MUNI
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Annual PhD Conference in Biomedical Sciences

Book of Abstracts

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MASARYK UNIVERSITY CAMPUS, BRNO

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FOREWORD

The Annual PhD Conference in Biomedical Sciences serves as a platform to bring together doctoral students from diverse disciplines within the biomedical sciences. The conference offers an excellent opportunity for participants to present their research to a broad and interdisciplinary audience, fostering scientific communication, constructive feedback, and open dialogue among peers. Each participant contributes actively to the event, promoting a spirit of collaboration and facilitating the exchange of knowledge and experience across research areas.

Since 2021, the conference has encompassed students from all three specializations under the guiding principle “More People, More Science, More Fun.” Building upon the success of previous editions, this format continues to encourage communication and collaboration among various departments and research groups, reinforcing the sense of a cohesive academic community.

The 2025 conference will span two days and feature a diverse scientific program. In total, eleven students will deliver oral presentations as part of their doctoral state examinations, four students will present their progress reports, and thirty-two students will showcase their research through posters across two poster sessions. These sessions are designed to provide a dynamic environment for scientific exchange and networking.

We are confident that this event will provide each participant with valuable feedback and new perspectives on their research projects. On behalf of the Organizing Committee and the Doctoral Board, we extend our best wishes for a productive and intellectually enriching conference experience.

Prof. RNDr. Ondřej Slabý, Ph.D.
Program Director

LIST OF SPEAKERS - DOCTORAL STATE EXAMS

Specialization Biochemistry and Molecular Biology

1. Papatheodorou Ioanna
2. Vepřková Jana
3. Lešková Anna
4. Koždoňová Kateřina

Specialization Molecular Medicine

5. Medaglia Alejandro
6. Hlaváč Kryštof

Specialization Cell and Tissue Morphology

7. Emamiaref Parisa
8. Molina Gambin Francisco
9. Portakal Türkan
10. Kročka Erik
11. Rábová Anna

LIST OF POSTERS

Poster session A (1st to 3rd year students)

1. Aldabash Bacel
2. Aniyan Bhavana Neeraj Bhavani
3. Grutnová Tereza
4. Kaštánková Nikola
5. Kostohryz Viktor
6. Koutná Jana
7. Kytková Lucia
8. Liang Shimin
9. Orviská Petra
10. Satková Miriam
11. Stylianou Filio

12. Suryanarayanan Gokula Narayanan
13. Torkashvand Marzie
14. Trybala Zuzanna
15. Vaňatková Kateřina
16. Zahornacká Saša

Poster session B (4th to 8h year students)

17. Beckerová Deborah
18. Belisová Denisa
19. Brezak Matea
20. Cesnaríková Soňa
21. Čimborová Katarína
22. Dostálová Lenka
23. Dudeja Pooja
24. Feiser Felicity
25. Holomková Kateřina
26. Chochola Václav
27. Jongen Vincent
28. Krishna Shwetha
29. Poovakulathu Abraham Sara
30. Smolková Karolína
31. Ursarchi Vlad-Constantin
32. Vadovičová Natália

1. IL-18 defines recovery and inflammation resolution in orthopedic surgery and sepsis

Ioanna Papatheodorou[1,2], Gabriela Blažková[1], Veronika Bosáková[1], Zuzana Tomášiková[1,3], Erin Spearing[1], Robin Klieber[4], Pavel Ostašov[4], Julie Štíchová[5], Martina Dvončová[6], Alexandra Mýtniková[1,6], Jan Emmer[7], Kamila Bendíčková[1], Tomáš Tomáš[7], Vladimír Šrámek[6], Petros Kolovos[8], Monika Holubová[4], Martin Helán[1,6], Marcela Vlková[5], Jan Frič[1,9,10*], Marcela Hortová-Kohoutková[1,10*]

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5 Institute of Clinical Immunology and Allergology, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Brno, Czech Republic.
6 Department of Anesthesiology and Intensive Care, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Brno, Czech Republic.
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8 Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece.
9 Institute of Hematology and Blood Transfusion, Prague, Czech Republic.
10 International Clinical Research Center, Faculty of Medicine, Masaryk University, Brno, Czech Republic.

Surgery as well as infection activate innate immunity and can progress to systemic inflammatory response syndrome (SIRS) or sepsis. Timely immune resolution is crucial for recovery, yet its mechanisms remain unclear. We studied orthopedic surgery (ORT) patients (n=33) at T0 (pre-surgery), T1 (24h), and T2 (3d post-surgery), with cytokine profiling, monocyte immunophenotyping and bulk transcriptomics. Findings were validated *in vitro* by comparing IL-18 and TNF-α effects on monocytes, and in 3D human intestinal organoids (IOs), with scRNA-seq. IL-18 exclusively characterized the cytokine signature in T2. Immunophenotyping showed elevated CD11b, CD64, and CD86 levels on ORT monocytes in T2, while transcriptomics showed upregulation of integrins, chemokines (monocyte migration) and SCL/TAL1 (differentiation) —without pro-inflammatory gene upregulation. Similar IL-18–driven effects, including elevated Akt but not NFκB phosphorylation, were validated in healthy donor monocytes. TNF-α attenuated these changes. IL-18 further induced monocyte–macrophage transition and migration in IOs. Finally, the TNF-α/IL-18 ratio showed high predictive value of clinical severity in septic patients. We emphasize the value of cytokine signature analysis for patient stratification, and propose IL-18 as a key mediator of successful ORT recovery, via a novel role on monocyte migration and macrophage transition.

2. Reproducible *In Vitro* Co-Culture Model for Evaluating T Cell-Mediated Cytotoxicity Across Cancer Types

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T cells are central to cancer immunotherapy, yet the lack of standardized *in vitro* models limits reproducibility and translational relevance in immune-cancer interaction studies. To address this, we developed a robust co-culture model using peripheral blood mononuclear cells (PBMCs) from healthy donors and luciferase-expressing cancer cell lines. PBMCs were co-cultured with or without cancer cells („educated" or „naive" PBMCs, respectively) in IL-2 supplemented media for two weeks, enabling functional priming.

Real-time luminescence assays revealed enhanced cancer cell killing in the educated condition. Single-cell RNA sequencing showed expansion of $CD8^+$ cytotoxic T cells and reduced $CD4^+$ helper T cell representation. Educated $CD8^+$ T cells expressed high levels of *Granzyme B* and *Perforin*, indicating cytolytic activity. Flow cytometry confirmed a stable $CD8^+$ T cell population with increasing CD107a expression over four weeks and slight IFNγ upregulation, while naïve cultures showed no change.

Initially validated with pancreatic cancer (PANC-1), the model was extended to lung (A549), triple-negative breast (MDA-MB-231) and multiple myeloma (AMO-1) cancer cell lines. Despite MHC mismatch, PBMCs exhibited 40-80% increased killing of the specific educated targets.

This platform provides a reproducible system for studying T cell-mediated cytotoxicity and optimizing immunotherapies, with future applications in characterizing tumor-intrinsic mechanisms of immunotherapy resistance.

3. Downregulation of Translation Sustains ERK–mTORC1 Signaling Under Amino Acid Deprivation in Malignant Melanoma

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Malignant melanoma is a highly aggressive type of skin cancer that often becomes resistant to standard treatments. Its rapid growth is mainly driven by activating mutations in key signaling pathways, particularly those involved in regulating cell proliferation and metabolism, such as the ERK and mTORC1 pathways [1].

In this project, we used melanoma cell lines with BRAF and NRAS mutations to explore how cells respond to metabolic stress caused by prolonged amino acid deprivation — specifically of methionine, glutamine, or asparagine. Interestingly, we found that under these conditions, ERK signaling becomes even more activated. This increase in ERK activity also stimulates mTORC1 signaling, which is known to play a central role in coordinating cellular metabolism and protein synthesis. However, despite this enhanced mTORC1 activity, global protein translation decreases.

To better understand this apparent uncoupling, we focused on DUSP6 — a rapidly degraded phosphatase that negatively regulates ERK [2]. After amino acid withdrawal, DUSP6 protein levels dropped significantly. A similar reduction was observed in components of the GATOR1 complex, which normally inhibits mTORC1 in response to amino acid deprivation [3].

