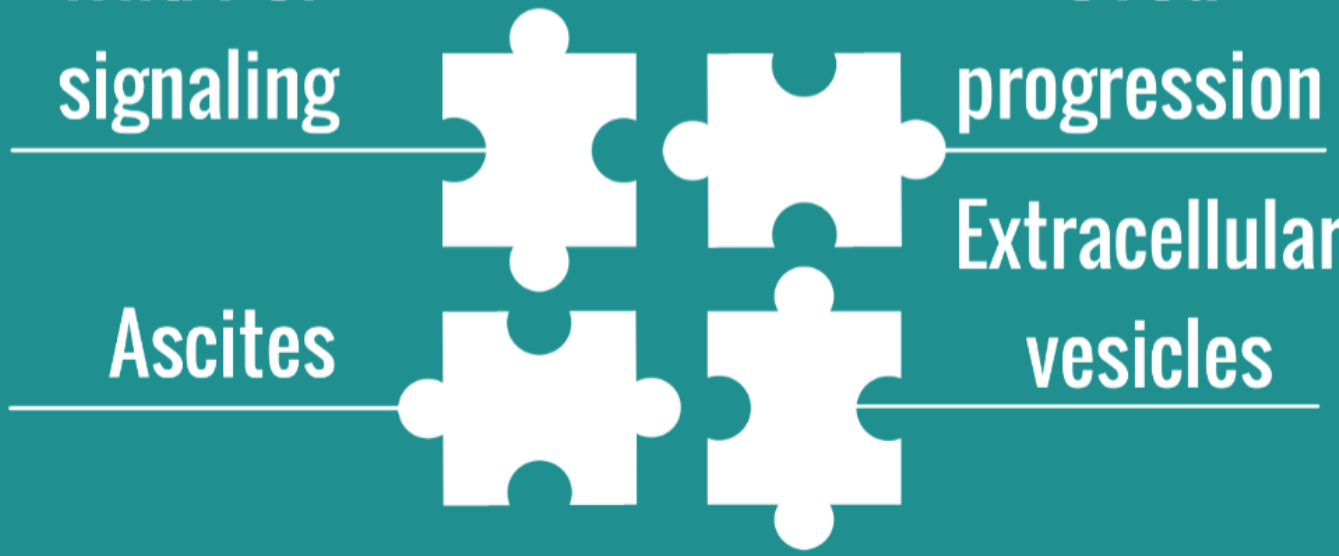
OvCa (OVarian CAncer) club

of Bryja lab

Ovarian cancer (OvCa)

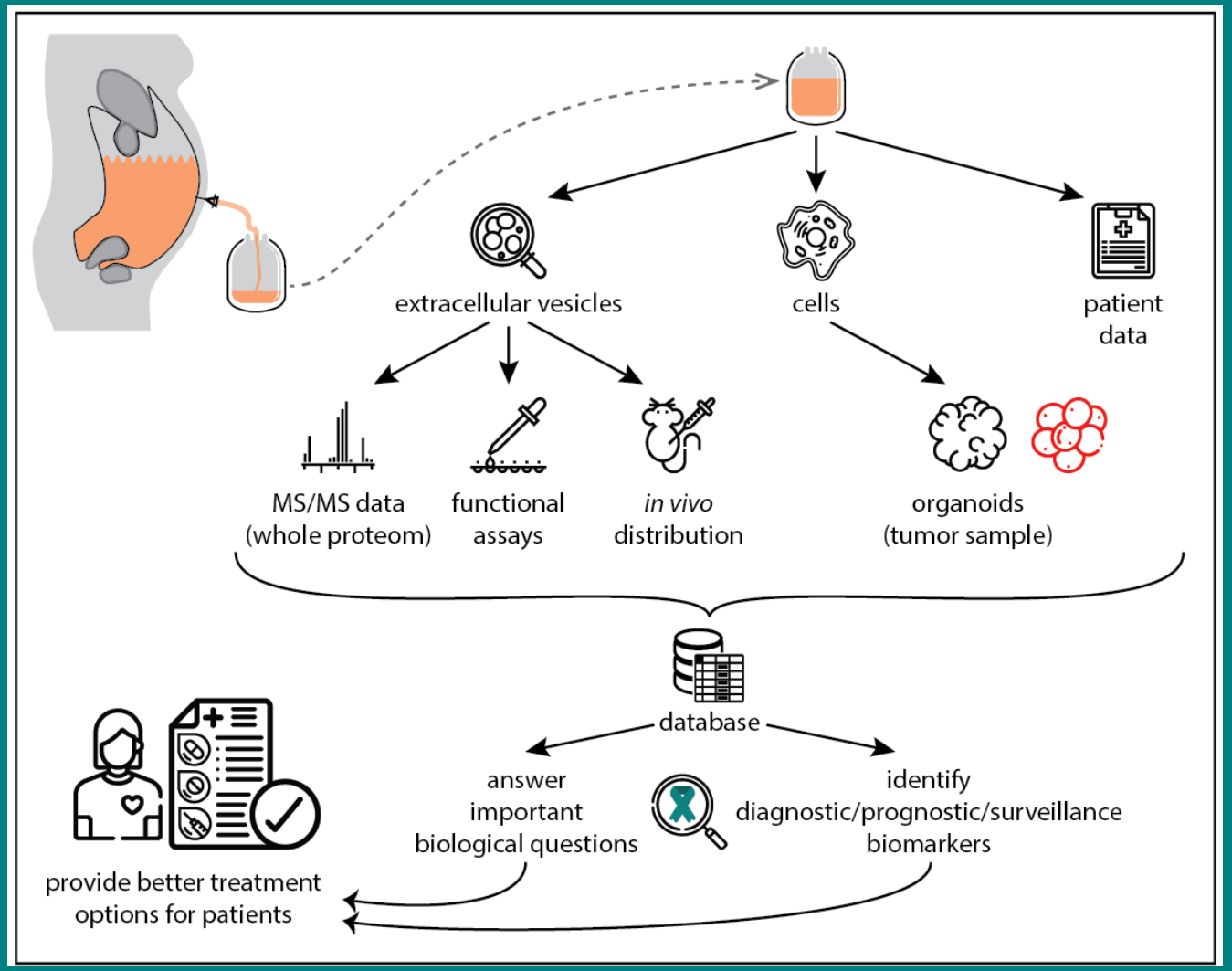
- Deadliest gynecological malignancy
- Late diagnosis (metastases already developed)
- Frequent recurrence of the disease
- No significant achievements in earlier diagnosis and better options of treatment (with the exception of PARP inhibitors)
- High-grade serous ovarian carcinoma (**HGSOC**)
  - 70 % of ovarian carcinomas
  - 5-year survival rate at the stage of diagnosis – less than 30%
- Fast progression - Intraperitoneal dissemination (**ascites**)
  - Rarely lymphatic or hematogenous dissemination

We Make Connections



To better understand OvCa biology to fight it more effectively

Research scheme



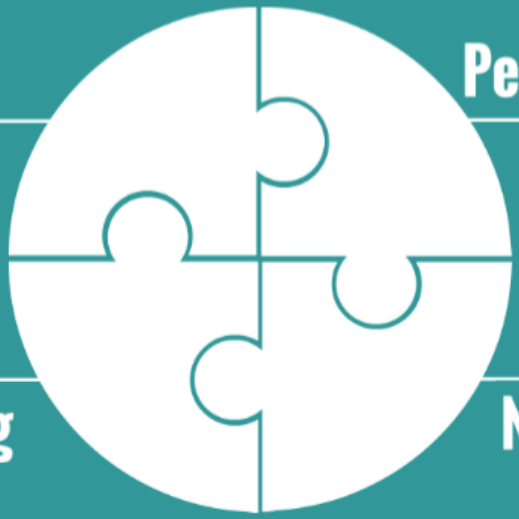
We are looking for Bachelor student(s) in 2nd year

Smart

Creative

Personal integrity

Motivation



Focused

Hard working

Interdependent

Nice personality

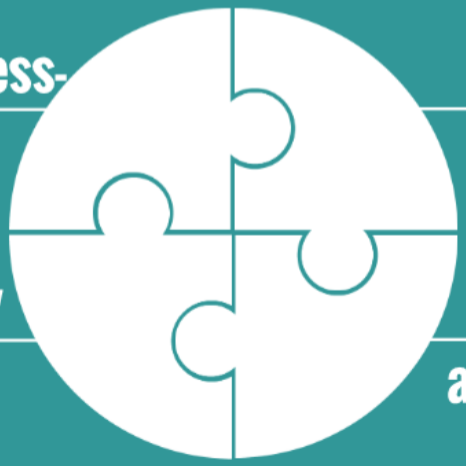
We offer

Young and success-full group

International environment

Good laboratory practice

Friendly atmosphere



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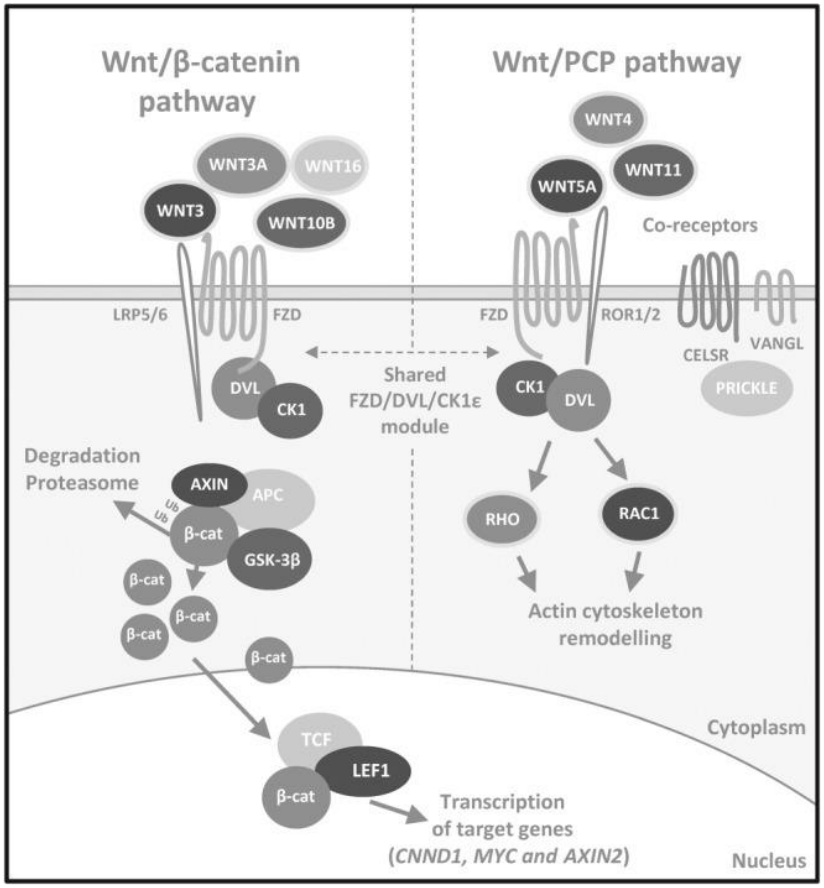
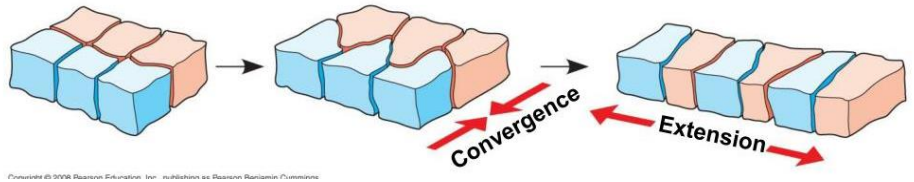
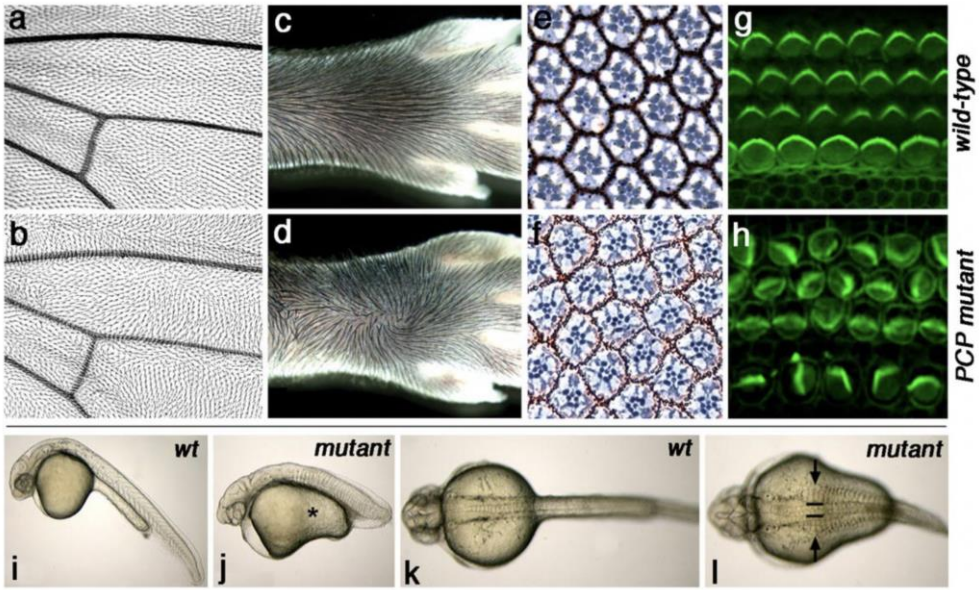
Bryja lab

