

Poster presentations

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German Stem Cell Network

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Max Delbrück Center
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Thursday, 10 September

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- P111** Improving cross-species comparability of human and cynomolgus macaque iPSCs
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- P113** Electroporation of cortical organoids for functional investigation of neurogenesis
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- P116** Modelling the molecular basis of human speech evolution
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Gökçe Nihan Yıldırım
- P135** Biobanking and stem cell technologies across species for conservation and basic research
Vera Zywitzka

Abstract No. P088**Development of a scalable stirred-tank bioprocess for large-scale manufacturing of human iPSC-derived cardiac organoids***Ebru Aksoy*, Sarkawt Hamad, and Kurt Pfannkuche*

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*Presenting author

Scalable manufacturing of human induced pluripotent stem cell (hiPSC)-derived cardiac organoids remains a major bottleneck for their translational application. We established an in-house cardiac organoid differentiation protocol and successfully scaled the process from a 100 mL to a 1 L working volume in stirred-tank bioreactors.

Dissolved oxygen (DO) and pH were continuously monitored throughout differentiation to characterize process dynamics during scale-up. Daily morphological assessment, flow cytometry and whole-mount immunostaining were used to evaluate organoid development and differentiation.

With the optimized process, we could reliably produce about 70,000 spontaneously beating cardiac organoids. Online monitoring helped us understand how DO and pH changed during differentiation, which will help us improve the process further. Flow cytometry and whole-mount immunostaining showed that cardiac organoid differentiation was successful after scaling up, and the organoids kept their shape and function even after increasing the working volume tenfold.

This study presents a scalable stirred-tank process for making hiPSC-derived cardiac organoids and establishes a basis for standardized large-scale production to support future regenerative medicine applications.

Abstract No. P089**Generation of vascularized human induced pluripotent stem cell (hiPSC) derived lung organoids**

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Lung organoid models derived from human and murine stem cells have become a powerful tool to study lung development and disease. Recently, the cellular and structural complexity of lung organoid models has increased significantly. However, a lung organoid system with a vascular compartment is still missing. Here, we describe a method to generate hiPSC-derived lung bud tip organoids with an integrated endothelial compartment. hiPSC were differentiated into CXCR4, CKit, FOXA2 and SOX17 expressing endoderm, further polarized into anterior foregut endoderm followed by the induction of lung progenitors. Lung progenitors were then enriched by sorting for NKX2.1+ cells. In parallel, hiPSC were differentiated into functional, tube forming CD31+CD144+CD34+CD105+ endothelial cells (ECs). Co-culture of sorted NKX2.1+ lung progenitors with ECs resulted in the formation of complex branched lung organoids containing NKX2.1+SOX2+SOX9+ lung bud tip progenitors, as well as differentiated cells, such as MUC5A+ goblet cells and SFTPC+ alveolar epithelial type 2 cells (AEC2). After a few days, organoids were surrounded by complex, lumen forming endothelial networks, closely interacting with the organoid. Co-culture of the sorted lung progenitors with ECs significantly increased organoid size, indicating epithelial-endothelial cell crosstalk.

To study the role of the lung vascular compartment during cancer, the vascularized organoids were microinjected with either non-small-cell-lung cancer or melanoma cells. Seven days after microinjection, tumour cells were engrafted, forming several tumours within the organoid. Furthermore, injected melanoma cells exhibited characteristic angiotropism by closely aligning with the endothelial network around the organoids.

In summary, we were able to generate complex hiPSC-derived lung organoids containing a vascular compartment which can be used to study epithelial-endothelial interactions and tumour-lung-endothelial cell interactions.

Abstract No. P090**Improving reproducibility and scalability in human organoid culture using AI-engineered proteins and a defined synthetic hydrogel***Volker Arps*

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Three-dimensional cell and organoid culture systems are key platforms for therapeutic screening and disease modeling. These models rely on animal-derived matrices, notably basement membrane extract (BME), and unstable growth factors such as FGF-10 and Wnt-3a1. These reagents pose manufacturing challenges, poor reproducibility, high costs, and no opportunities for clinical translation due to risk of contaminants. To address these limitations, a two-pronged strategy was used: (1) a novel variant of FGF-10 and a Wnt/RSPO (R-Spondin) agonist⁴ with improved thermostability and solubility were developed through AI-driven protein engineering and mutation library screening and (2) a fully defined matrix, Cultrex Synthetic Hydrogel, was adopted to replace BME.