Taken together, these findings suggest that the observed upregulation of ERK signaling under amino acid deprivation may be linked to impaired protein synthesis and a subsequent drop in DUSP6 levels. This may represent part of an adaptive response that allows melanoma cells to cope with nutrient stress under metabolically unfavorable conditions.

[1] Valdez-Salazar F, Jiménez-Del Rio LA, Padilla-Gutiérrez JR, Valle Y, Muñoz-Valle JF, Valdés-Alvarado E. Advances in Melanoma: From Genetic Insights to Therapeutic Innovations. Biomedicines. 2024; 12(8):1851. https://doi.org/10.3390/biomedicines12081851.
[2] Valcikova B, Vadovicova N, Smolkova K, Zacpalova M, Krejci P, Lee S, Rauch J, Kolch W, von Kriegsheim A, Dorotikova A, Andrysik Z, Vichova R, Vacek O, Soucek K, Uldrijan S. eIF4F controls ERK MAPK signaling in melanomas with BRAF and NRAS mutations. Proc. Natl. Acad. Sci. U.S.A. 2024; 121(44): e2321305121, https://doi.org/10.1073/pnas.2321305121.
[3] Yao Y, Jones E, Inoki K. Lysosomal Regulation of mTORC1 by Amino Acids in Mammalian Cells. Biomolecules. 2017; 7(3):51. https://doi.org/10.3390/biom7030051.

This work is supported by the Czech Science Foundation (GA25-17766S), the European Union – Next Generation EU – the project National Institute for Cancer Research (Programme EXCELES, Project No. LX22NPO5102), and Brno city municipality (Brno Ph.D. Talent Scholarship).

4. Limitations of *In Vitro* Models in Evaluating Carbonic Anhydrase IX Inhibitors

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Carbonic anhydrase IX (CAIX) is a key enzyme involved in pH regulation, especially under hypoxic conditions. It is commonly overexpressed in advanced tumors, where it serves as a negative prognostic marker. Therefore, numerous research groups are attempting to discover the most selective CAIX inhibitor [1].

These inhibitors typically exhibit high efficacy *in vitro*, with nanomolar concentrations inhibiting the purified CAIX enzyme. However, much higher concentrations (hundreds of micromolar) are required to observe the effect on cancer cell growth in standard 2D cultures.

To address this discrepancy, we tested a series of CAIX inhibitors on glycolytic cancer cell lines; however, we observed no significant increase in sensitivity to CAIX inhibitors under hypoxia compared to normoxic conditions. More importantly, we generated cell lines with the CAIX gene knocked out using the CRISPR-Cas9 system. None of these CAIX-null lines exhibited a significant growth defect when cultured *in vitro*, whereas the growth of CAIX-null cells in a mouse *in vivo* model was significantly reduced compared to controls.

Our findings confirmed the crucial role of CAIX in tumors growing *in vivo*, but also showed that standard cell cultures are not suitable for assessing the biological activity of CAIX inhibitors, indicating that a better model is needed.

[1] McDonald et al. A Phase 1 Study of SLC-0111, a Novel Inhibitor of Carbonic Anhydrase IX, in Patients With Advanced Solid Tumors. 2020. American Journal of Clinical Oncology 43(7): 484-490

This work was funded by the European Union – Next Generation EU - the project National Institute for Cancer Research (Programme EXCELES, Project No. LX22NPO5102), and Masaryk University (MUNI/G/1002/2021 and MUNI/LF-SUp/1392/2024).

5. From Genomic Variants to Microbial Communities: Scalable Frameworks for Clinical and Translational Research

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High-throughput sequencing has opened new opportunities for both clinical genomics and microbiome research, yet challenges remain in producing accurate, interpretable, and actionable results. Here, we present two complementary developments that address critical needs in medical and biological applications.

A first development addresses the detection of germline copy number variants (CNVs), which are essential for diagnosing inherited disorders and assessing genetic predispositions. Existing algorithms often produce inconsistent calls and high false-positive rates, restricting their clinical usefulness. To mitigate these limitations, a consensus-based pipeline was designed to integrate multiple state-of-the-art CNV detection methods. The combined approach improves sensitivity and specificity while generating standardized and reproducible reports, thus providing a more solid basis for clinical interpretation and genetic counseling.

In parallel, a reproducible metagenomics pipeline was implemented in Snakemake and complemented with an interactive visualization platform. This environment enables real-time exploration of microbial community structures, including taxonomic distributions, clustering, and sample comparisons. By facilitating dynamic study of microbiome profiles, it supports potential applications in infection monitoring, biomarker discovery, and precision medicine.

Altogether, these results underline the value of integrative pipelines and accessible visualization in linking sequencing outputs to practical applications, providing scalable and reproducible frameworks that can strengthen genomic and metagenomic research in healthcare.

6. The role of FoxO1 transcription factor in response to targeted therapy in chronic lymphocytic leukemia

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FoxO1 transcription factor has an indispensable and highly context-dependent role in B cell biology which is often dysregulated in related malignancies [1]. Apart from its general functions in cell metabolism, DNA damage and others, FoxO1 has been found to promote the survival of chronic lymphocytic leukemia (CLL) cells in various ways which include homing capacity towards lymph node microenvironment or adaptation to B cell receptor (BCR) signaling inhibitor treatment [2,3]. We hypothesize that FoxO1 operates on a larger scale to support CLL cells survival in both basal and therapy-induced conditions. We have discovered that FoxO1-regulated transcriptional network also affects pathways highly relevant to the microenvironment stimuli which were previously proven to have a major pro-proliferative and protective role in context of disease progression and targeted therapy resistance, respectively [4,5]. In our project, we investigate how FoxO1 activity impacts responsiveness to these stimuli through which CLL cells gain pro-survival support, induce proliferation and acquire resistance to targeted therapy. Our findings serve as a base for preclinical testing of FoxO1 inhibition combined with approved small molecule inhibitors currently used in CLL therapy.

[1] Hlavac, K, Pavelkova, P, Ondrisova, L, Mraz, M. FoxO1 signaling in B cell malignancies and its therapeutic targeting. 2024. FEBS Lett. https://doi.org/10.1002/1873-3468.15057.
[2] Seda V, Vojackova E, Ondrisova L et al. FoxO1-GAB1 axis regulates homing capacity and tonic AKT activity in chronic lymphocytic leukemia. Blood. 2021; 138:758-772. doi: 10.1182/blood.2020008101.
[3] Ondrisova L, Seda V, Hoferkova E et al. FoxO1/Rictor axis induces a nongenetic adaptation to ibrutinib via Akt activation in chronic lymphocytic leukemia. 2024. J Clin Invest. 2024;134(23):e173770. https://doi.org/10.1172/JCI173770.
[4] Hoferkova E, Seda V, Kadakova S et al. Stromal cells engineered to express T cell factors induce robust CLL cell proliferation in vitro and in PDX co-transplantations allowing the identification of RAF inhibitors as anti-proliferative drugs. 2024. Leukemia. doi: 10.1038/s41375-024-02284-w.
[5] Haselager MV, Thijssen R, Bax D et al. JAK–STAT signalling shapes the NF-KB response in CLL towards venetoclax sensitivity or resistance via BCL-XL. 2023. Mol Oncol, 1878–0261. doi: 10.1002/1878-0261.13364.

This project was supported by the Ministry of Health of the Czech Republic, grant no. NW24-03-00369. This work was also supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU, and institutional support: MUNI/A/1685/2024.

7. The reaction of the choroid plexus to chemotherapy

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2 Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen

Paclitaxel, a widely used anti-cancer drug, stabilizes microtubules to block cell division but also induces neuropathic pain through immune-related mechanisms. This study investigates its effects on the choroid plexus (CP), which regulates the blood–cerebrospinal fluid barrier and immune cell entry into the brain, with a focus on Kolmer cells (KC).
In our previous work, male Wistar rats treated with paclitaxel developed neuropathic pain behaviors, including reduced paw force tolerance and altered thermal sensitivity. Immunohistochemistry showed increased activated and resident KC without Ki67 colocalization, suggesting recruitment from peripheral immune cells. In vitro, paclitaxel disrupted CP barrier integrity by altering tight junction proteins: Occludin levels rose at 6–24h before declining at 48–72h, while ZO1 displayed a biphasic pattern.