https://www.sci.muni.cz/ofiz/en/bryja/

WNT / Planar Cell Polarity Pathway

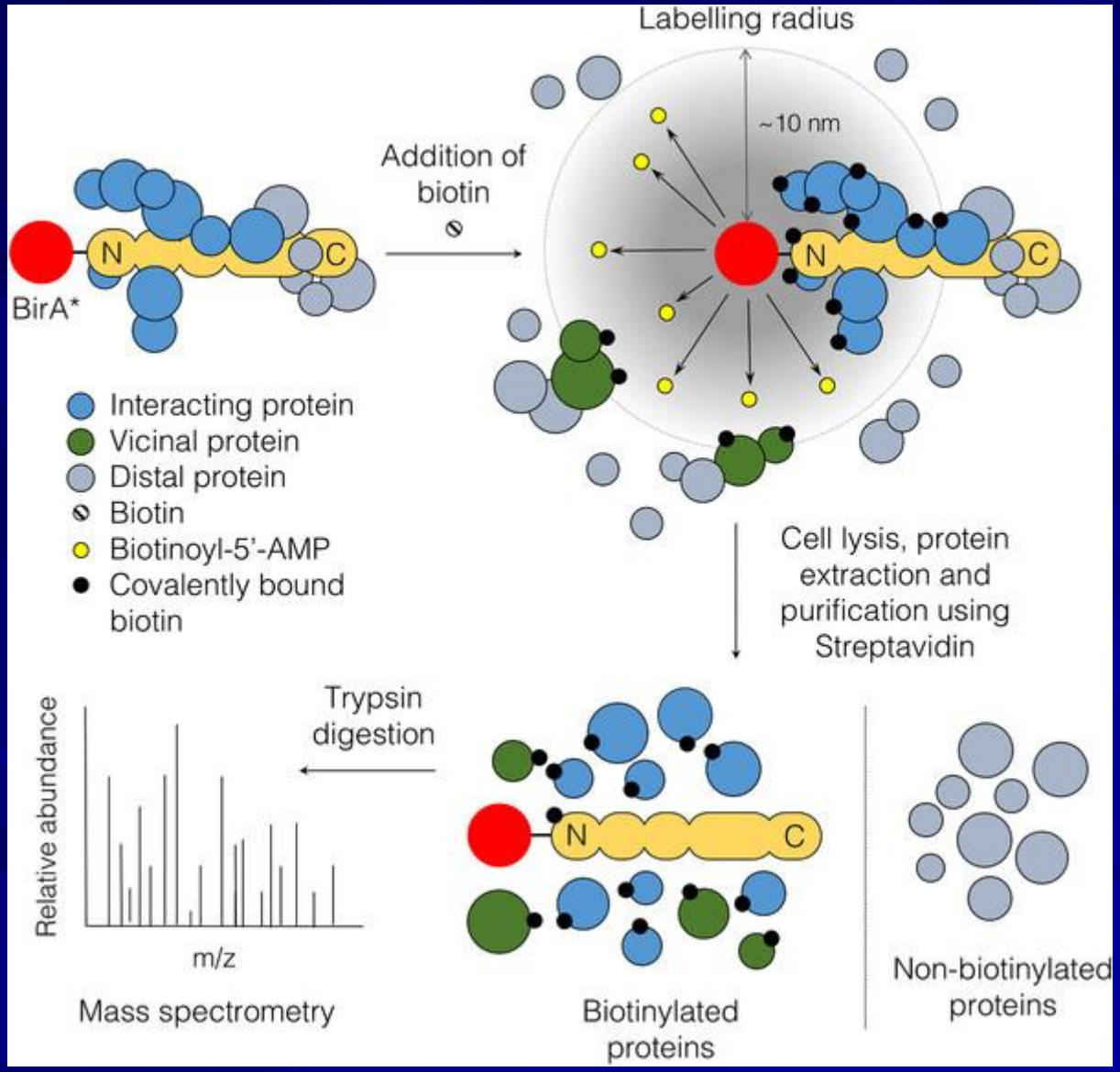




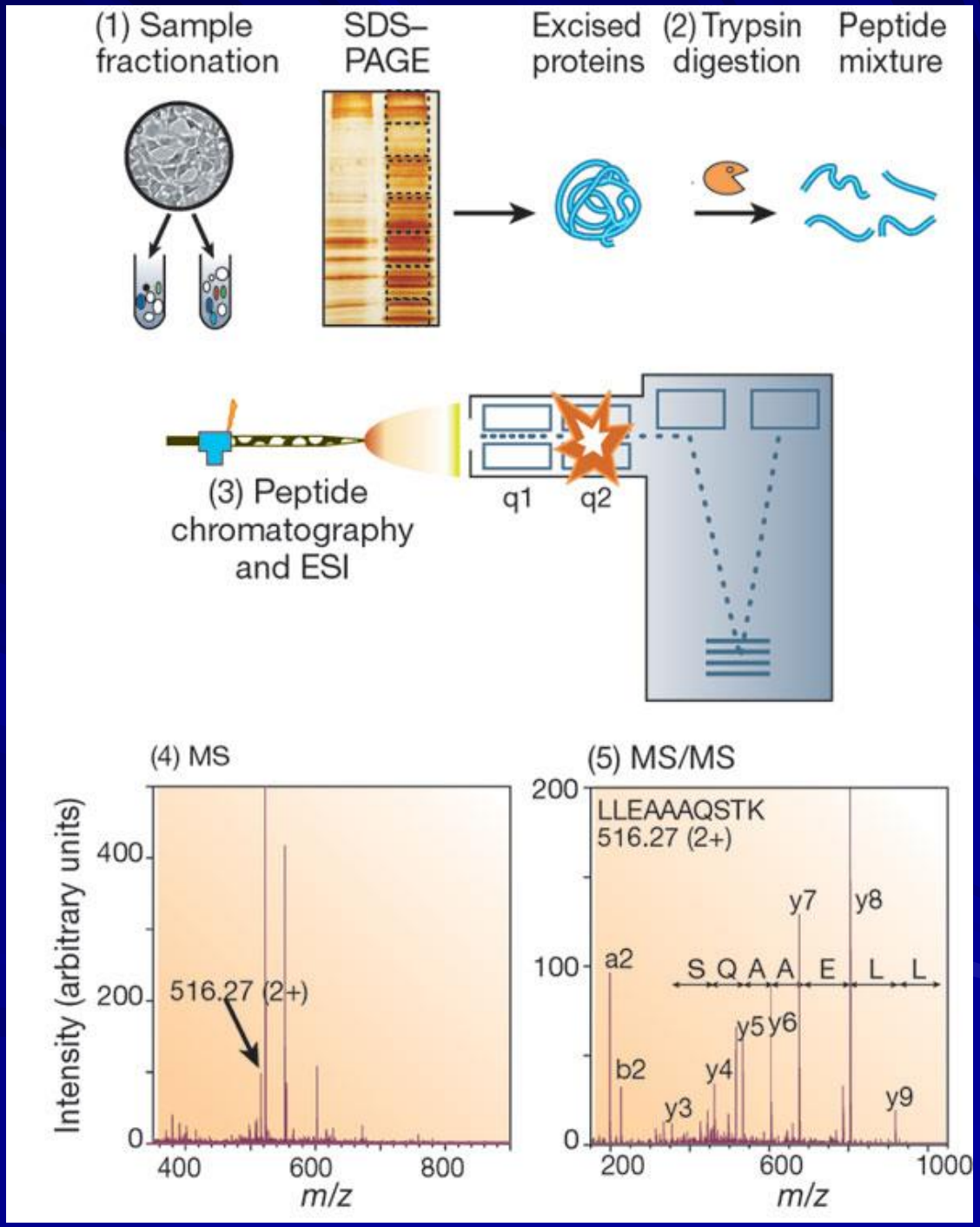


Metoda: BioID a hmotnostní spektroskopie

BioID – proximity labeling

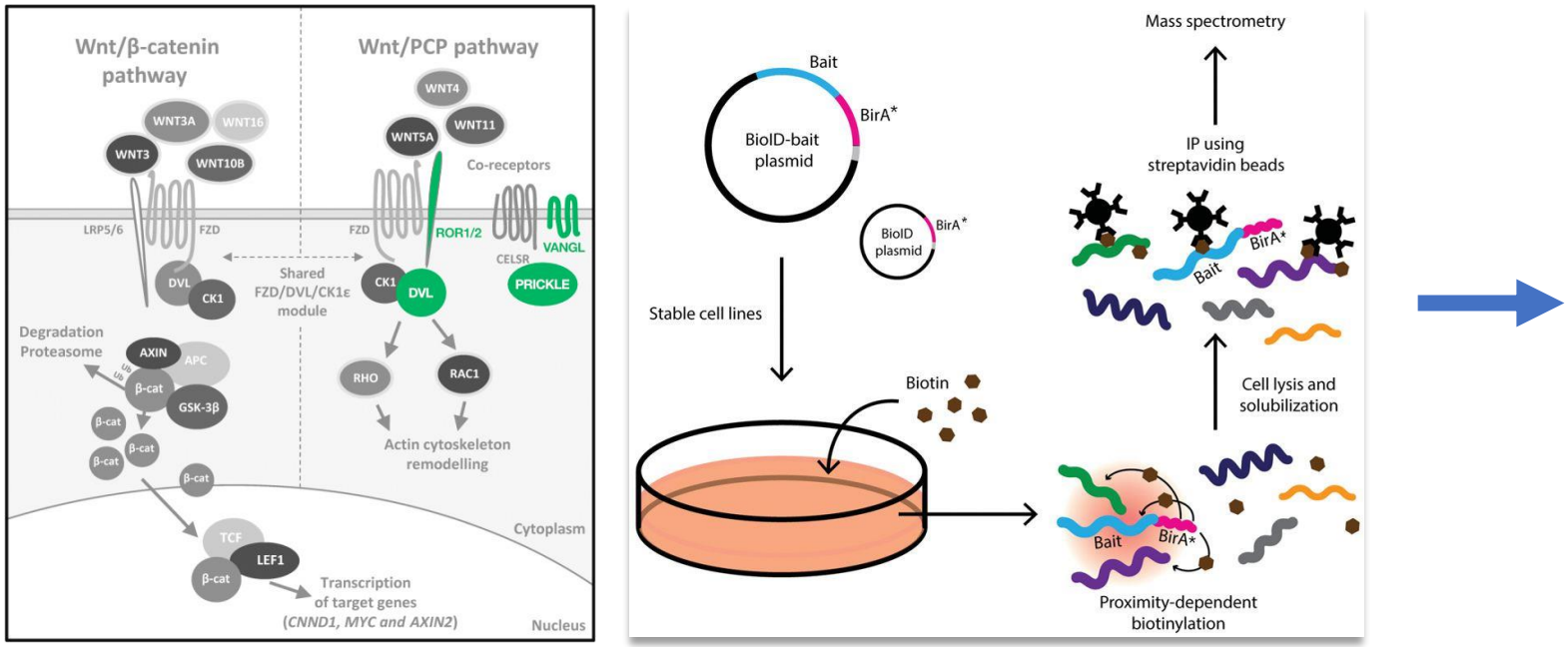


Hmotnostní spektroskopie (Mass Spec)



PCP BioID – finding new potential WNT/PCP proteins

BioID and MS/MS analysis

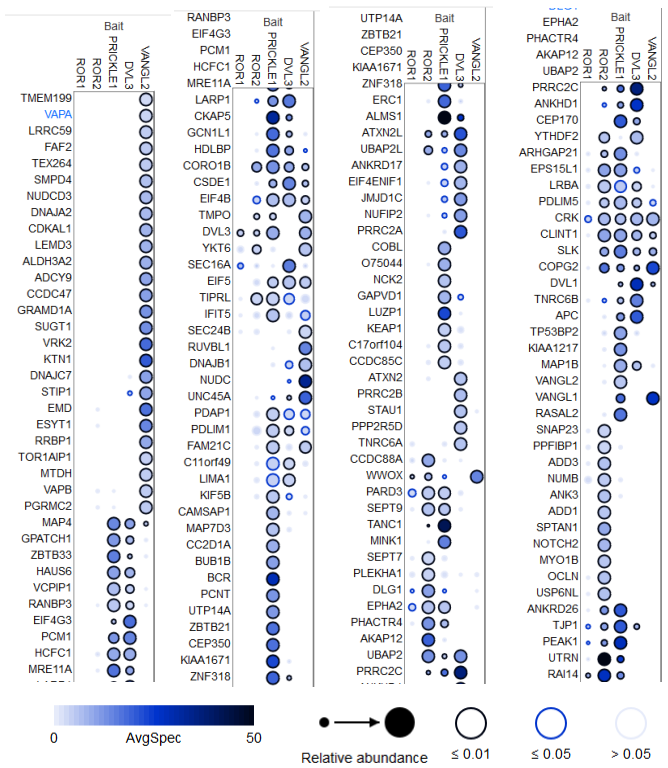


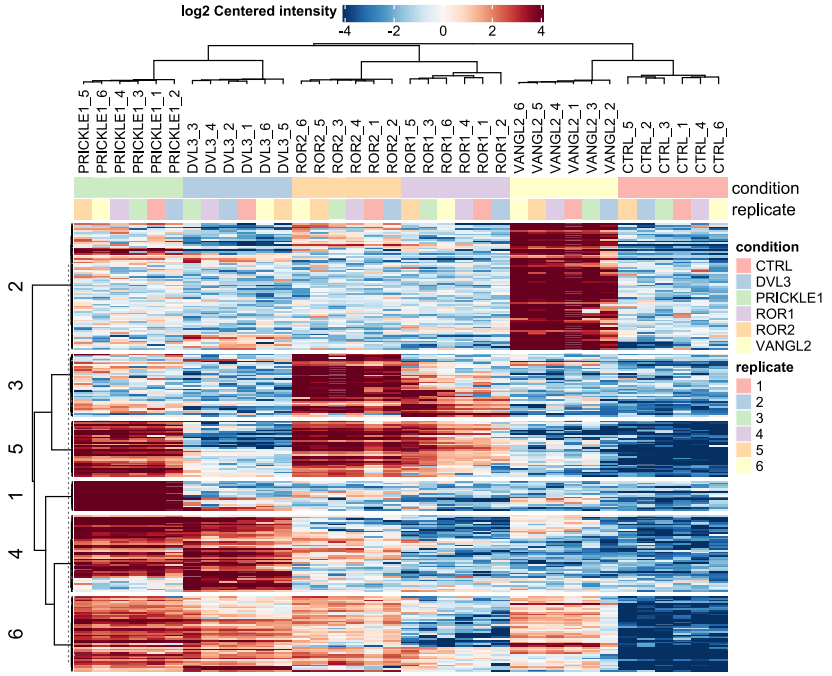
Baits: DVL3, ROR1, ROR2, PRICKLE1, VANGL2

Credit: T. W. Radaszkievicz and P. Paclíková

PCP BioID – finding new potential WNT/PCP proteins

Bioinformatics analysis





Testing in zebrafish

CLUSTER 5
PRICKLE1, ROR2, ROR1

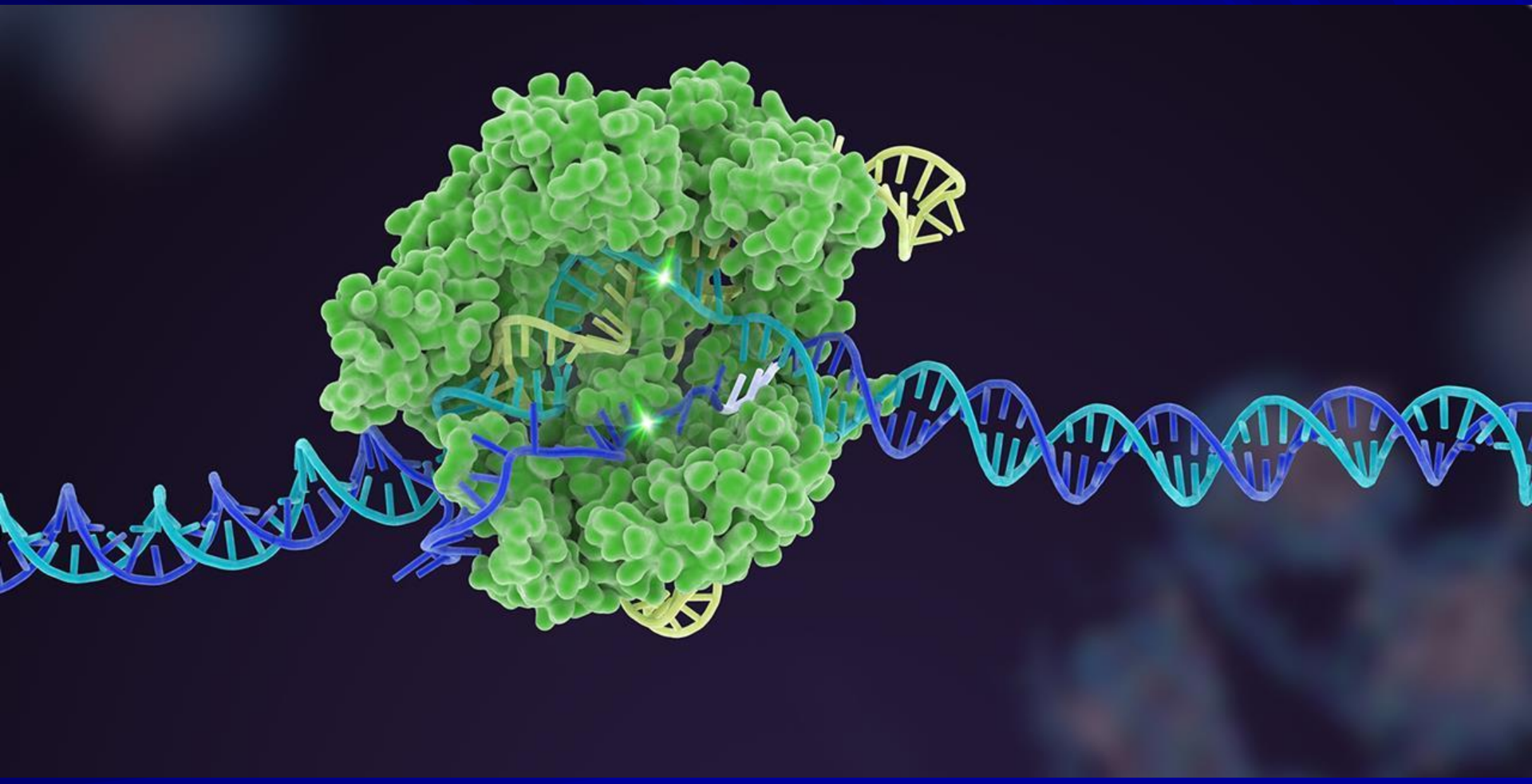
RASAL2	PTPN13
MAP4K4	TJP1
ERBB2IP	DLG1
PARD3	DLG5
PEAK1	EPB41L3
UTRN	EPB41L2
RAI14	CCDC88A
MARK2	EPB41
PHACTR4	AHNAK2
AKAP12	SEPT9
EPHA2	KIAA1217
SCRIB	ARHGAP21
AHNAK	RAPH1
	DST