Human adult intestinal, liver, and lung organoids and iPSC-derived pancreatic duct-like organoids (PDLOs) were cultured with these reagents. The data revealed robust growth across multiple organoid types while maintaining key markers and morphology. The Wnt-agonists more efficiently induced β -catenin signaling at reduced concentrations relative to wild-type Wnt-3a. Further, thermostable FGF-10 supported healthy organoid culture without needing additional supplementation. These findings support the advantages of combining thermostable FGF-10 and Wnt agonists with a synthetic hydrogel to generate supportive growth environments for diverse organoid models.

Abstract No. P091**Patient-derived hiPSC sensory neurons reveal increased neuronal activity in paroxysmal extreme pain disorder**

Lena Braun^{1, 2,*}, Esther Eberhardt^{1, 2}, Marcelo A Szymanski de Toledo^{3, 4}, Anika Neureiter^{1, 2}, Petra Hautvast^{1, 2}, and Angelika Lampert^{1, 2}

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Paroxysmal Extreme Pain Disorder (PEPD) is a rare inherited pain syndrome caused by gain-of-function variants in *SCN9A*, encoding the voltage-gated sodium channel Nav1.7. Previous studies in heterologous expression systems have linked PEPD-associated variants to impaired channel inactivation, enhanced resurgent sodium currents, and increased neuronal excitability^{1,2}.

Here we investigate patient-specific human induced pluripotent stem cell (hiPSC)-derived sensory neurons (iSNs) carrying the heterozygous missense variant Nav1.7 p.V1299F. The patient presents with typical clinical PEPD symptoms, including recurrent episodes of severe pain and skin flushing. We generated an isogenic wild-type hiPSC line using CRISPR/Cas12a-mediated genome editing and differentiated hiPSCs into iSNs by forced NGN1 overexpression. iSNs were electrophysiologically analyzed using whole-cell patch-clamp.

Patient-derived iSNs showed increased spontaneous activity compared with isogenic control neurons (33%, n = 66 vs. 20%, n = 54). Furthermore, patient-derived neurons exhibited a significantly reduced rheobase and a trend toward higher action potential firing frequencies in response to current injection. Ongoing voltage-clamp experiments aim to determine whether these changes are associated with altered Nav1.7 channel properties, particularly enhanced resurgent sodium currents.

In summary, patient-derived sensory neurons recapitulate features of the PEPD gain-of-function phenotype, including increased neuronal activity and reduced rheobase. This hiPSC-based model provides a human platform to study the contribution of mutant Nav1.7 channels to pain pathophysiology and to explore novel therapeutic strategies.

Theile, J. W., Jarecki, B. W., Piekarz, A. D. & Cummins, T. R. Nav1.7 mutations associated with paroxysmal extreme pain disorder, but not erythromelalgia, enhance Nav64 peptide-mediated resurgent sodium currents. J. Physiol. 589, 597–608 (2011).

Dib-Hajj, S. D. et al. Paroxysmal Extreme Pain Disorder M1627K Mutation in Human Nav 1.7 Renders DRG Neurons Hyperexcitable. Mol. Pain 4, 1744-8069-4–37 (2008).

Abstract No. P092/T42**Modeling primate atherosclerosis using iPSC-derived macrophages***Eva Briem^{1,*}, Alex L. Starr², Dana Lopez¹, Hunter Fraser², and Wolfgang Enard¹*¹LMU Munich, Faculty of Biology²Stanford University, Department of Biology, California, USA

*Presenting author

Atherosclerosis is a chronic inflammatory disease characterized by lipid-driven plaque formation within the arterial wall and is one of the leading causes of death worldwide. Early stages of plaque development involve macrophages, which become lipid-laden foam cells upon uptake of oxidized low-density lipoprotein (oxLDL).

While the cellular and molecular mechanisms of atherogenesis have been extensively studied in mice and humans, far less is known about how these processes differ across primate species and how they have evolved. Non-human primates (NHPs), particularly chimpanzees, are of special interest in this context due to their close evolutionary relationship to humans and reported differences in susceptibility to atherosclerotic plaque formation.