In the present study, further analysis revealed higher CCR7 expression in the paclitaxel group compared with vehicle, peaking at day 7. MHC II expression was also elevated, particularly on days 7 and 14. Colocalization of Ki67 with MHC II was highest on day 14, suggesting that proliferation and immune activation contribute to the response.
In conclusion, paclitaxel-induced neuropathic pain is linked to KC activation, CP barrier disruption, and enhanced immune activation, highlighting immune barrier interactions as key contributors to neurotoxicity.

[1] Xu H, Lotfy P, Gelb S, Pragana A, Hehnly C, Byer LIJ, et al. The choroid plexus synergizes with immune cells during neuroinflammation. Cell. 2024 Sep 5;187(18):4946-4963.e17.
[2] Thompson D, Brissette CA, Watt JA. The choroid plexus and its role in the pathogenesis of neurological infections. Fluids Barriers CNS. 2022 Sep 10;19(1):75.

8. Elucidating the molecular mechanisms of Bardet-Biedl Syndrome using retinal models

Francisco Molina Gambin[1], Canan Celiker[1], Eva Hruba[1], Katarína Cimborová[1], Eman Elrefaay[2], Michaela Bosaková[2] and Tomas Barta[1]

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Bardet-Biedl Syndrome (BBS) is a rare autosomal recessive ciliopathy and the most common non-lethal ciliopathy, typically diagnosed during early childhood. A set of proteins, the chaperonin-like BBS proteins, account with a 30% of the BBS total cases, and are associated with the most severe and aggressive retinal degeneration phenotype. However, studying the retinal defects associated with BBS presents several significant challenges, including limitations in the number of relevant tissue samples, the inability to study the human retina on a cellular and molecular level, the lack of patient-specific drug testing platform, and the limitations of animal models reproducing human pathophysiology. Addressing these challenges is key for making progress towards our comprehension of BBS-related retinal degeneration and developing effective treatments for this condition.

Here we aimed to study the role of *BBS6* gene in rod and cone dystrophy. We edited *BBS6* using CRISPR/Cas9 technology to introduce knockout mutations underlying the BBS phenotype into a fully characterized hiPS cell line from a healthy individual. We differentiated them into retinal pigmented epithelial (RPE) cells to characterize the ciliary defects and to retinal organoids (RO) to define the photoreceptor phenotype. Our results show significant differences in cilia length and impaired phagocytosis function in RPE cells. In addition, light stimulation of *BBS6*-deficient RO revealed differences in the expression of light-responsive genes after different light exposure time frames, indicating that ROs are a suitable tool with potential for the elucidation of BBS-related retinal anomalies.

This study was supported by the Ministry of Health of the Czech Republic, grant nr. NU22-07-00380

9. Lipopolysaccharide induces retention of E-cadherin in the endoplasmic reticulum and promotes hybrid epithelial-to-mesenchymal transition of human embryonic stem cells-derived expandable lung epithelial cells

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Lipopolysaccharide (LPS), a bacterial endotoxin, is known to induce strong inflammatory responses in the lungs. However, its direct effects on lung epithelial tissue are poorly understood due to the lack of suitable non-cancer models.

This study examined the responses of epithelial progenitor cells to LPS using expandable lung epithelial (ELEP) cells derived from human embryonic stem cells [1]. LPS application was found to have no effect on cell viability but to direct the cells to a hybrid epithelial-mesenchymal-like state. This state was characterized by the loss of membrane localization of E-cadherin and its retention in the endoplasmic reticulum (ER), activation of the unfolded protein response (UPR), and intracellular accumulation of β-catenin. These molecular changes were accompanied by increased cell migration.

Tunicamycin, used to induce ER stress, produced similar effects, while the application of the chemical chaperone TUDCA partially regulated E-cadherin localization, decreased UPR markers, and reduced migration. In 3D cultures, LPS disrupted spheroid structure, while tunicamycin suppressed growth. Both effects were partially reversed by TUDCA.

These findings demonstrate that LPS induces a non-lethal but transformative stress response in lung epithelial precursors, that ER stress plays an important role in epithelial plasticity, and that ELEP cells provide a suitable model for studying inflammation-induced epithelial remodeling.

[1] Kotasova H. et al. Tissue Eng Regen Med, 2022, 19(5):1033-1050. doi: 10.1007/s13770-022-00458-0
Portakal T, Havlíček V, Herůdková J, Pelková V, Gruntová T, Çakmakci RC, Kotasová H, Hampl A, Vaňhara P. Lipopolysaccharide induces retention of E-cadherin in the endoplasmic reticulum and promotes hybrid epithelial-to-mesenchymal transition of human embryonic stem cells-derived expandable lung epithelial cells. Inflamm Res. 2025 May 24;74(1):82. doi: 10.1007/s00011-025-02041-4. PMID: 40413286; PMCID: PMC12103375.

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10. Changes of the Brain Barriers Induced by Diabetes Mellitus

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Diabetes mellitus (DM) is chronic metabolic disease affected the whole organism including the central nervous system. One of the lesser-explored diabetic complication is diabetic encephalopathy (DE), where structural and functional alterations of the brain are evident. Pathophysiology of the DE is complex and there is certainly involvement of the blood-brain barrier and blood-cerebrospinal fluid barrier (BCSFB).

We investigated the inflammatory response of the choroid plexus as a major part of the BCSFB in streptozotocin-induced rat model of DM. Glycemia and weight were measured periodically and three weeks after application of streptozotocin or vehiculum, animals were euthanized using CO_2 inhalation, followed by perfusion with Zamboni's fixative. Brains were collected and processed for coronal sectioning. Immunohistochemical staining of the lateral ventricular choroid plexus was performed using markers of macrophage/microglial activation and polarization: CD68, CD163, CCR7, CD206, CD11b/c, and MHC-II. The number of positive cells per mm^2 was examined and statistically evaluated.

Our results demonstrate a significant increase in immune cell infiltration and activation in the choroid plexus of diabetic rats compared to controls. This inflammatory response could lead to disruption of brain microenvironment and development of DE. Therefore, the choroid plexus may represent a potential therapeutic target for modulating neuroinflammation in DM.

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11. Regulation of the delta peripheral opioid receptor in pain conditions

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Primary sensory neurons (PSNs) transmit nociceptive input to the spinal dorsal horn (SDH) and contribute to the development of neuropathic pain (NPP) after nerve injury. Delta opioid receptor (DOR), expressed in PSNs and SDH neurons, achieves its functional efficacy when localized at the plasma membrane, where ligand binding suppresses neuronal excitability and attenuates NPP.

Using a spared nerve injury (SNI) mouse model of NPP, we quantified DOR immunofluorescence (DOR-IF) intensity at the plasma membrane and within the cytoplasm of individual subpopulations of PSNs. The cellular distribution of DOR in SDH was determined by double immunostaining with specific markers. We performed a semi-quantitative analysis comparing DOR-IF intensity in lumbar spinal segments of naïve, sham-operated, and SNI-operated animals at 7 and 21 days post-surgery.

Our findings demonstrate that plasma membrane-localized DOR undergoes dynamic, injury- and time-dependent changes across PSN subpopulations. In the SDH, DORs were detected at presynaptic terminals, postsynaptic neurons, and activated glia. Both sham and SNI surgery resulted in bilaterally increased DOR-IF intensity in the lumbar SDH at both survival periods, more pronounced at 7 days.
These results indicate that changes in DOR levels and distribution after nerve injury influence NPP signaling and the analgesic potential of DOR ligands.

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1. The Role of YAP in Cardiomyocyte Structure and Sarcomere Function

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Heart failure and many other cardiomyopathies lead to a weakened heart. With subsequent fibrosis and/or hypertrophy, the cardiac output is lowered, making the heart and other organs susceptible to complications over time. Recent breakthroughs have highlighted that an approach centered around increasing the contractile strength of the heart is feasible[1].