Credit: K. Gömöryová

Metoda: Crispr/Cas9

2014: Crispr/Cas9-mediated gene editing

METHOD OF THE YEAR

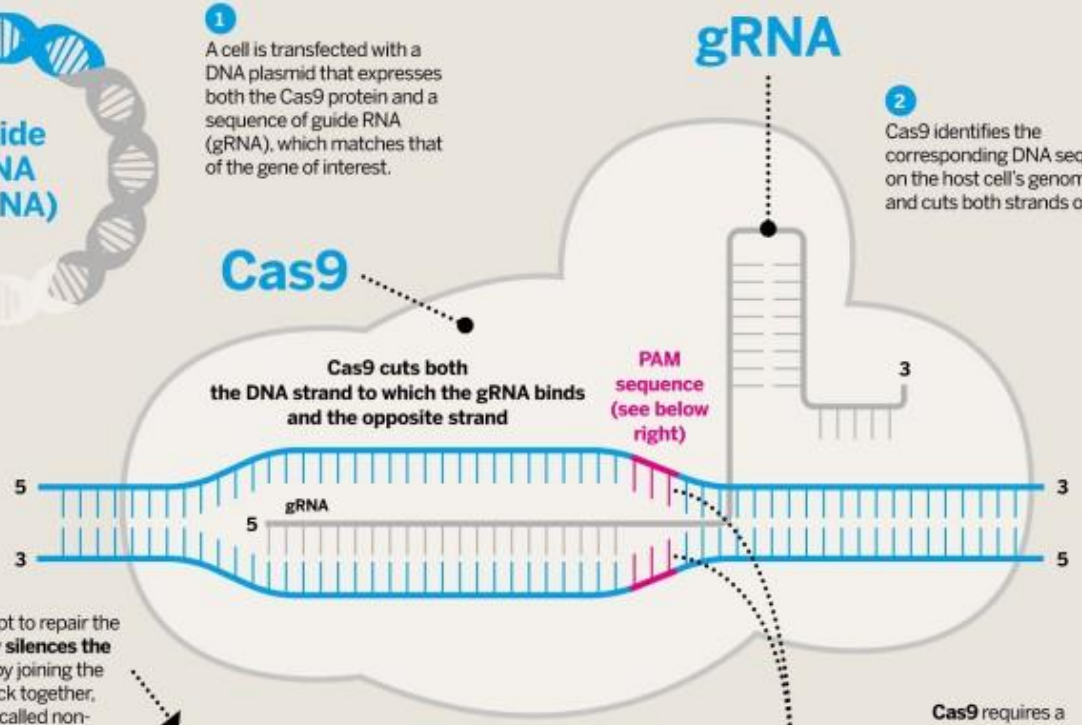


CRISPR-Cas9

How the genome editor works

1 A cell is transfected with a DNA plasmid that expresses both the Cas9 protein and a sequence of guide RNA (gRNA), which matches that of the gene of interest.

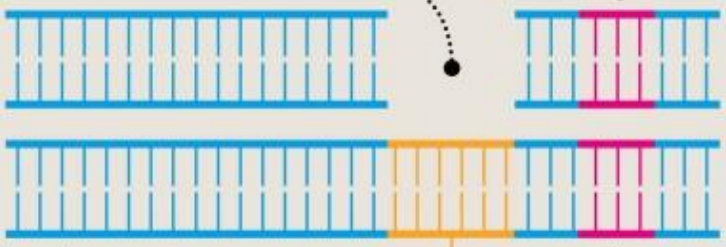
2 Cas9 identifies the corresponding DNA sequence on the host cell's genome, and cuts both strands of DNA.



3a The cell's attempt to repair the break effectively **silences the targeted gene** by joining the cleaved DNA back together, using a process called non-homologous end joining (NHEJ).

OR

3b A **faulty gene can be 'corrected'** with a replacement segment of DNA, or **a new gene altogether can be introduced**. If a modified piece of DNA whose flanking regions match the target sequence is also supplied, then there is a good chance that it will recombine with the host DNA when the cut is made, thus introducing a new or replacement gene. This pathway is known as homology directed repair (HDR).



Cas9 requires a simple and common sequence of base pairs called a **PAM sequence** to actually bind to target DNA. This feature means bacteria can prevent Cas9 from chopping up important 'memorised' sequences of foreign DNA in their own genome – by ensuring there are no PAM sequences in those regions.

What next?

Testing in zebrafish – CRISPR/CAS9

List of genes

CLUSTER 5
PRICKLE1, ROR2, ROR1

RASAL2	PTPN13
MAP4K4	TJP1
ERBB2IP	DLG1
PARD3	DLG5
PEAK1	EPB41L3
UTRN	EPB41L2
RAI14	CCDC88A
MARK2	EPB41
PHACTR4	AHNAK2
AKAP12	SEPT9
EPHA2	KIAA1217
SCRIB	ARHGAP21
AHNAK	RAPH1
	DST

zebrafish orthologous

- Epha2a
- Epha2b
- Map4k4a
- Map4k4a
- Erbin
- Akab12a
- Akab12b
- Phactr4a
- Phactr4b
- Pard3aa
- Pard3ab
- Pard3ba
- Pard3bb
- Peak1

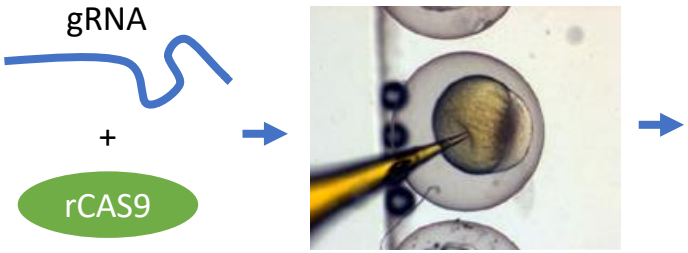
gRNA design

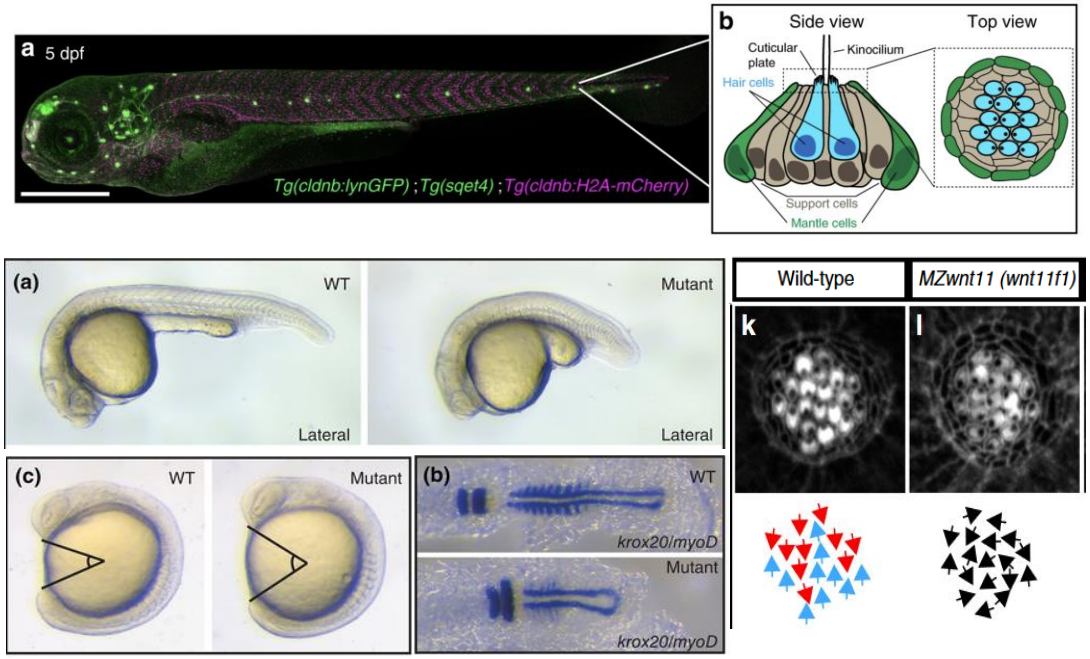
CHOPCHOP

Preparing RNAs

PCR and
in vitro transcription

Testing for WNT/PCP phenotypes



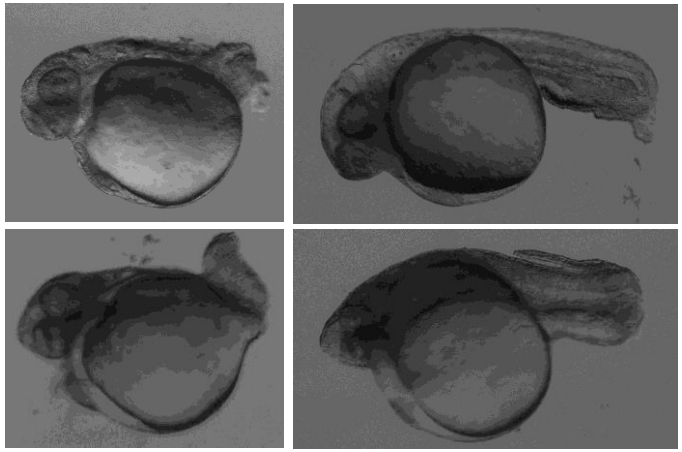


Testing for WNT/PCP phenotypes

Total body lenght

Uninjected control 2dpf

rai14 gRNA injected



LIST OF GENES

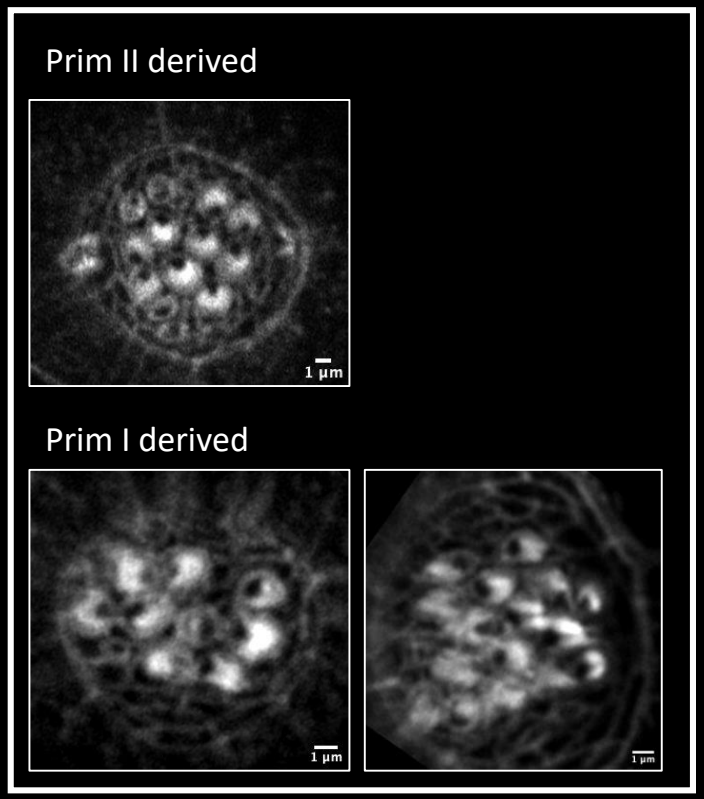
RASAL2	SCRIB	TJP1
MAP4K4	AHNAK	DLG1
ERBB2IP	ALMS1	DLG5
PEAK1	RASAL2	EPB41L3
PARD3	PEAK1	EPB41L2
UTRN	ERBIN	CCDC88A
RAI14	PLEKHA5	EPB41
MARK2	USP6NL	AHNAK2
PHACTR4	GIGYF2	SEPT9
AKAP12	TANC1	KIAA1217
EPHA2	MINK1	ARHGAP21
PTPN13	DST	RAPH1

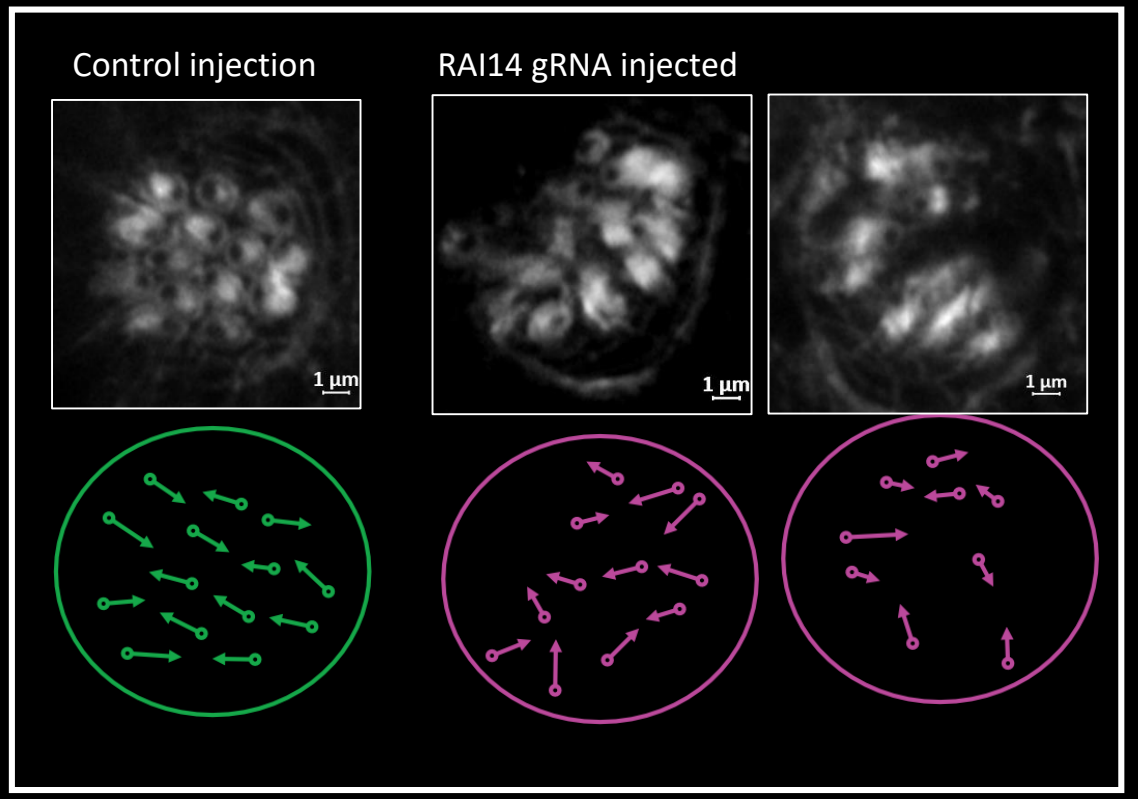
POTENTIAL HITS

TESTED

EXCLUDED

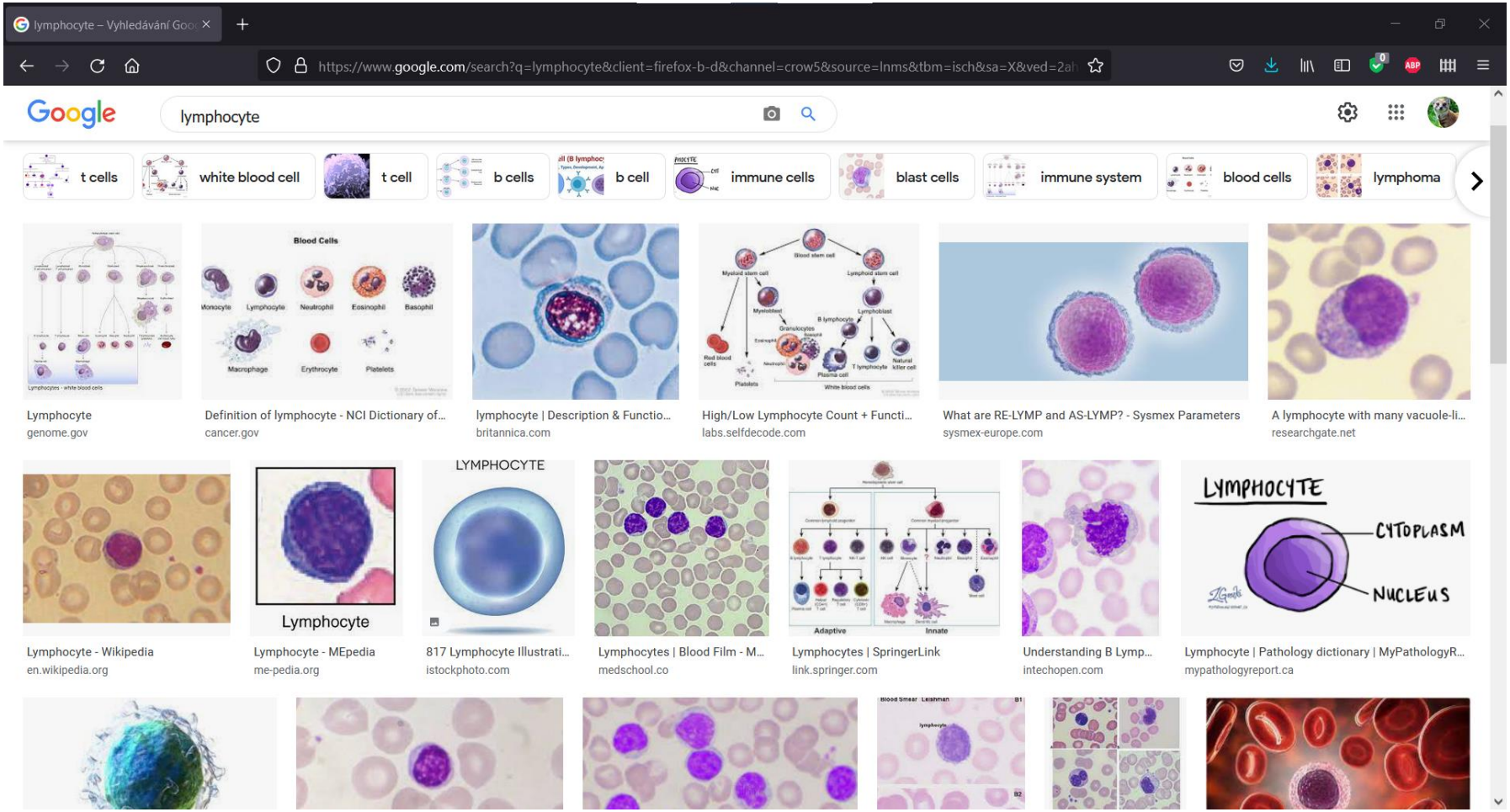
Preliminary data – Kinocilia orientation