Yes-Associated Protein(YAP) is a well-studied transcriptional co-activator that is part of the Hippo pathway. Its proproliferative effects were utilized to obtain more favorable outcomes in animal infarction models[2]. However, knockout experiments have shown that it also plays a developmental role, promoting better sarcomere alignment and cellular morphology. Within this study, we will be isolating the nuclear and cytoplasmic functions of YAP as well as its interactome. We aim to illustrate the interaction mechanism that leads YAP to directly or indirectly alter the alignment, length, and strength of myofibrils.

[1] Farmakis D, Agostoni P, Baholli L, et al. A pragmatic approach to the use of inotropes for the management of acute and advanced heart failure: An expert panel consensus. Int J Cardiol. 2019;297:83-90. doi:10.1016/j.ijcard.2019.09.005
[2] Lin Z, von Gise A, Zhou P, et al. Cardiac-specific YAP activation improves cardiac function and survival in an experimental murine MI model. Circ Res. 2014;115(3):354-363. doi:10.1161/CIRCRESAHA.115.303632

2. Functional characterization of novel meiotic factors in *Caenorhabditis elegans*

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Meiosis ensures accurate segregation of homologous chromosomes, producing haploid gametes in sexually reproducing animals. It is vital for genetic diversity, species survival, and genome stability. *Caenorhabditis elegans* serves as a powerful model for dissecting meiotic processes due to its transparent gonad, rapid life cycle, and conserved genetic pathways. Its spatially organized germline enables detailed analysis of DNA replication, homolog pairing, synaptonemal complex (SC) formation, and double-strand break (DSB) repair. [1] As meiotic mechanisms are conserved between worms and mammals, *C. elegans* offers an insightful system for uncovering processes relevant to human health.

HIM-17, a chromatin-associated protein, regulates the topoisomerase-like enzyme SPO-11, which initiates meiotic DSBs.[2] Here, we investigate the gene *C30G12.6*, an uncharacterized gene conserved in nematodes, that was identified as a putative HIM-17 interactor via mass spectrometry. Using genetic mutants of *C30G12.6* in combination with established meiotic regulators, we assess its role in DSB induction, repair, SC assembly, and crossover stabilization. Preliminary data suggest that *C30G12.6* may influence genome integrity by playing a role in DSB repair and/or CO resolution. This work aims to uncover novel aspects of conserved meiotic regulation with implications for fertility and chromosome stability in higher organisms.

[1] Yeh, S. D., Doering, J. L., & Goldstein, B. (2017). Caenorhabditis elegans as a model organism for studying meiosis. Scientific Reports, 7, 2641.
[2] Raices, M., Subramanian, V. V., & Villeneuve, A. M. (2024). HIM-17 promotes meiotic DNA double-strand break formation through chromatin regulation. eLife, 13, e96458.

3. Differentiation of iPSCs from donors suffering from pulmonary fibrosis and from healthy donors into ELEP cells

Tereza Gruntová[1], Vendula Pelková[1, 2], Jarmila Herůdková[1, 2], Marianna Štefániková[2], Martina Doubková[2], Aleš Hampl[1,2], Petr Vaňhara[1,2]

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Pulmonary fibrosis (PF) is a chronic, progressive interstitial lung disease characterized by excessive extracellular matrix (ECM) deposition and aberrant fibroblast activation, leading to scarring and stiffening of lung tissue. The consequences of these pathological processes on lung epithelial cells and progenitors remain poorly understood. To address this, we employed *in vitro* differentiation of PF patient-derived and control human induced pluripotent stem cells (iPSCs) into expandable lung epithelial progenitors (ELEPs), previously established at the Department of Histology and Embryology [1].

Peripheral blood samples obtained from patients with familial PF and healthy donors at the University Hospital in Brno were used to isolate peripheral blood mononuclear cells (PBMCs), which were reprogrammed into iPSCs and subsequently differentiated into ELEPs. These progenitors express NK2 Homeobox 1 (NKX2.1), a key marker of early lung epithelial lineage. Differentiation protocols were optimized to ensure robust induction of NKX2.1 expression and concomitant downregulation of pluripotency markers including SOX2, OCT4, and NANOG, thereby confirming successful lung epithelial specification.

This model enables direct comparison of ELEPs derived from PF patients and healthy controls, providing a platform to investigate epithelial contributions to pulmonary fibrosis pathogenesis.

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4. Circulating histone signatures in biofluids: toward a non-invasive diagnostic tool for pediatric brain tumors

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Pediatric brain tumors (PBTs)—including medulloblastoma and diffuse midline glioma (DMG)—represent a leading cause of cancer-related mortality in children. Current diagnostic strategies rely on imaging and surgical biopsies, which are often invasive, risky, or infeasible due to tumor location or patient fragility. This project aims to develop a non-invasive diagnostic platform by investigating free circulating histone variants and their complexes in blood and cerebrospinal fluid (CSF) as novel biomarkers for early detection, tumor characterization, and disease monitoring.

Building on preliminary findings, we hypothesize that distinct histone signatures (e.g., H2A, H2B, H3, H4 and macroH2A isoforms) correlate with tumor type and progression. Using high-resolution imaging flow cytometry, we analyzed histone profiles in clinical samples from pediatric patients and healthy controls[1], [2]. In parallel, in vitro experiments with representative PBT cell lines we explored the underlying mechanisms of histone release, focusing on the roles of histone chaperones and cell death, including apoptosis, necroptosis, and necrosis.

By integrating biomarker discovery with mechanistic studies, this research seeks to establish circulating histone variants as reliable, minimally invasive indicators of tumor biology. Such an approach could revolutionize PBT diagnostics by enabling earlier, safer, and more precise clinical decision-making—ultimately improving patient outcomes and treatment personalization.

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5. Non-viral gene therapy using episomal vectors

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Gene therapies have existed since the 1990s and still, they are plagued with the same problems that have been in the field since its inception. These problems are 1) immunogenicity of the vector, 2) its instability and subsequent integration into the genome and also 3) the immense costs and hardships of the production of the vector. All of these issues are in part due to the fact, that most vectors used in approved gene therapies are based on the platform of viral vectors.

Because of the problems of viral vectors, the genetic disorders treated so far have been mostly limited to the brain, which is uniquely resilient to immunogenicity of the vector.

These problems are the motivation behind the attempts to replace viral vectors with non-viral alternatives. One of these alternative vectors is the S/MAR episomal minicircle. This type of vector is working on the basis of the aforementioned S/MAR sequence which provides replicative stability of the vector across the cell cycle in dividing cells[1].

To further build on the capabilities of the vector we are trying to better describe and understand the role of the S/MAR sequence in the episomal establishment process.

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6. Polygenic risk score and lipoprotein(a) in relation to low-density lipoprotein cholesterol levels in Czech newborn cohort

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Elevated low-density lipoprotein cholesterol (LDL-C) levels in childhood is a major risk factor for premature cardiovascular disease (CVD). While monogenic familial hypercholesterolemia is well recognized, a significant proportion of patients with elevated LDL-C levels have polygenic cause of their condition. Polygenic risk score (PRS) that aggregates the impact of multiple common variants associated with LDL-C levels is commonly used to characterize polygenic hypercholesterolemia [1].

Lipoprotein(a) (Lp(a)), an LDL-like particle containing apolipoprotein(a), represents additional CVD risk [2]. Elevated Lp(a) can contribute to measured LDL-C and potentially confound its interpretation.

In this study, we analyzed umbilical cord blood samples from pilot newborn screening project. For nearly 6000 newborns, the LDL-C levels were measured at study enrolment and LDL-C level percentiles were ascertained. For individuals with low (0-15th percentile), medium (40-60th percentile) and high (75-100th percentile) LDL-C levels, we analyzed the PRS and Lp(a) values. PRS and Lp(a) were assessed as independent genetic risk factors for elevated LDL-C. Preliminary results show statistically significant differences in PRS and Lp(a) levels between groups with low, medium, and high LDL-C. Results indicate that evaluation of PRS and Lp(a) measurement are important, as both may contribute to explaining elevated LDL-C levels in individuals with hypercholesterolemia.

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7. Expression of Fas ligand and Fas receptor in calvarial and long bone-derived cell models

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During calvaria development, osteoprogenitors differentiate directly into osteoblasts and osteocytes via intramembranous ossification, unlike endochondral ossification in long bones, which involves a cartilage intermediate. Fas/FasL signaling influences these processes, initiating osteoblast-induced osteoclast apoptosis and exerting both catabolic and anabolic effects on bone [1, 2, 3, 4]. The aim of the investigation was to follow the expression of Fas ligand (FasL) and Fas receptor in two calvarial and long bone-derived cell models. Calvaria-derived primary osteoblasts, the MC3T3-E1 cell line, long bone-derived primary osteoblasts, and the IDG-SW3 cell line were collected at weekly intervals during osteogenic differentiation. The dynamic mRNA expression of Fas receptor was quantified by qPCR analysis. FasL expression was assessed by flow cytometry at selected time points.

Fas expression varied across models. In calvaria-derived systems, Fas peaked at day 21. In long bone-derived models, Fas expression increased from day 7 to 21 in primary cells and remained elevated in the IDG-SW3 cell line through day 28. FasL was not detected in either the MC3T3-E1 or IDG-SW3 cell lines; however, it was present in calvaria-derived primary osteoblasts. Our results reveal differences between ossification types, primary cultures, and cell lines, which will be considered in ongoing experiments aimed at investigating the mechanisms of Fas/FasL signaling in bone.

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8. Characterisation of RECQ4-RAD51 interaction inDNA replication and genome integrity

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Genomic instability is a hallmark of cancer and can result from errors during DNA replication or repair. Cells utilise mechanisms to stabilise and restart stalled replication forks, thereby maintaining genomic integrity. RAD51, a core factor in DNA break repair, is a key regulator of this process. RAD51 mutations and overexpression have been associated with various cancer progression and chemotherapy resistance [1]. Another emerging factor in replication fork processing is RECQ4, a DNA helicase whose dysfunction is linked to genetic disorders with cancer predisposition [2]. Novel evidence suggests that RECQ4 contributes to replication fork stability and may cooperate with RAD51 [2]. However, whether and how RECQ4 and RAD51 interact, how this is regulated, and how it affects the recruitment and activity of repair complexes under replication stress remain unclear.

This project aims to elucidate the molecular crosstalk between RECQ4 and RAD51 during replication stress. Specifically, it will determine whether RECQ4 directly interacts with RAD51, how this interaction is regulated, and the functional consequences of disrupting this interplay, particularly under replication stress. Given the essential role of RAD51 in replication fork integrity and frequent dysregulation in cancers, uncovering its coordination with RECQ4 could reveal novel targets to overcome therapy resistance.

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9. Choroid plexus and neuroinflammation in Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by cognitive decline. Key pathological features include amyloid-beta (Aβ) deposition, tau hyperphosphorylation, and neuroinflammation. The choroid plexus, which constitutes the blood–cerebrospinal fluid (CSF) barrier, may contribute to AD progression by regulating immune activity in the central nervous system. This work investigates alterations in choroid plexus function in AD. An in vitro AD model was established using Z310 choroid plexus epithelial cells treated with Aβ(42) peptide for different time periods. Gene and protein expression were analyzed, with a focus on inflammatory markers. Cellular changes were evident as early as one day following Aβ exposure. Modifications in proteins such as phosphorylated tau, amyloid precursor protein, and inflammatory mediators were identified. Altogether, the results demonstrate that Aβ compromises choroid plexus function and barrier properties, highlighting its contribution to early AD pathology and its potential as a therapeutic target.

10. Investigating *SORL1* mutations in Alzheimer's disease through iPSC-derived glial models

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by the accumulation of amyloid-β plaques and Tau neurofibrillary tangles. While most cases are sporadic, about 5% are associated with mutations in *APP, PSEN1, PSEN2*, and *SORL1*. The SORLA protein, encoded by the *SORL1* gene, is essential for intracellular APP trafficking. Its dysfunction has been extensively characterised in neurons, where it impairs endosomal function and APP recycling. In contrast, much less is known about SORLA and its pathogenic mutations in glial cells, despite their critical role in maintaining brain homeostasis and providing neuroprotection.

To address this gap, we developed unique human iPSC-based models for studying SORLA function in glial cells. Our platform includes well-validated 2D astrocytes and 3D astrocyte-enriched spheroids, both expressing the full set of mature glial markers, providing a physiologically relevant context. We hypothesize that pathogenic *SORL1* mutations impair essential glial functions and thereby contribute to Alzheimer's disease pathogenesis. We aim to define how these mutations alter glial biology, to investigate neuron-glia interactions and to uncover novel glia-specific mechanisms of neurodegeneration. By combining robust marker-verified 2D and 3D systems with CRISPR/Cas9-engineered mutations, we seek to expand understanding of the non-neuronal contributions to Alzheimer's disease.

11. Investigation of Alternative Therapeutic Options for Acute Myeloid Leukemia Patients Resistant to Venetoclax

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Acute myeloid leukemia (AML) is one of the most challenging hematological cancers to treat due to its aggressive nature and heterogeneous genetic aberrations [1]. Venetoclax (Ven) is a BCL-2 inhibitor used in combination with the hypomethylating agent Azacitidine as a standard care for AML patients unfit for chemotherapy [2, 3]. Despite promising responses, resistance to BCL-2 inhibitors emerges in many cases, emphasizing the need for alternative therapies, identifying biomarkers and understanding the underlying mechanisms leading to resistance.
To gain insight into alternative therapies for Ven/Aza resistant patients, we assembled a large drug library covering 300 clinically relevant oncology-targeted FDA-approved drugs, in three different concentrations, on a collection of AML primary samples that failed on Ven/Aza therapy. In addition, we performed quantitative real-time PCR to compare the expression profile of candidate genes at the time of diagnosis and relapse. Also, bulk RNA-sequencing was utilized to study the general gene expression profiles and dysregulation of individual gene sets in Ven-resistant samples.

This study provides an overview of the drugs that could potentially be proposed as novel therapeutic targets for patients unresponsive to Ven. Differentially expressed genes conferring resistance will be further validated on additional Ven-resistant samples and the top compounds from the drug screening will be tested on patient-derived AML xenograft mouse models.

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12. The effect of biomechanical stress on the RBM20-induced dilated cardiomyopathy

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Dilated Cardiomyopathy (DCM) is the most prevalent non-ischemic cause of heart failure, affecting roughly 1 in 250 individuals worldwide (1). Specifically, the mutations in splicing regulator RNA-binding motif 20 (RBM20) are known to cause an aggressive form of DCM (2). RBM20 mutants directly impact the splicing of the sarcomeric protein Titin, leading to the expression of the fetal isoform of Titin, which is one of the main contributors to the disease progression (3). One of the key hallmarks of DCM is the pathological remodeling of extracellular matrix (ECM) components, leading to myocardial fibrosis (4). Our lab recently demonstrated that ECM turmoil in the failing heart affects the nuclear localization of heterogeneous nuclear ribonucleoprotein C (hnRNPC), which in turn affects the alternative splicing of key mRNAs involved in cardiomyocyte function (5). Hitherto, no link between mechanical stress and the splicing protein RBM20 has been reported. We propose to use an Induced pluripotent stem cell lines derived Cardiomyocytes (iPSC-CMs) model to explain the effect of ECM-associated stress on RBM20 and its effect on titin splicing. We believe the outcome will enable mechanotherapy to mitigate DCM.

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13. Fabrication of GelMA/ColMA Hydrogel Microfluidic Model to study vascular endothelial cells

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On-chip vascular microfluidic models are powerful tools for studying vascular biology under controlled conditions. While conventional glass or plastic devices provide defined microchannel geometry, their stiffness and low permeability limit physiological relevance. Hydrogels such as Gelatin Methacryloyl (GelMA) and Collagen Methacryloyl (ColMA) offer bioactivity, porosity, and tunable mechanics, better resembling native extracellular matrix. We developed a method combining stereolithography and casting to fabricate transparent GelMA/ColMA hydrogel chips with smooth, cylindrical channels (800 µm diameter).