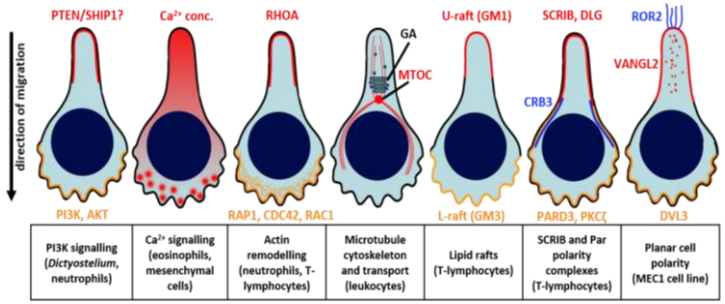


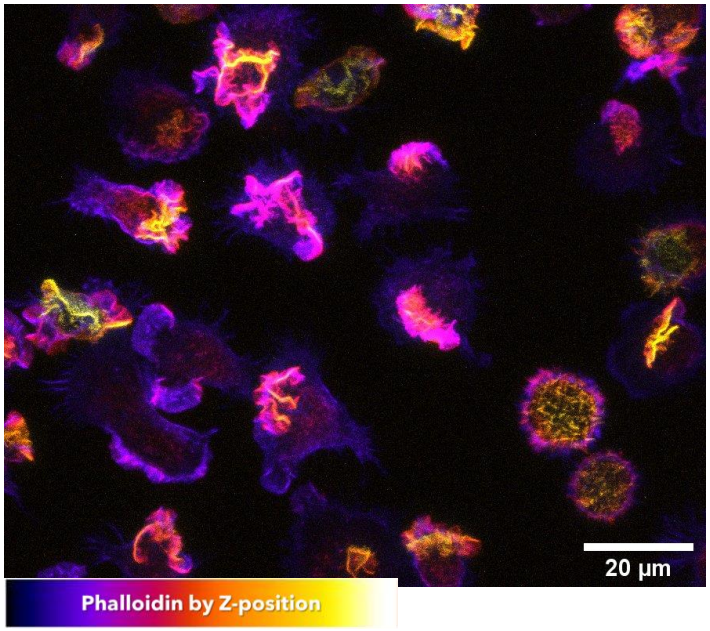
Role nekanonické Wnt dráhy v polarizaci lymfocytů

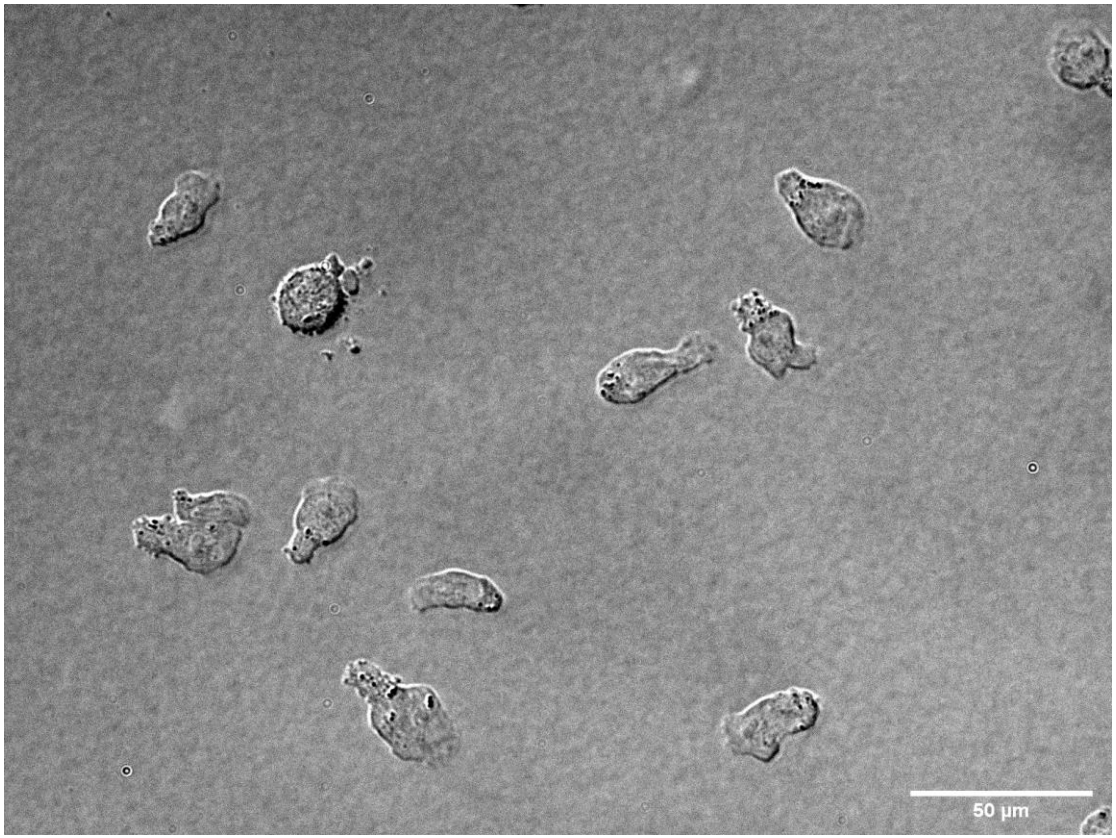
What does a lymphocyte look like?



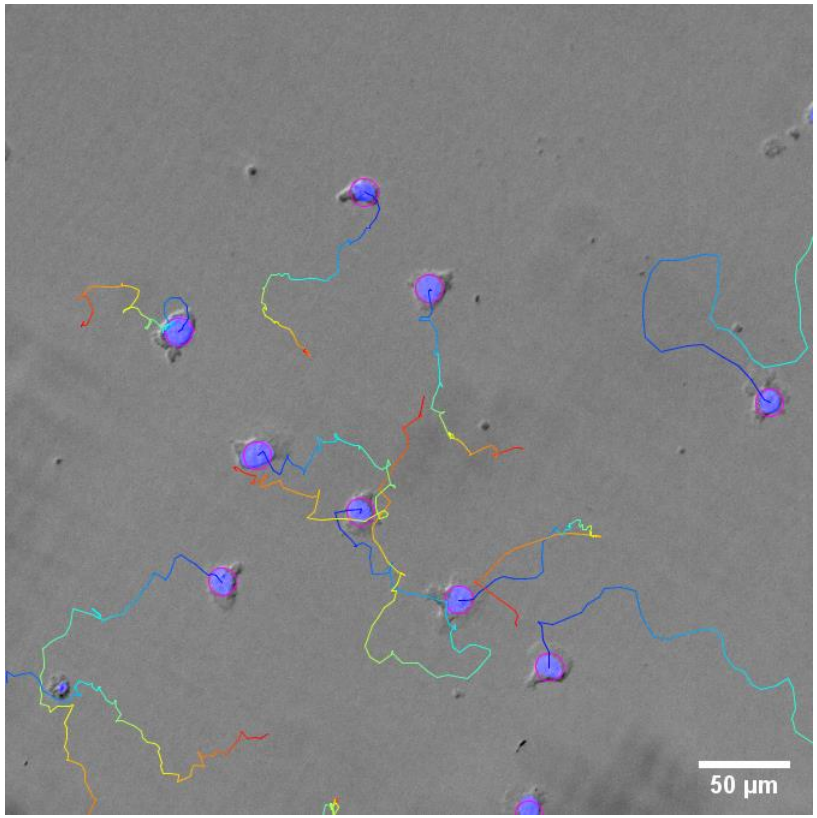
Active lymphocytes are highly polarized cells





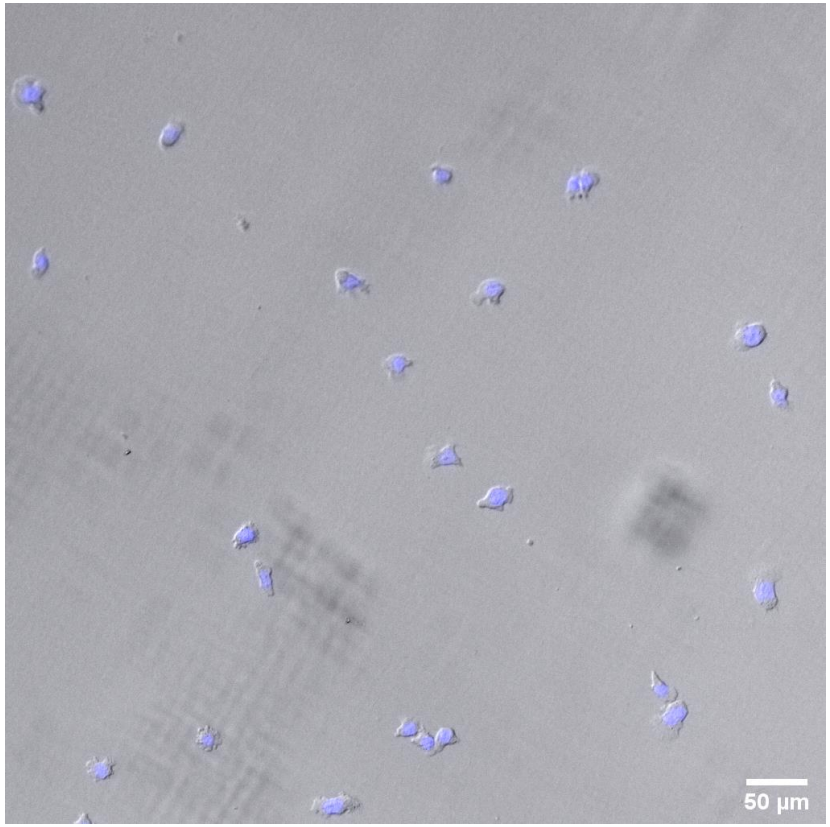


Automated tracking of CLL cell migration using under-agarose assay

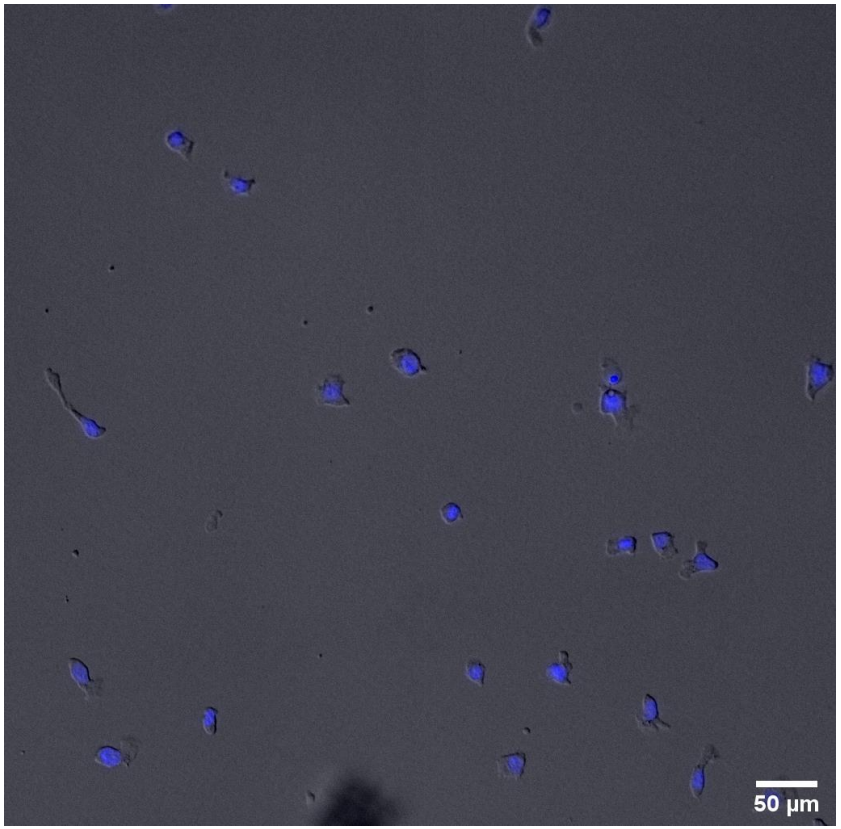


CK1 inhibitor inhibits migratory properties of CLL cell lines differently from ROCK inhibitor

DMSO



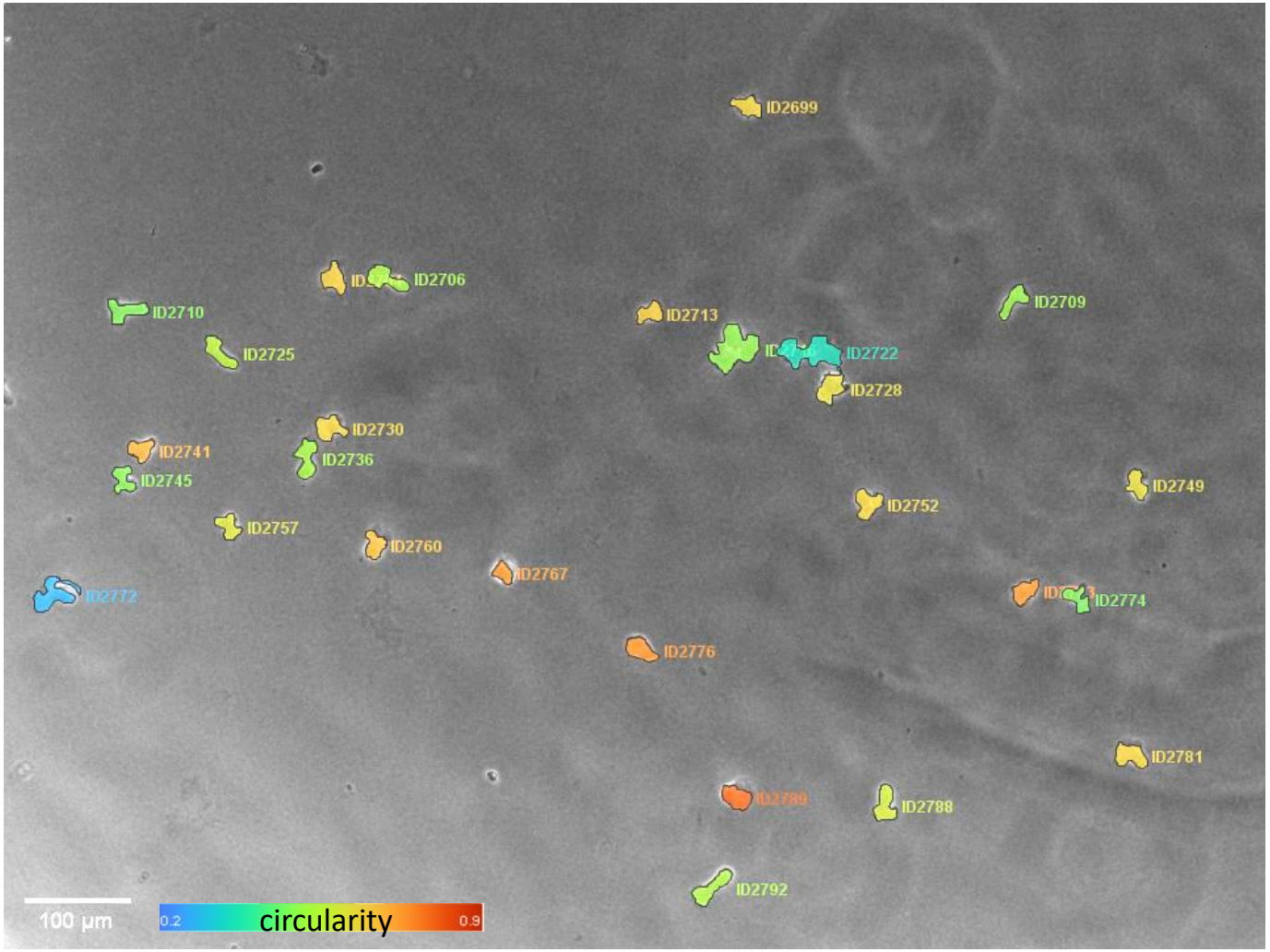
Y27632



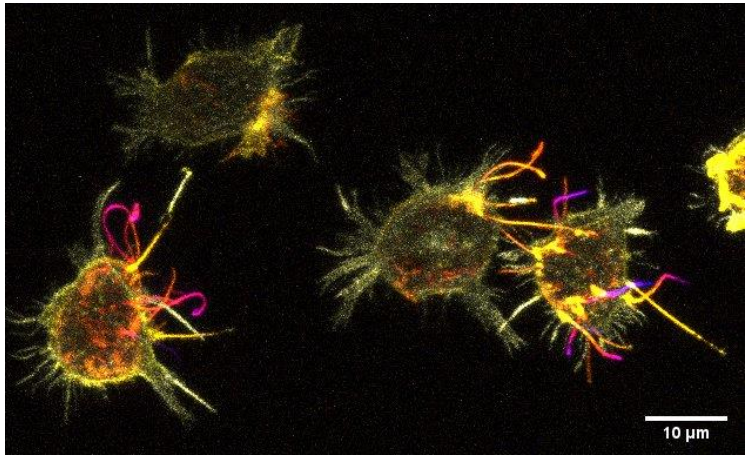
Future aims

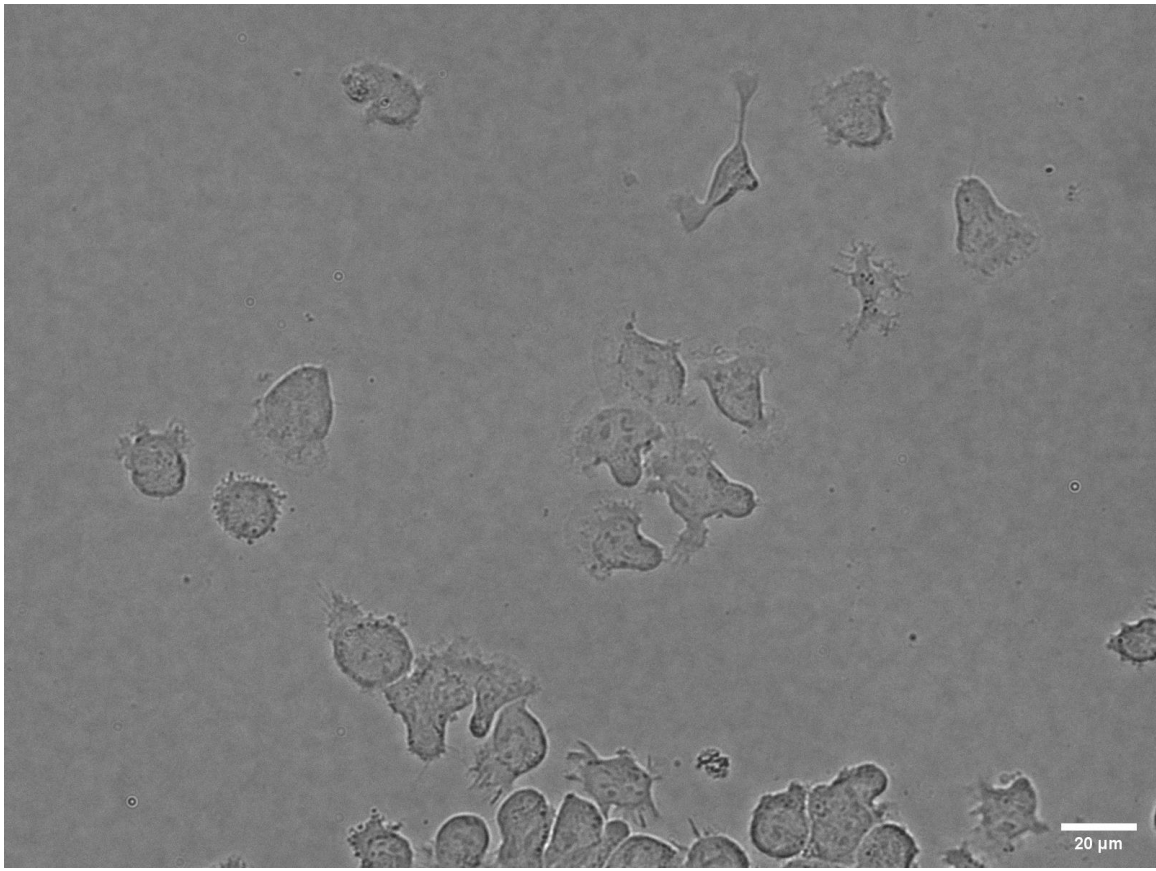
ilastik

trackmate



Future aims





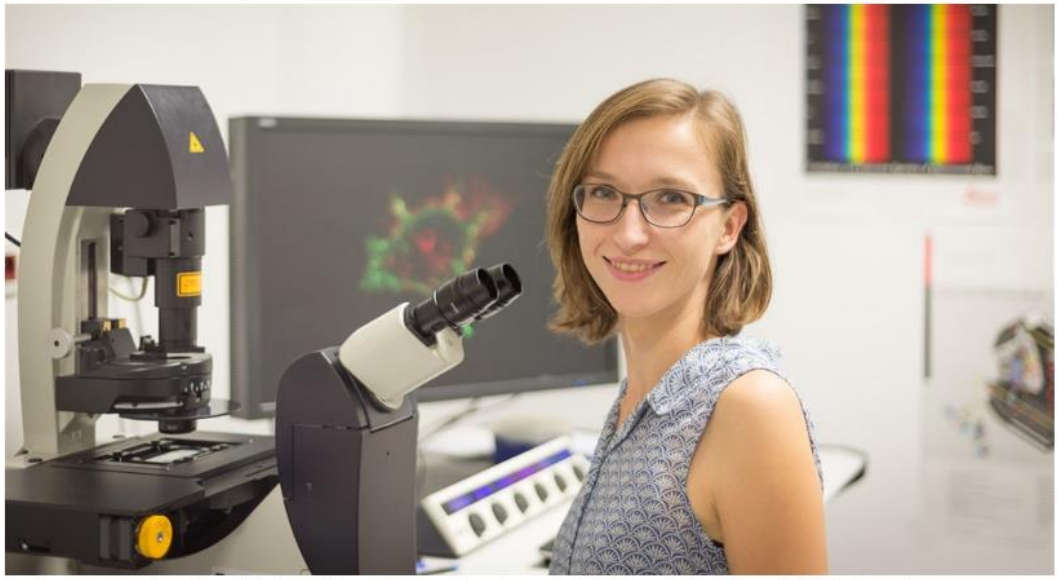
Role nekanonické Wnt dráhy v rozvoji chronické lymfocytární leukémie

Dr. Pavlína Janovská

Snažíme se zamezit šíření nádorových buněk v těle, říká oceněná bioložka

7. července 2018 18:38

Mladé bioložce z Fulneku Pavlíně Janovské nedávno poblahopřál nositel Nobelovy ceny za chemii Francouz Jean-Marie Lehn. A to při příležitosti předávání prestižní Ceny Sanofi za farmacii, kterou Janovská letos získala. Talentovaná vědkyně se ocenění dočkala za výzkum možností usnadnění léčby rakoviny.



Pavlína Janovská dostala prestižní ocenění za výzkum možností usnadnění léčby rakoviny. | foto: Archiv P. Janovské

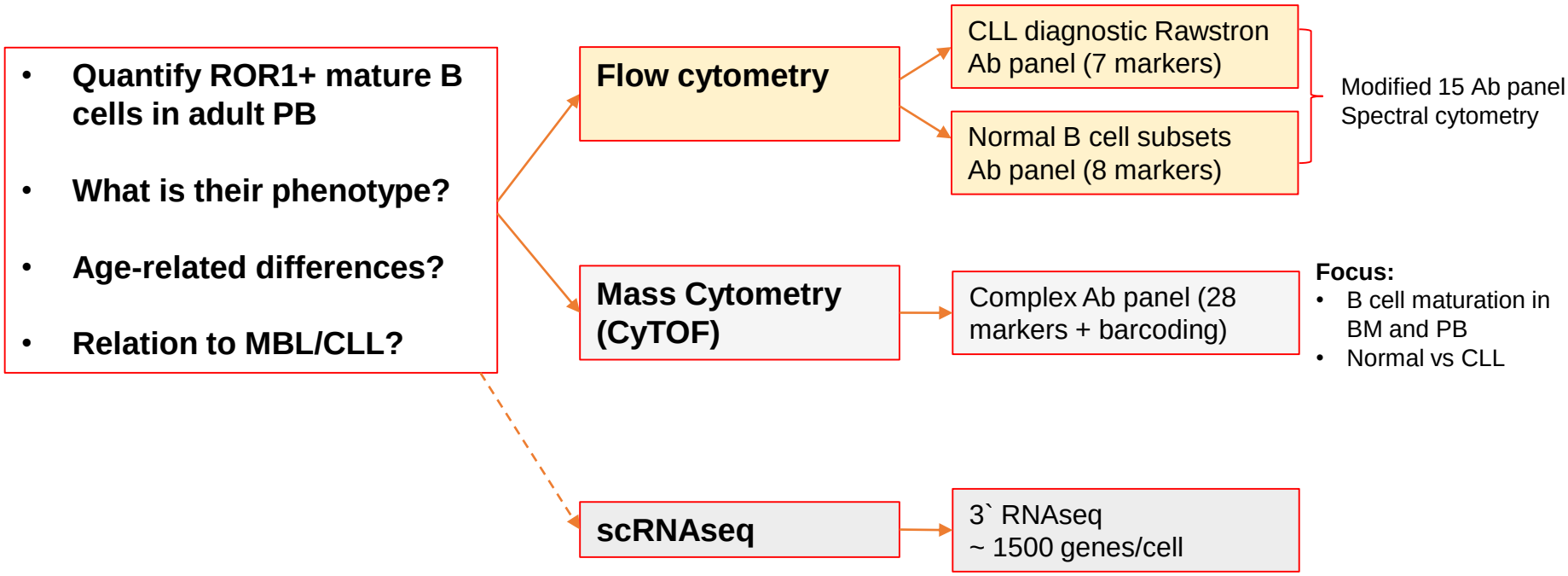
Our hypothesis:

- Rare ROR1+ B cell subsets in adults represent the origin of CLL

Our goals (ongoing project):

- To describe **phenotype** of ROR1+ B cells in adult PB
- Describe relation with **increasing age**
- **Model the relation of these cells to MBL and CLL cells**
- Validate the observations by other methods
- Describe functional characteristics of ROR1+ B cells (anergy? self-reactive? stereotypic BCRs? cycling capacity? acquisition of typical driver mutations?)
- Validate the findings on larger cohort, with focus on early MBL cases

Methodological approach:

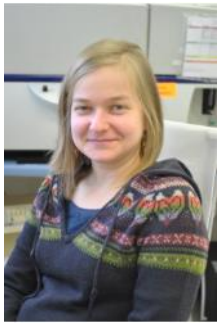


Samples:

PB B cells	• Young healthy donors (<40) • Older donors (>60) • MBL patients • CLL patients
Bone marrow (BM) B cells	• Young donors (<40) (child, no B cell malignancy) • Older donors (>60) (MDS, no B cell malignancy)

List of markers

Mgr. Jitka Stančíková, Ph.D.



SURFACE

marker	B cell maturation	CLL vs normal
CD38	yes	yes
CD19	yes	
IgM	yes	yes
CD81	yes	yes
IgD	yes	
CD20	yes	yes
CD34	yes	
CD5	yes	yes
Anti- Human IgG lambda	yes	yes
CD21	yes	
CD45RA	yes	
CD10	yes	
pStat3[Y705]		yes
aROR-1 PE	yes	yes
Anti- Human IgG kappa	yes	yes
CD9		yes
CD95	yes	yes
CD27	yes	yes
CD24	yes	yes
PE	Ror1 detection	
Biotin	depletion of non-B cells	
T-bet	yes	
HLA-DR	yes	
CD184	yes	yes

INTRA

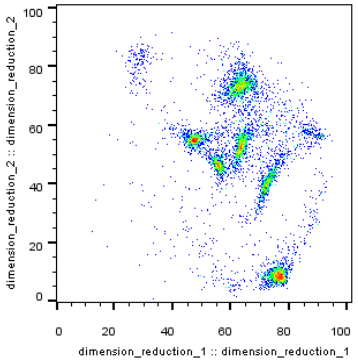
marker	B cell maturation	CLL vs normal
i_cPARP	apoptosis	
i_BCL-2	yes	yes
CD79B	yes	yes
i_Ki-67	proliferation	
i_IgM	yes	yes

Data analysis – Mgr. Jan Stuchly, Ph.D.

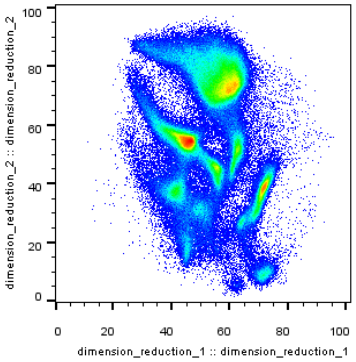
Dimension reduction >>

Mgr. Jan Stuchly, Ph.D.

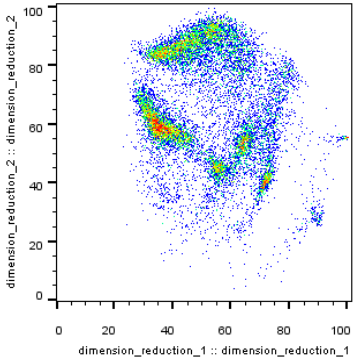
BM CD19+



PB B CD19+ unsorted



ROR1+ PB B cells

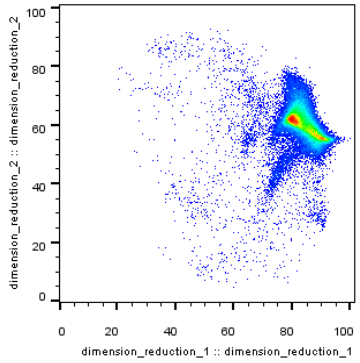


Differences ROR1+/-?

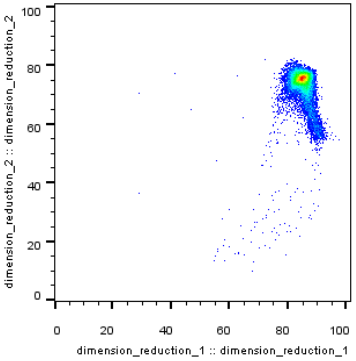
Phenotype?