GelMA (5, 10, 15%) and ColMA (0.5, 1, 1.5%) hydrogels were synthesized and characterized by compression, swelling, and porosity assays. Endothelial cell viability (MS1, HUVEC) was evaluated on different formulations. The 15% GelMA/1.5% ColMA hydrogel showed superior compressive modulus, reduced swelling, and stable porosity, supporting long-term endothelial culture. Needle-assisted casting produced robust lumens with surface roughness ≤1 µm, stable under shear stresses up to 70 Pa. Endothelial cells seeded into the channels aligned and elongated in response to flow, confirming functional mechanobiological responses.

This platform provides a robust, transparent hydrogel-based chip with tunable properties, enabling reproducible studies of endothelial mechanobiology under physiologically relevant flow.

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14. Uncovering Vulnerabilities in Oesophageal Adenocarcinoma

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Oesophageal adenocarcinoma (EAC) is an aggressive and increasingly common malignancy of the upper gastrointestinal tract, characterized by late diagnosis, poor prognosis, and limited treatment options. Resistance to therapy remains one of the main obstacles to improving patient outcomes, and its molecular basis is still not fully understood.

As part of a collaboration with Leipzig University, chemoresistant EAC cell lines were developed through long-term exposure to 5-fluorouracil, carboplatin, oxaliplatin, and paclitaxel. These models, together with their parental counterparts, were processed using the Filter-Aided Sample Preparation (FASP) protocol and analyzed by data-independent acquisition (DIA) mass spectrometry. The resulting datasets enabled quantitative comparison of global protein abundance profiles across resistant and sensitive phenotypes.

The analysis revealed clear proteomic separation between resistant and parental cell lines, with numerous differentially expressed proteins previously described in relation to chemoresistance, as well as several novel candidates potentially involved in treatment failure. These data provide a comprehensive proteomic resource for understanding treatment adaptation and form a basis for future validation and functional studies aimed at overcoming chemoresistance in EAC.

15. The use of micro-CT in paleopathology

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For paleopathologists, paleoepidemiologists, and clinical research specialists, understanding the pathology and epidemiology of diseases that affected human populations in the past is important. This knowledge contributes to our understanding of modern diseases. As micro-CT has become more accessible, paleopathological studies have begun utilising micro-CT to diagnose historical diseases evident in skeletal remains. This relatively new method offers novel approaches to diagnosis and could lead to faster and more accurate assessments of individual cases. The selected bone pathologies originate from archaeological material dating from various historical periods and are caused by different aetiological processes, including tumours, metabolic diseases, infectious diseases, and trauma. Scanning was performed using an experimental in-house CT scanner (TORATOM) at the Institute of Theoretical and Applied Mechanics of the Czech Academy of Sciences. Scanning resulted in a large number of images of bone microstructure, providing the possibility of 3D imaging of samples suitable for structural analysis of bone tissue pathology. A micro-CT examination provides a detailed view of the microstructure of bone tissue, which is useful for identifying osteolytic/osteoplastic lesions, examining bone tissue cavities and their potential connections, analyzing the effects of injury, and assessing bone tissue density. This study aims to improve the diagnosis of bone pathologies.

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16. Genomic and phenotypic analysis of *Escherichia coli* strains with probiotic potential

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Probiotics are widely used beneficial microbes that support human health. Several preparates include *Escherichia coli* strains, with *E. coli* Nissle 1917 (EcN) being the most established. Although *E. coli* is usually a beneficial gut commensal, certain strains, including probiotics, carry pathogenic traits, raising concerns about their use.

In our laboratory, four human *E. coli* isolates (B1172, B771, 582, H22) demonstrated antagonistic activity against enteric pathogens *in vitro* and *in vivo* [1,2], suggesting their probiotic potential. This study aims to evaluate the safety profile of four experimental strains compared to EcN. Complete genome sequences of experimental *E. coli* strains [3] and EcN were analyzed to identify virulence-associated genes (VAGs) and antimicrobial resistance determinants. Among all analyzed strains, strain 582 carries the fewest VAGs. Strains B1172 and B771 carry genes for antibiotic-inactivating β-lactamases on conjugative or mobilizable plasmids, with B1172 also harboring chromosomal genes for sulfamethoxazole (*sul2*) and tetracycline (*tet(B)*) resistance. Phenotypic characterization included *in vitro* adhesion and invasion assays using three different colorectal cancer cell lines (Caco-2, HT-29 and SW620). All four experimental strains displayed adhesion capacities similar to EcN, while strains 582 and B771 exhibited equal or lower invasion into epithelial cells compared to EcN, indicating low pathogenic potential. Based on invasion assay, virulence and resistance gene content, strain 582 appears to be the most promising probiotic candidate.

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17. Cardiac progenitors in Duchenne muscular dystrophy show elevated DNA damage and accelerated maturation

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Duchenne muscular dystrophy (DMD) is a rare, X-linked neuromuscular disorder caused by dystrophin mutations, which result in progressive skeletal muscle loss followed by cardiomyopathy. Current medical care is palliative; interventions and improved therapies require further research. We previously showed that DMD already affects the pluripotent stem cell (PSC) stage through elevated DNA damage and mutagenesis, at least partially caused by deregulated nitric oxide synthase (NOS) and production of reactive species. Given these results, we questioned how that affects cardiac tissue development.

DMD cardiovascular progenitor (CP) population in developing cardiac organoids shows an altered transcriptional program during differentiation. CP markers activate earlier, followed by earlier onset of maturation-related gene expression and ongoing inflammation. DMD CPs present higher levels of DNA damage and lowered proliferation. These early deregulations are followed by impaired cardiac differentiation efficacy with higher rate of cardiomyocyte (CM) death as well as increased and accelerated collagen deposition, thus recapitulating the phenotype of *mdx* mouse and human DMD hearts. NOS inhibition attenuates DNA damage in CPs, rescues CP proliferation and improves formation of beating organoids; however, it does not prevent CM death or significantly affect transcription of cardiac development-related genes. Thus, with further study, NOS inhibition may complement current medical care.

18. S100A4+ stromal cells in development and function of skin derivatives

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During the embryonic development, seemingly homogenous epidermis transforms into multilayered skin with a complex structure, including skin derivatives. Specialized nipple skin is anatomically and functionally connected to a skin appendage, the mammary gland. Together they are exclusively found in mammals and play an irreplaceable role in nourishing the newborns.

In this work, we used experimental mouse model *S100a4-Cre; DTA* to investigate the role of S100A4+ stromal cells in mammary gland development and function. We found that while gland development proceeds normally through puberty and pregnancy, *S100a4-Cre; DTA* lactating dams exhibit nursing failure due to defective nipples. These nipples lack proper protrusion, preventing pups from grasping and suckling. We found that observed nipple defect is associated with abnormal collagen deposition and insufficient dermal proliferation. Bulk RNA sequencing further revealed molecular signatures of ongoing cell depletion in *S100a4-Cre; DTA* nipple tissue. Also, we analyzed available data from scRNAseq of the skin and mammary gland and performed immunofluorescence labeling to define S100A4+ cell population, which resulted to be comprised of immune cells and fibroblasts.

In summary, our work characterizes S100A4+ cells heterogeneity and describes their role in the development of the nipple and the mammary gland. We believe that our work may contribute to advancing research on nipple pathologies and breastfeeding medicine.

19. Assessing the effects of the ERK biosensor EKAREV-NLS on mouse mammary gland development

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Molecular biosensors are powerful tool for monitoring protein activity in vivo, yet their expression can perturb native signaling and lead to developmental or physiological side effects. The EKAREV-NLS mouse, which carries a FRET biosensor reporting ERK activity, has been applied across many tissues and cell types. In this study, we uncover a pronounced defect in mammary epithelial development in female EKAREV-NLS mice. These animals show markedly reduced mammary epithelial outgrowth accompanied by systemic abnormalities, like, disrupted estrous cycles, impaired ovarian follicle maturation, and anovulation. Notably, estrogen supplementation restored mammary epithelial expansion in EKAREV-NLS females. We also verified sensor responsiveness in hormone-treated tissues using time-lapse imaging of primary mammary epithelial cells. Together, these findings underscore the need for thorough phenotypic vetting of biosensor strains prior to their use. Further, this work highlights the strong hormonal control of mammary gland development and emphasizes the importance of careful strain selection for studies of the mammary epithelium.