Subsets?

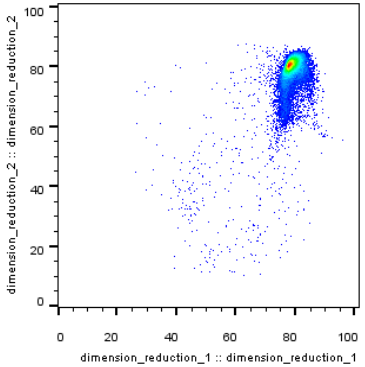
MBL PB B cells



CLL 1



CLL 2

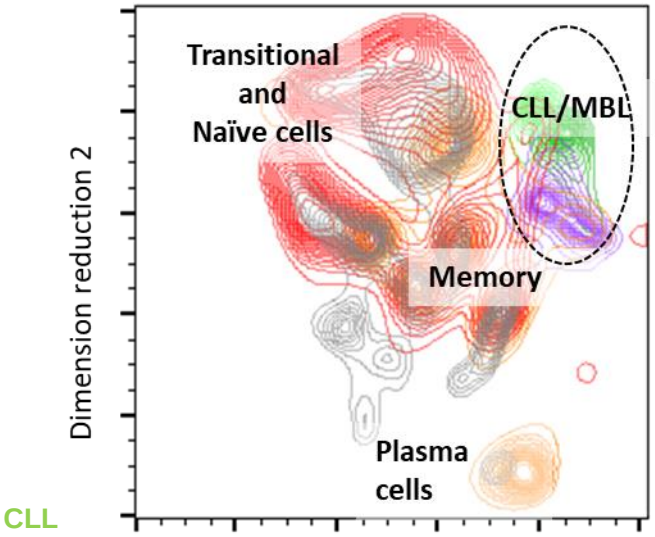


MBL clone + remaining B subsets

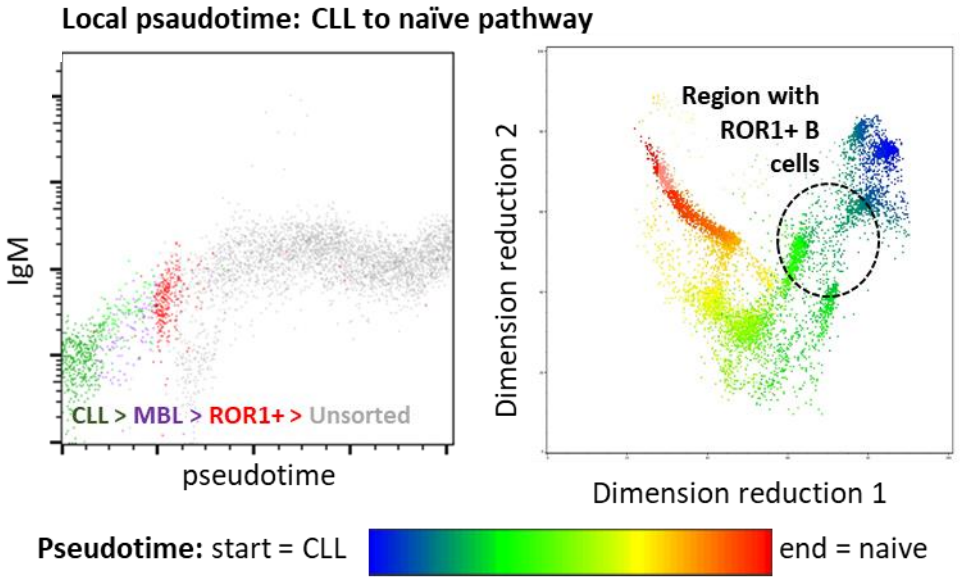
Are they different…?! – Not analyzed yet

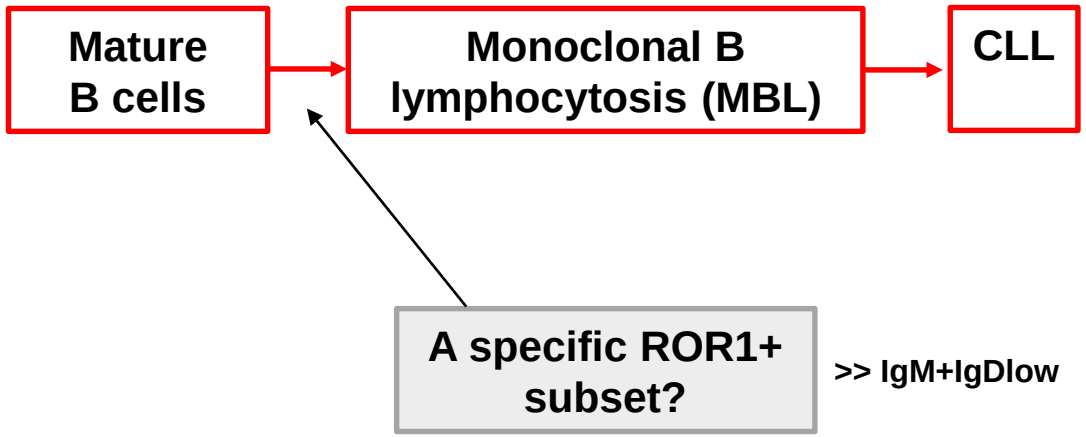
Plan for scRNAseq

Modeling of most probable developmental trajectories



CLL
MBL
ROR1+ B cells
Unsorted B cells
BM B cells





PHD WORKSHOP 2022

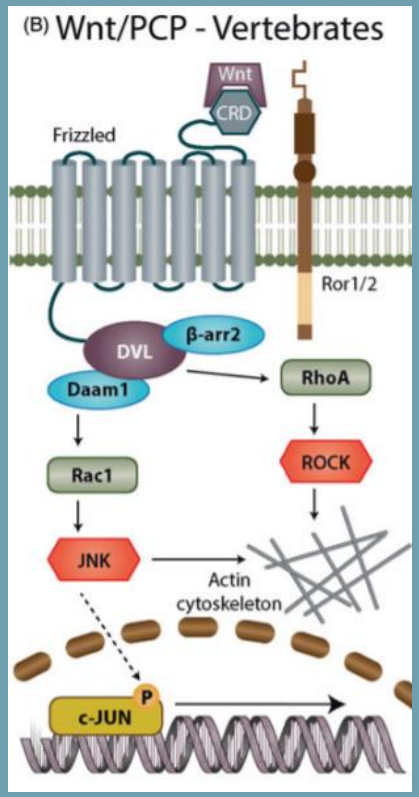
MGR. PETRA KOMPANÍKOVÁ

Wnt5-induced signaling in the tissue morphogenesis

molecular, cellular and evolutionary aspects

SUPERVISOR: PROF. V. BRYJA, PH.D.

Wnt5a



Bryja *et al.*, 2017

Metoda: Genetické modifikace myší

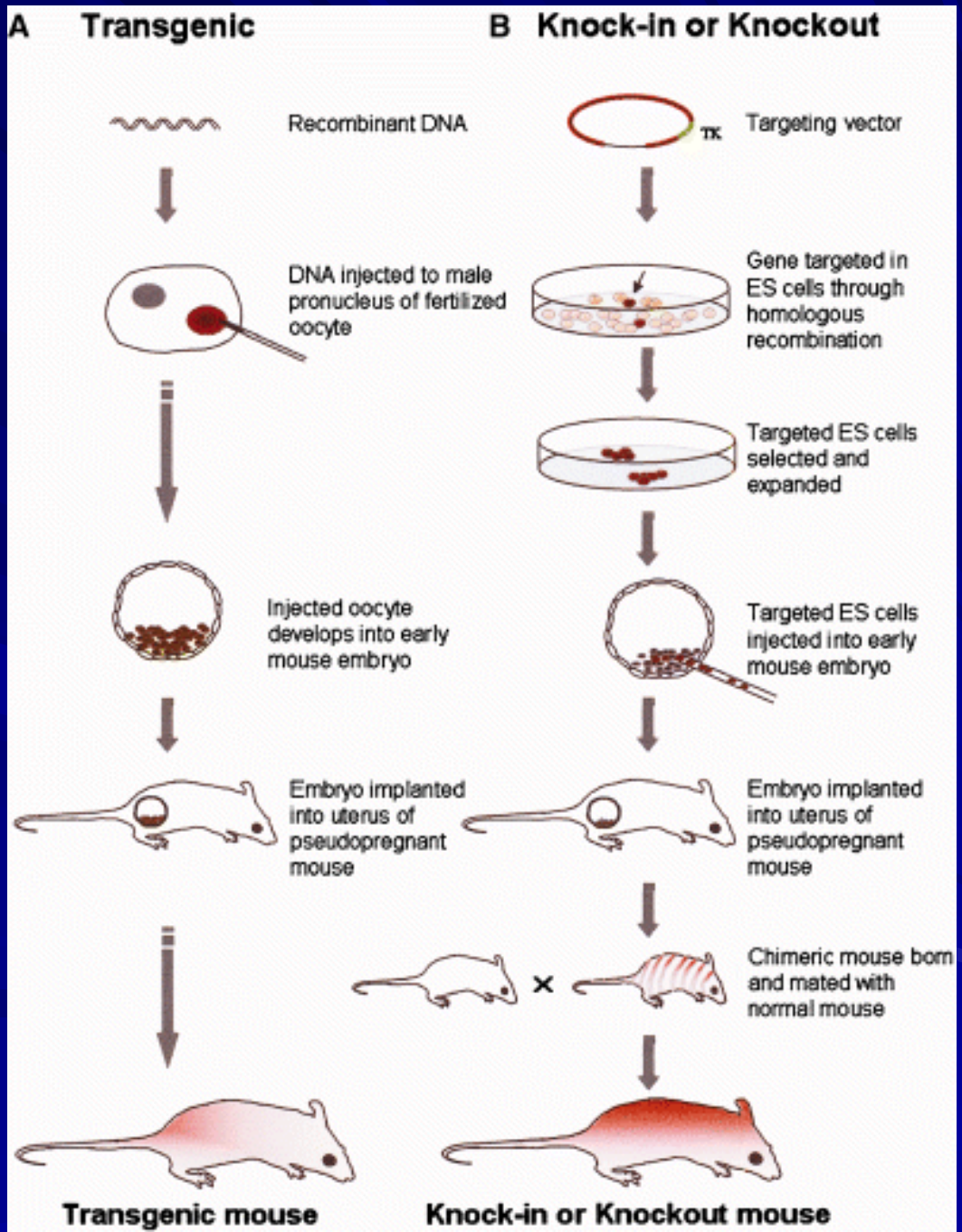
Transgenní myš

Nobelova cena 2007

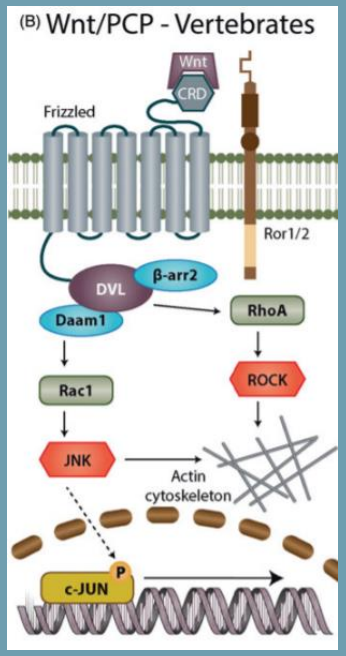
Mario R. Capecchi, **Martin J. Evans** and **Oliver Smithies**

za

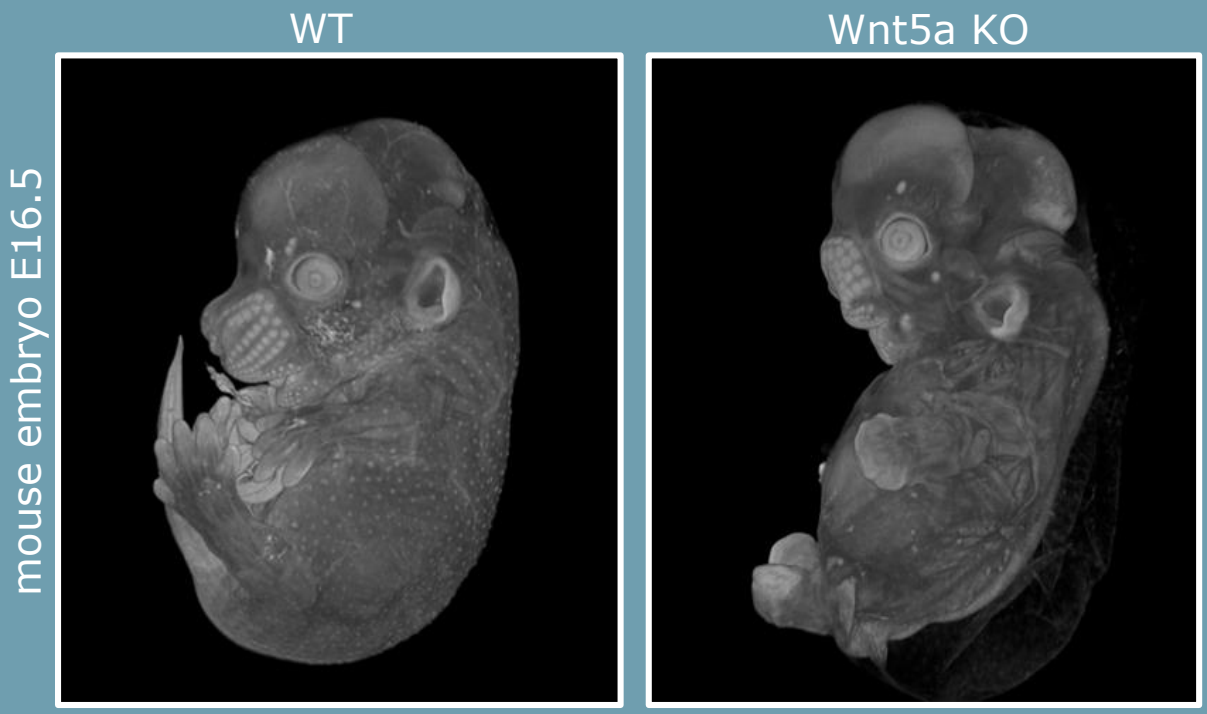
„principles for introducing specific gene modifications in mice by the use of embryonic stem cells“



Wnt5a



Bryja *et al.*, 2017

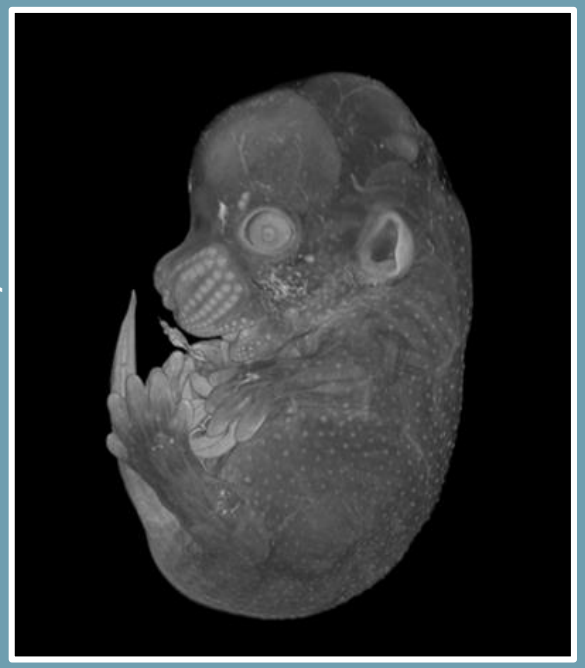


J. Procházka

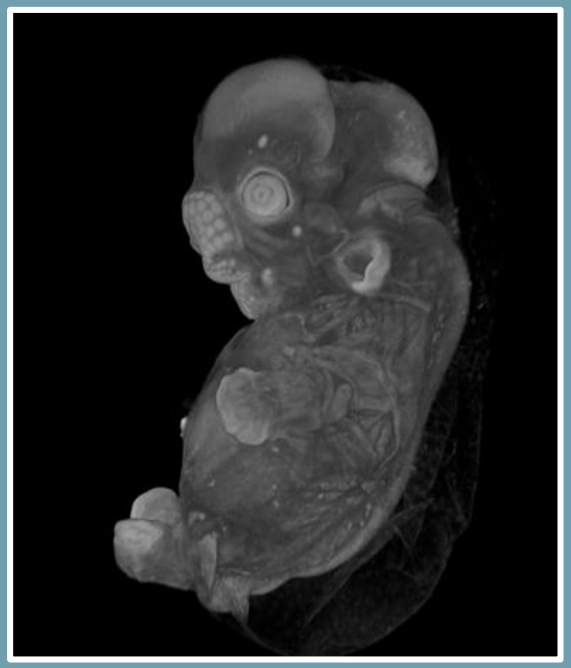
Wnt5a

mouse embryo E16.5

WT

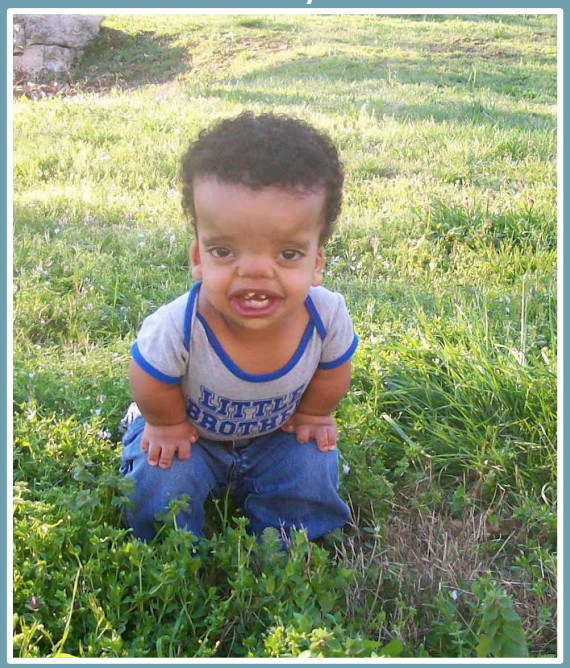


Wnt5a KO



J. Procházka

Robinow syndrome



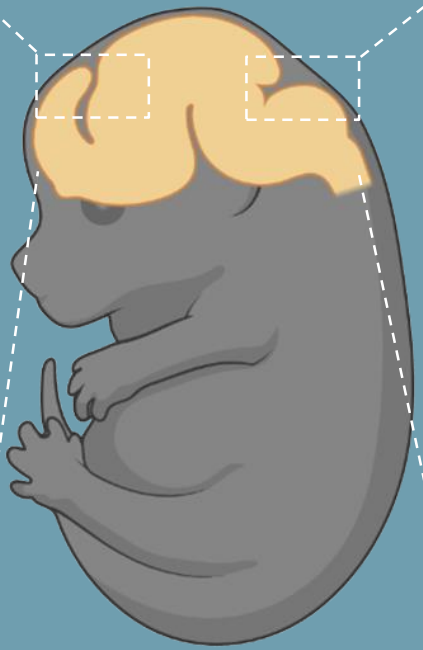
www.robinow.org/

Choroid plexus

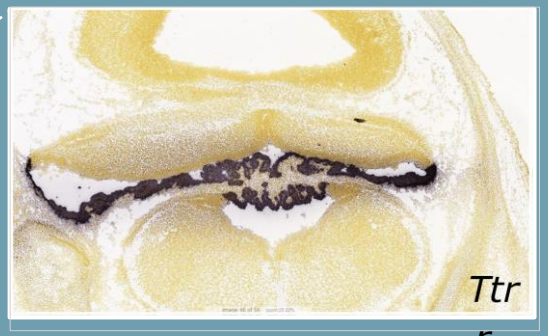
Lateral & 3rd Choroid Plexus

Coronal section

Sagittal section



Hindbrain Choroid

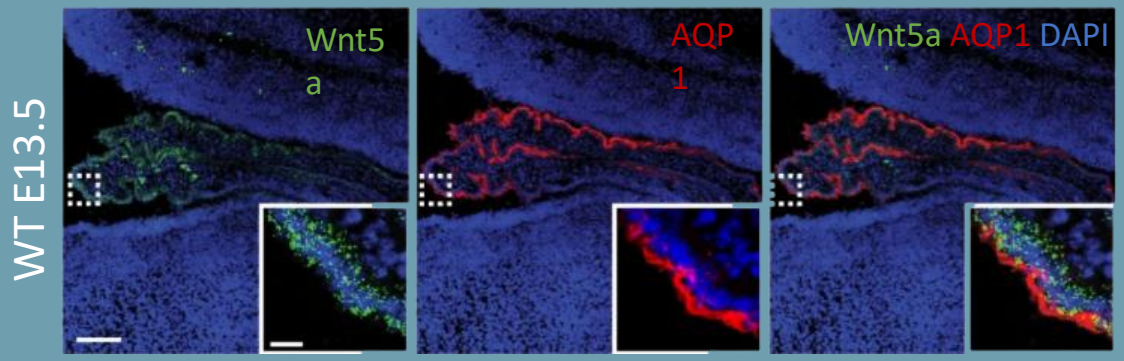


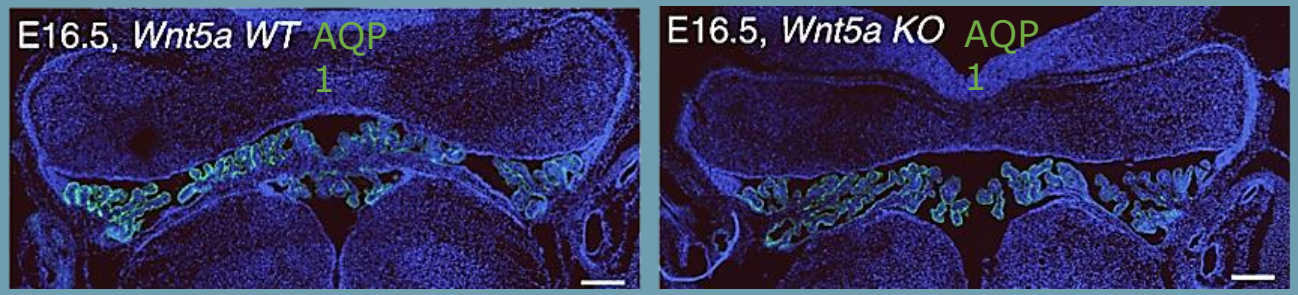
In situ credit*:* Allen brain institute

Ttr – epithelial marker of choroid plexus

E14.5

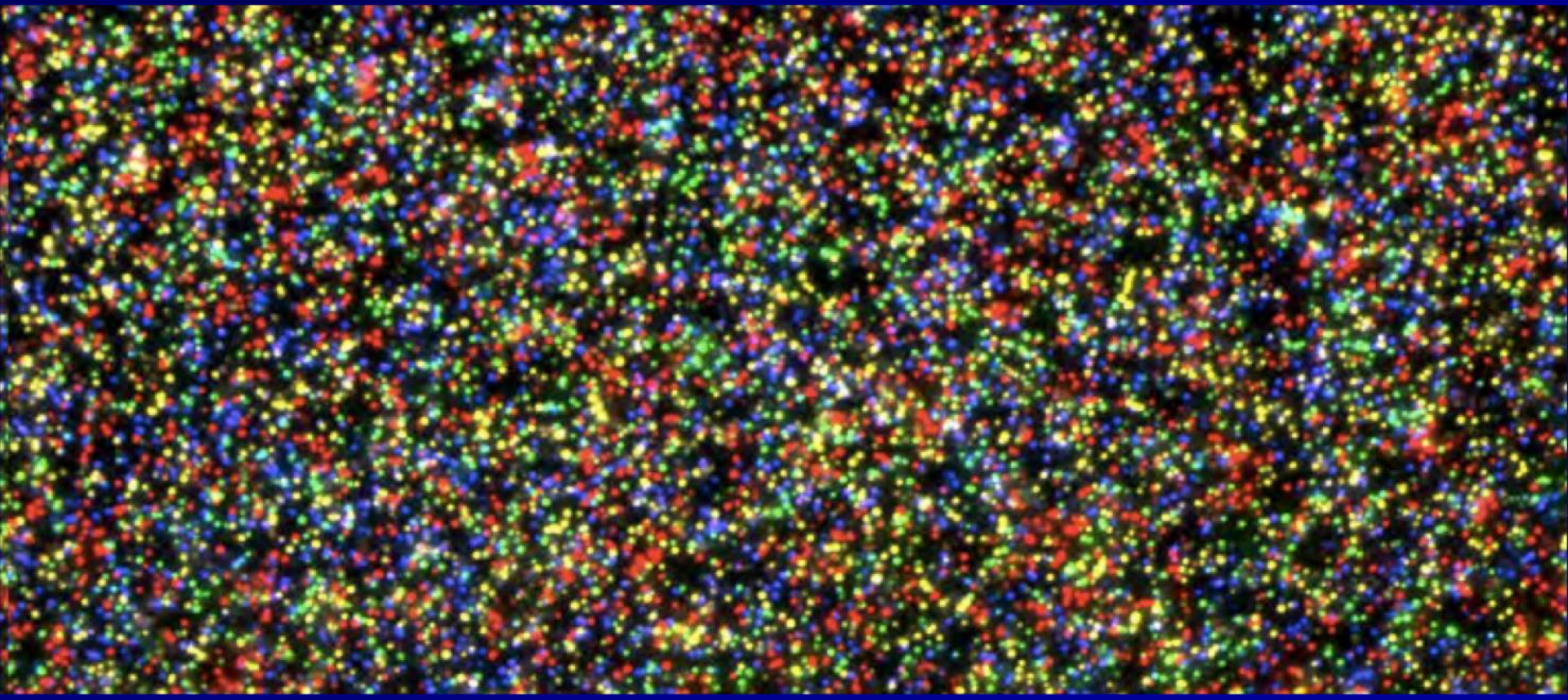
Choroid plexus & Wnt5a



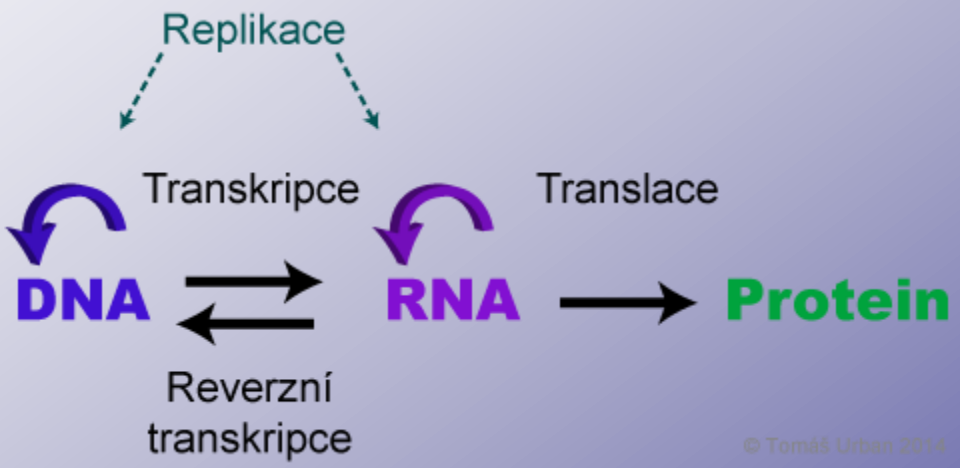


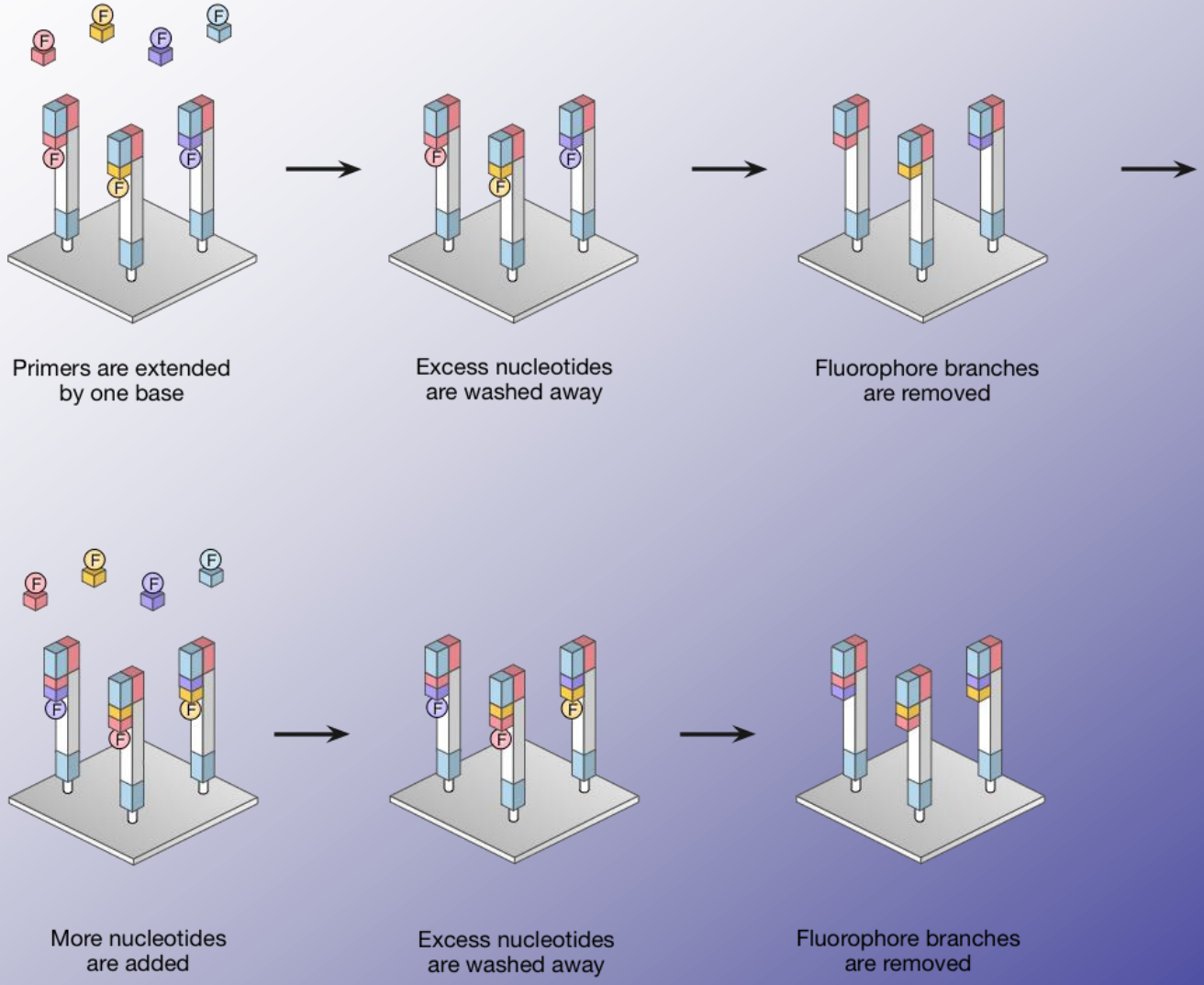
Kaiser *et al.*, 2019

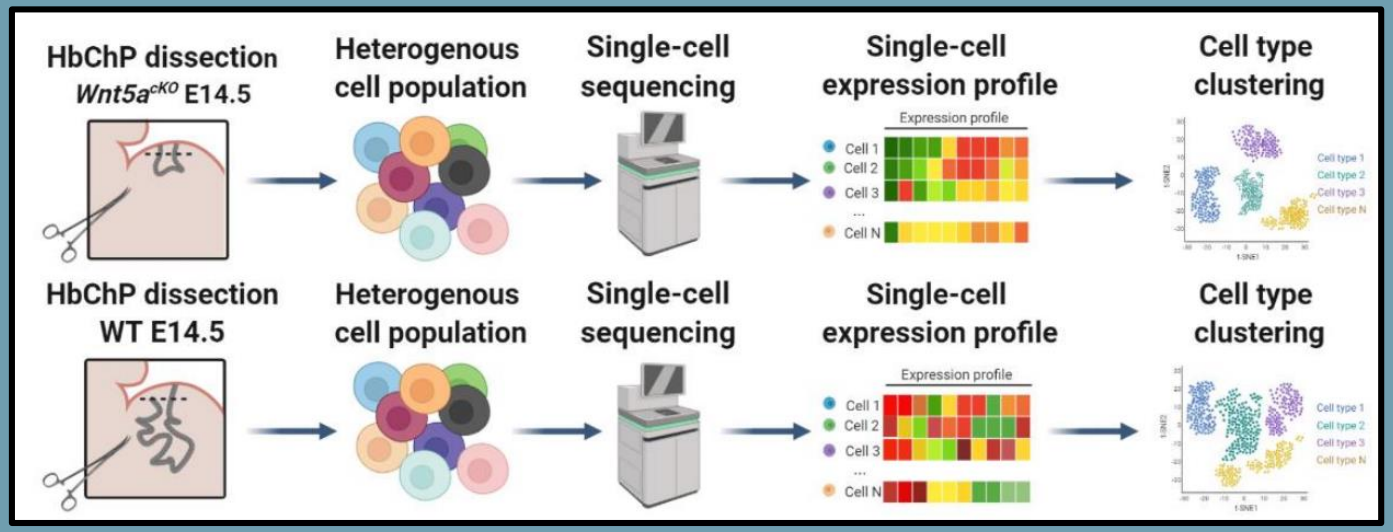
Metoda: RNA sekvenování (RNA Seq)