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20. From Neurodevelopment to Neurodegeneration: Insights from Cerebral Organoids

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The human cerebral cortex develops through a balance between progenitor maintenance and neuronal differentiation. While well studied in animal models, these processes are less understood in humans. Mutations in PSEN1, which cause familial Alzheimer's disease (fAD), are known for altering amyloid precursor protein (APP) processing, but PSEN1 is also a key component of the γ-secretase complex, which cleaves other substrates, including the Notch receptor. Postmortem studies reveal early cortical abnormalities in fAD, such as reduced thickness, disrupted layering, and impaired neural stem cell renewal. iPSC-derived models, including ours, show premature neuronal differentiation and depleted progenitor pools in PSEN1-mutant cells, suggesting that early developmental defects may increase later disease vulnerability.

In this study, we modeled early human neurodevelopment using iPSC-derived cerebral organoids (COs) from healthy donors. Previous studies with fAD patient COs carrying PSEN1 mutations showed altered morphogenesis and disrupted NOTCH and BMP signaling. To probe these pathways, control COs were treated with the γ-secretase inhibitor DAPT (blocking NOTCH) or the BMP inhibitor LDN193189. Inhibition of either pathway induced morphological and molecular changes similar to PSEN1-mutant phenotypes. These findings indicate that early disruption of neurodevelopmental signaling may contribute to AD pathogenesis and highlight potential targets for therapeutic intervention.

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21. Lost cells, retained structure: Proteomic mapping of decellularized matrisome across species

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The extracellular matrix (ECM) is a fundamental, yet often overlooked, component of tissue architecture and a key regulator of cellular behavior and organ homeostasis. Through complex biochemical and biomechanical signals, it undergoes continuous remodeling to adapt to environmental changes, while its dysregulation drives pathological processes such as fibrosis, tumor progression, metastasis, and impaired repair.

While many studies have explored the ECM in individual species, cross-species comparisons remain scarce, limiting our understanding of conserved versus divergent features and the translational relevance of animal models to human biology. To address this, we performed a detailed proteomic analysis of lung ECM from mouse, pig, and human tissues. By employing the method of decellularization[1], we isolated the ECM from the lung tissues of each species, ensuring the removal of cellular components and genetic material while preserving the intricate ECM structure and composition. We validated the approach using histological, immunofluorescent, biochemical, and ultrastructural analyses. This strategy enabled accurate detection of less abundant ECM proteins that are often masked by cellular contaminants, allowing a more comprehensive mapping of the lung matrisome.

Our analysis revealed both conserved and species-specific ECM components, providing new insights into lung matrix biology and evolutionary conservation. These findings underscore the value of comparative ECM studies for evaluating the relevance of animal models to human biology and advancing translational strategies in tissue engineering, regenerative medicine, and therapies for ECM-related diseases.

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22. CRISPR screen reveals regulators of CD20 surface expression

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Anti-CD20 monoclonal antibodies (mAb) such as rituximab (RTX) have been used in therapy of B-cell malignancies for more than two decades. However, therapy is often hampered by downregulation of CD20 antigen on the surface of malignant cells, resulting in resistance to mAbs and disease relapse. Therefore, it is crucial to study CD20 downregulation in B-cell malignancies to enhance the efficacy of anti-CD20 mAb. This project aims to identify genes involved in regulation of CD20 surface level using whole-genome CRISPR/Cas9 knockout screening.

We performed CRISPR/Cas9 screening was performed in RTX-resistant $CD20^{low}$ B-cell line using GeCKO lentiviral library to identify genes whose disruption increased CD20 surface expression in cells. Based on their statistical significance and biological relevance, several genes identified by the screening were selected for validation. Among selected there are genes involved in B-cell receptor pathway (*CSK*, *PTEN*) – an essential pathway in B cells, *CD37*, encoding immune cell specific protein, and genes associated with endoplasmic reticulum (*SSR1–4*, *STT3A*). Validation of these genes has been ongoing, and the mechanism of action being investigated. The understanding of underlying mechanisms could provide a way for a potential enhancement of anti-CD20 therapy in the clinic.

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23. Biased signaling of fibroblast growth factor receptor 3

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Fibroblast Growth Factor (FGF) signaling serves an essential role in embryonic development and regulates various biological processes, including tissue maintenance, regeneration, repair, and metabolism. The FGF family comprises of 18 FGF ligands (signaling proteins) and four fibroblast growth factor receptors (FGFR1-4) (tyrosine kinase receptors). Several FGFs can bind to the same FGFR yet elicit different cellular response. This phenomenon is known as ligand bias. However, ligand bias in the FGF family remains poorly understood, particularly in context of FGFR3, a receptor involved in several skeletal dysplasia and cancers. Here, FGFR3-expressing cells are treated with different FGFs and the effect of FGF on the activation of downstream FGFR3 signaling pathways and on the cellular responses to the FGF stimulus are analyzed, such as loss of extracellular matrix, induction of premature senescence, and growth arrest. Our findings reveal distinct FGF-specific responses, providing clear evidence for ligand bias in FGFR3 signaling. These insights advance the understanding of FGFR3 signaling dynamics and open new avenues for the development of more selective FGFR3-targeted therapeutic strategies.

24. Cancer-associated RAD51 S296L mutant is defective in strand engagement and leads to genome instability

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Homologous Recombination (HR) is crucial for repairing DNA double strand breaks (DSBs) and the processing of stalled replication forks. Defects in HR lead to genomic instability and cancer. RAD51, a critical component of HR, forms nucleoprotein filaments on single-stranded DNA (ssDNA) that protect nascent DNA, promote pairing with homologous dsDNA and strand exchange, and catalyse fork reversal. Here, we investigate cancer-associated RAD51 S296L mutation, identified in a patient with Head and Neck Squamous Cell Carcinoma. Mouse embryonic stem cells carrying this mutation exhibit elevated fork stalling, defects in fork restart and impaired DSB repair, leading to replication stress-induced chromosomal aberrations and mutagenesis due to reliance on error-prone pathways. Biochemical analysis revealed differences in ATP coordination, ssDNA binding, filament formation and structure. These abnormalities impaired D-loop formation due to a defect in pairing of RAD51 filaments with homologous dsDNA. Importantly, increased Ca^{2+} or the accessory factor HOP2-MND1 partially restored mutant activity, indicating compensatory mechanisms. Our findings illustrate how RAD51 S296L undermines HR and fork recovery, driving genome instability, with implications for targeted diagnostics and therapies.

25. Mapping of sensory innervation in developing teeth to search for the molecular mechanisms behind

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Besides the pain sensation there is increasing evidence that the neurosensory system has a role in pulp regeneration, immune response, angiogenesis and dental pulp tissue maintenance. In the first mouse molar, the most common model to study odontogenesis, sensory nerves penetrate the dental pulp by postnatal (P) day 3-4.

This work aimed to contribute to recent knowledge by transcriptomic analysis comparing developmental stages of sensory innervation.

To collect the exact stages for transcriptomic evaluation, immunohistochemistry on the mouse molar section at the stage P2, P3, P4, P5 and P6 was performed. The sensory nerves firstly appeared inside of the dental pulp at P3. Nerves were seen more prominent in the pulp core at P4 and reached the cusp tips by P6.

The samples of the dental pulp tissue at the stage P2, P4 and P6 were delivered for RNA sequencing. The most enriched biological processes were Neuron projection development in P2vsP4 comparison and Nervous system development in P4vsP6. Genes *Clu* and *Rgma* were downregulated in P2vsP4 and upregulated in P4vsP6 comparisons, influencing neuron outgrowth and axonal guidance, respectively.

The insights into transcriptomic changes during crucial stages of dental innervation provide findings that could be beneficial for functional pulp regeneration during regenerative endodontics procedures.

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26. Synthetic materials for 3D cell culture, bioprinting and vascularized tissue models

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Synthetic materials play an important role in rapidly developing fields of biological research, such as tissue engineering, bioprinting or 3D organoid cell culture. Often specific requirements on material properties from mechanical, chemical and biological point of view are constantly driving the research of novel tunable materials.