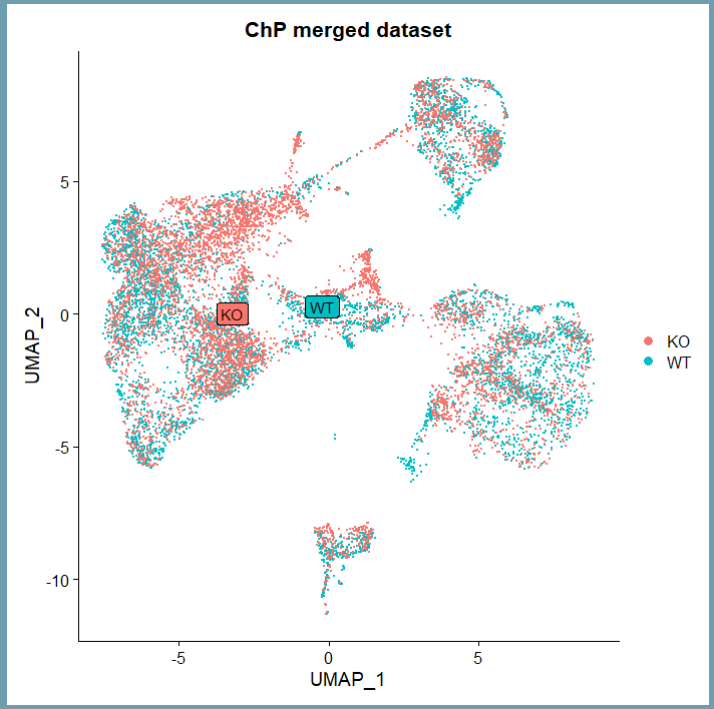


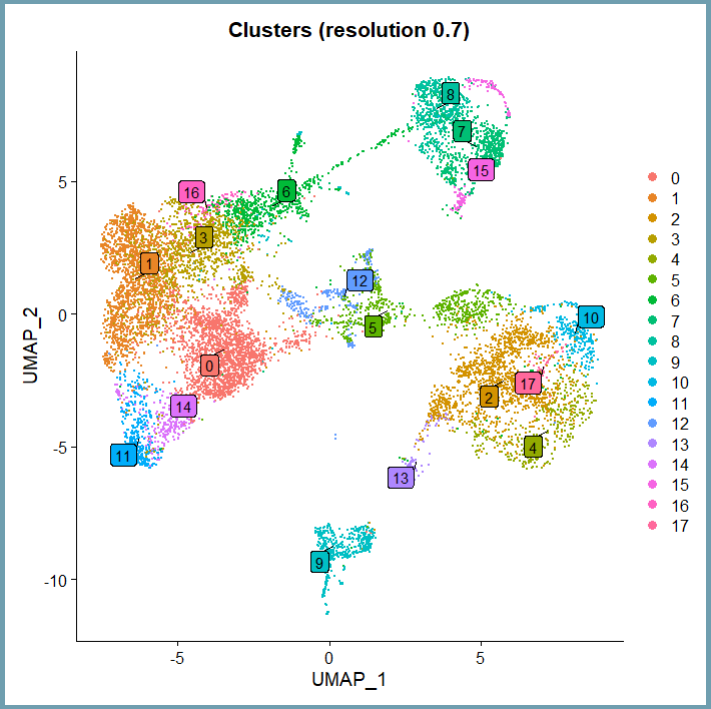
Centrální dogma molekulární biologie



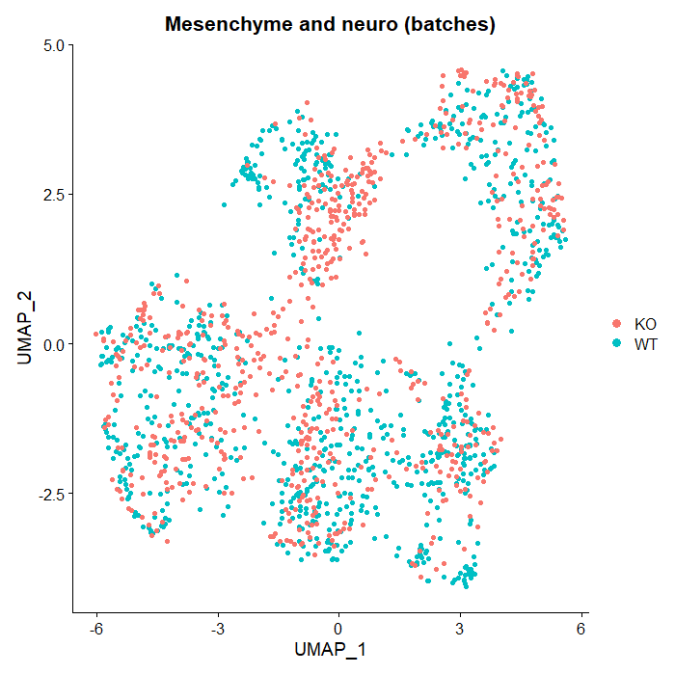

Primers are extended by one base
Excess nucleotides are washed away
Fluorophore branches are removed
More nucleotides are added
Excess nucleotides are washed away
Fluorophore branches are removed
A
C
Fluorophore
G
T

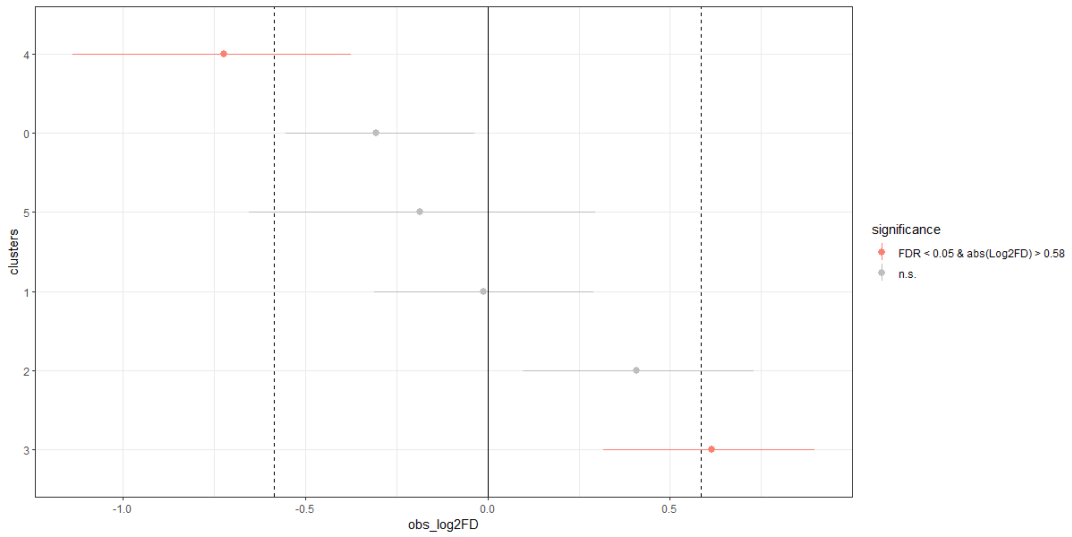

HbChP dissection
Wnt5a^cKO E14.5
Heterogenous cell population
Single-cell sequencing
Single-cell expression profile
Expression profile
Cell 1
Cell 2
Cell 3
...
Cell N
Cell type clustering
Cell type 1
Cell type 2
Cell type 3
Cell type N
HbChP dissection
WT E14.5
Heterogenous cell population
Single-cell sequencing
Single-cell expression profile
Expression profile
Cell 1
Cell 2
Cell 3
...
Cell N
Cell type clustering
Cell type 1
Cell type 2
Cell type 3
Cell type N


ChP merged dataset
UMAP_2
UMAP_1
KO
WT
KO
WT


Clusters (resolution 0.7)
UMAP_2
UMAP_1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

A. Mikulová

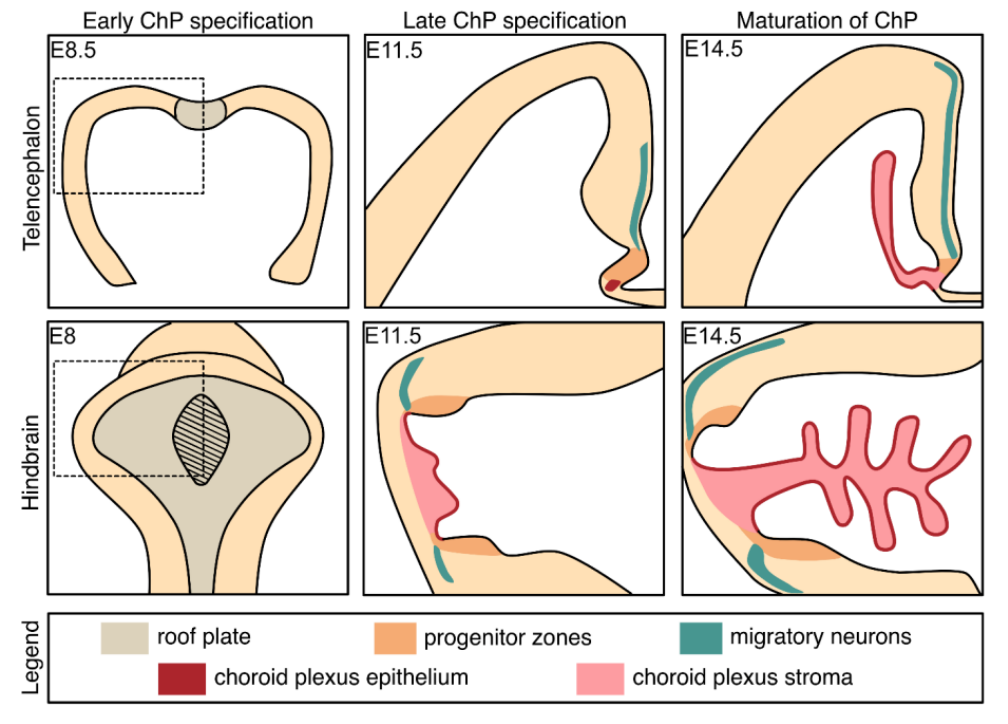




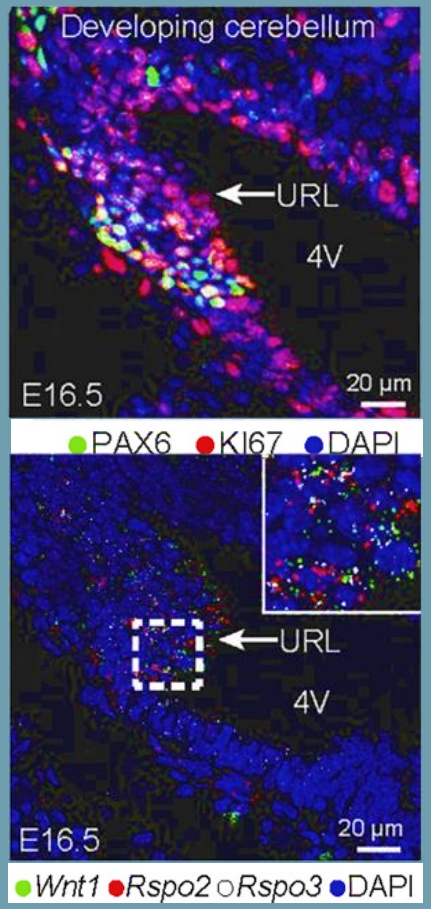
A. Mikulová

Mesenchymal and neuro/glial cells (res. 0.3, 1604 cells)		
Cluster no.	*Celltype*	*Markers*
0	?	Kcnj8, Gucy1a1, Rgs5, Abcc9
1	pericytes	Kcnj8, Gucy1a1, Rgs5, Abcc9
2	4V fibroblasts	Id2 Igfbp7
3	meningeal fibroblasts	Apod Nnat Igfbp2
4	pericytes	Kcnj8, Gucy1a1, Rgs5, Abcc9

Wnt signalling and formation of ChPs

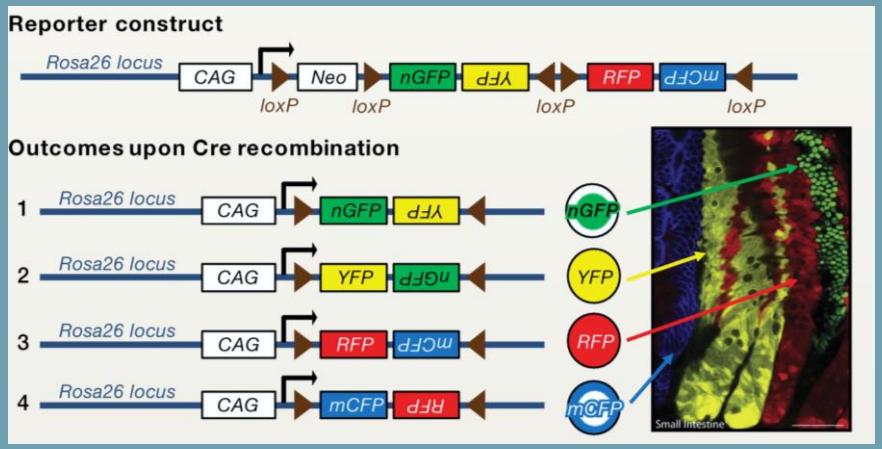


Kompaníková & Bryja, in revision



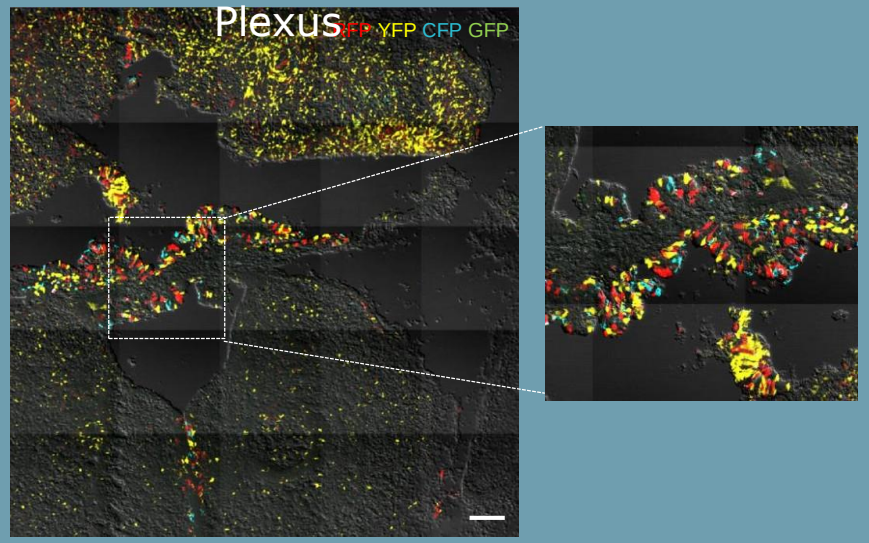
Dani *et al.*, 2021

Wnt1-Cre2/R26R Confetti



Hindbrain Choroid Plexus

E14.5 Wnt1-Cre2/R26R Confetti



Děkuji za pozornost!

Celogenomové techniky

Molekulární mechanismus

Celoproteomové techniky