During recent years, our focus shifted from an inert natural alginate, through chemically modified gelMA, to fully synthetic amino acid-based material. Our material can be modified with adhesive peptide motifs and offers tunable mechanical properties. These features, along with photocrosslinking capabilities, are especially useful for bioprinting. In this work we show some of our key optimizations that significantly improve wellbeing of cells encapsulated within this hydrogel, bringing us closer to fabrication of multicellular vascularized constructs.

Given these advancements, polyamino acids emerge as a promising candidate for further use in the field of biomedical sciences and regenerative medicine.

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27. Comparing Lung Organoids grown in Defined Media and Microfluidic Devices

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Both lung-on-chip and lung organoid models have been great additions as *in vitro* models for the study lung development and disease. Both models come with different advantages to fill a unique niche and allow their user to study specific mechanisms or processes. Organ-on-chip models allow for greater control of the cellular microenvironment as well as elaborate co-culture systems. This makes lung-on-chip models a useful tool to simulate the dynamic microenvironment of the respiratory system.

Organoids on the other hand can give great insight in the development of both healthy and diseased lung tissue, by forming the organoids from either embryonic stem cells or patient derived induced pluripotent stem cells. Organoids have great versatility because, depending on the specifics of the cultivation protocol, lung organoids can be used to create a variety of morphologies, ranging from intricate branching structures to single-cell layered alveolospheres.

In this work, we compare a variety of lung organoid cultivation protocols and investigate how different medium compositions influence lung organoid development. We then aim to design a microfluidic platform to cultivate the lung organoids in a controlled environment to create a more physiologically relevant *in vitro* model for lung development.

28. FTO m^{6}A RNA demethylase is important for proper DNA replication in human cells

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N6-methyladenosine (m6A) and N6-2'-O-dimethyladenosine (m6Am) are two highly prevalent and dynamic eukaryotic mRNA modifications that exert regulatory effects on multiple steps of mRNA metabolism (1). m6A, in particular, has also been shown to regulate a vast majority of different cellular processes, including their newly identified roles in the maintenance of genome stability (2).

Here, we demonstrate that FTO, an RNA demethylase of m6A and m6Am, is a critical player in replication fork dynamics. Our omics studies reveal physical and genetic interactions between FTO and DNA replication factors. We identify that FTO is physically present at sites of active replication, and its demethylation activity is critical for normal replication fork progression. Under conditions of replication stress, FTO exercises a protective role over stalled replication forks to prevent the degradation of nascent DNA. Prolonged replication stress in the absence of FTO was seen to result in DNA strand breaks, possibly due to the collapse of stalled replication forks. Collectively, our results demonstrate a previously unknown role of FTO in the maintenance of replication fork integrity and provide a potential link between RNA modifications and DNA replication.

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29. Ciliogenesis associated kinase 1 (CILK1) safeguards centriole cohesion and duplication

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Primary cilia and centrioles represent two closely related structures of eukaryotic cells. Centrioles are essential for organization and function of the mitotic spindle, also transform into the basal body to produce cilia. Proper function of cilia and centrioles is critical in development as well as homeostasis of the tissue. Ciliogenesis associated kinase 1 (CILK1) is an evolutionarily conserved and ubiquitously expressed serine/threonine kinase, with significant functions in development of multiple tissues. The so far reported mechanisms of CILK1 functions involve regulation of cilia architecture, intraflagellar transport, cell proliferation and signaling. In this study, we show that CILK1 is involved in centriole biology. Apart from the involvement in building primary cilia, CILK1 is also required for centriolar engagement, and for blocking ectopic centriolar amplification. The supernumerary centrioles produced due to loss of CILK1 fail to cluster during mitosis and result in multipolar spindles and aneuploidy. Altogether our data suggests a novel function of CILK1 and indicates its potential involvement in cancer.

30. A new reporter system for the identification of eIF4F inhibitors: validation of the compounds identified in the high-throughput screening

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The role of the eIF4F translation initiation complex in cancer is crucial. Its enhanced activity was identified as a nexus of therapy resistance and a promising target in melanoma [1,2]. However, the spectrum of available eIF4F inhibitors is limited, with none of them in clinical use. One reason could lie in the relative complexity of techniques used to identify such inhibitors [3].

Thanks to our finding that eIF4F functions in the negative regulation of the RAS/RAF/MEK/ERK mitogen-activated protein kinase (MAPK) signaling pathway [4], we were able to build a unique cell-based reporter system suitable for the high-throughput identification of novel eIF4F inhibitors in small-molecule compound libraries. The system's response to eIF4F inhibition is dose-dependent and is detected by changes in luciferase expression.

The reporter system was tested in a panel of cell lines, determining the impact of eIF4F inhibition on luciferase activity. Its applicability for the high-throughput screening (HTS) of bioactive compounds was verified in 384- and 1536-well format. Several highly diverse libraries comprising over 80 thousand compounds were assessed in the HTS. Surprisingly, apart from compounds potentially targeting eIF4F, the screening revealed possible new ways of ERK pathway regulation. The most potent compounds are currently undergoing validation in orthogonal assays and follow-up experiments.

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31. Modulation of fibroblast growth factor signaling for future therapies

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Fibroblast growth factor receptors (FGFRs) are transmembrane receptors that regulate many cellular processes such as growth, differentiation, metabolism and survival. The main downstream signaling pathway activated by FGFRs is the ERK MAP kinase pathway, through which FGFRs control gene expression. The FGFR family consists of four receptors (FGFR1-4), which are present in most cells from the early stages of embryonic development. Impaired FGFR signaling, which is often caused by an activating mutation in the receptor itself, leads to numerous developmental disorders and also to cancer. Tyrosine kinase inhibitors (TKIs) have been considered as a potential therapy, but their low specificity at high concentrations is problematic for cancer treatment. In contrast, low dose TKIs may be used to treat FGFR-related developmental disorders such as achondroplasia. In this project, we are investigating different approaches to interfere with FGFR-ERK signaling, including TKIs, FGFR-specific DNA aptamers or the induction of stress in the endoplasmic reticulum. Ultimately, our experiments also lead to better understanding of the signaling mechanisms of individual FGFR variants, which in turn may contribute to the development of more targeted drugs.

32. eIF4F controls AMPK activity in BRAF^{V600E}-mutant melanoma

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Malignant melanoma is an aggressive cancer often driven by MAPK/ERK pathway mutations. Although BRAF and MEK inhibitors improve survival, resistance emerges rapidly. The eukaryotic translation initiation complex eIF4F has been recently implicated as a key mediator of this resistence and combined BRAF and eIF4F inhibition showed synergistic anti-tumor effects [1].

We previously identified eIF4F as a critical regulator of ERK pathway signaling flux in malignant melanoma. eIF4F maintains normal cellular levels of DUSP6, a major negative regulator of ERK. Inhibition of eIF4F promoted a rapid decrease in DUSP6 and subsequent ERK hyperactivation [2]. Here, we report the unexpected concomitant activation of both ERK and AMPK pathways in BRAF-mutant melanoma cells following eIF4F inhibition, despite the known negative feedback between ERK and LKB1, canonical AMPK activator [3].

Treatment with eIF4F inhibitors induced AMPK activation and ERK hyperactivation *in vitro* and *in vivo*. AMPK activation occurred through a non-canonical, LKB1-independent mechanism, as demonstrated in LKB1-deficient cells and through LKB1-knockdown experiments. Moreover, eIF4F inhibition decreased the expression of both canonical and alternative AMPK regulators. Proteomic analysis identified PP2A as a key eIF4F target. Both pharmacological PP2A inhibition and targeted PP2A knockdown induced AMPK activation in melanoma cells, confirming the vital role of the eIF4F-PP2A axis in limiting AMPK activity.

Collectively, these results provide a new mechanistic insight into the anti-cancer activity of compounds targeting eIF4F and indicate a therapeutic potential for small molecule PP2A inhibitors to activate the growth-inhibitory AMPK signaling in melanoma regardless of the LKB1 status of cancer cells.

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