Here, I present an in vitro model of foam cell formation from induced pluripotent stem cell (iPSC)-derived macrophages. It enables the differentiation of NHP iPSCs into macrophages and the investigation of their oxLDL uptake in vitro. The use of fused human-chimpanzee iPSCs — a tetraploid composite cell line containing human and chimpanzee genomes — enables the investigation of regulatory activity within a shared trans-regulatory environment. This allowed us to identify gene expression differences between human and chimpanzee macrophages during oxLDL uptake, attributable to independent cis-regulatory changes between species. These changes were significantly enriched, especially in the cholesterol efflux pathway, with chimpanzee macrophages exhibiting elevated expression of multiple efflux-associated genes upon oxLDL exposure. These results indicate evolutionary changes in the regulatory architecture of lipid-handling pathways. Ongoing functional assays will determine whether these transcriptional differences translate into altered cholesterol efflux capacity.

This system provides a framework for investigating how regulatory development influences human susceptibility to atherosclerosis and could ultimately reveal conserved and species-specific mechanisms relevant to future prevention and treatment strategies.

Abstract No. P093**Synthetic peptide growth factors and cytokines enable generation of iPSC-derived immune cells**

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Manufacturing of cell therapy products typically relies on recombinant growth factors, cytokines, and serum-derived supplements to support cell expansion and differentiation. While these biologics have enabled significant advances, their batch-to-batch variability and dependence on animal- or cell-derived systems present challenges for process standardization, scalability, and reproducibility. Developing chemically defined alternatives that replicate essential signaling pathways is therefore important for more controllable cell manufacturing.

Here, we investigated synthetic peptide growth factor and cytokine mimetics as substitutes for conventional biologics. These peptides are designed to activate signaling pathways analogous to those triggered by native factors while offering advantages in chemical definition and stability.

As a demonstration, human iPSCs were differentiated into natural killer T (NKT) cells via a hematopoietic stem and progenitor cell (HSPC) intermediate. Synthetic peptide growth factors corresponding to Wnt3a, TPO, and VEGF were used to induce differentiation into HSPCs, followed by differentiation into NKT cells. Peptide conditions accelerated differentiation, shortening the process by one day to completion within 10 days, while approximately doubling cell yield without altering HSPC surface marker expression. The resulting NKT cells showed functional properties comparable to those generated using recombinant factors. Moreover, terminal maturation of NKT cells was achieved using an IL-15 alternative peptide, which promoted robust expansion and maturation comparable to recombinant IL-15.

These findings demonstrate that synthetic peptide mimetics can support differentiation and expansion of iPSC-derived immune cells in a defined system without serum or recombinant proteins, highlighting their potential for standardized cell therapy manufacturing.

Abstract No. P094**Community driven-consolidation of the vast expanse of stem cell differentiation protocol literature***Rafaella Buzatu*, Tiago Scarano, Christian Schröter, and Micha Drukker*

Leiden Academic Centre for Drug Research (LACDR), Leiden University, The Netherlands

*Presenting author

Humanity's knowledge of induced and embryonic pluripotent stem cell (i/ePSC) differentiation spans a vast and largely empirical literature that remains heterogeneous and poorly standardized. Unifying this fragmented knowledge could yield crucial insight into the molecular logic of stem cell differentiation decisions. As a first step towards this goal, we establish a community-driven harmonization effort to consolidate differentiation protocols for human i/ePSCs. We developed an online community platform to organize differentiation steps and experimental readouts into a harmonized database, populated using AI-assisted text extraction and curated by community volunteers. The platform captures protocol steps, durations, reagents, culture media, vendors, compound concentrations, and differentiation readouts. We applied this framework to ~200 curated articles, enabling systematic comparison of protocols across the literature and connecting them into a longitudinal branching differentiation landscape. Together, this work establishes a searchable database linking protocol parameters to cellular phenotypic outcomes, enabling data-driven and AI-assisted optimization of i/ePSC differentiation.

Abstract No. P095**Promoting survival and maturation of ASCL1-induced basket cell-like neurons by tuned ASCL1 expression and MEF2C overexpression**

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Efficient fate conversion of non-neuronal cells by overexpression of neurogenic transcription factors (TFs) such as *Ascl1* holds promise as a source of induced neurons (iNs) for basic and translational research purposes. Our ability to promote iN survival and to instruct the acquisition of molecular, morphological and functional properties typical of endogenous neurons is, however, still limited. Overexpression of a mutant form of *Ascl1* (*Ascl1SA6*) converts cortical glia to an inhibitory neuron-like state characterized by marker expression and functional features typical of cortical fast-spiking interneurons such as Parvalbumin-positive Basket cells, while retaining broadly immature morphological characteristics. To test the hypothesis that persistent overexpression of *Ascl1SA6* and lack of expression of TFs responsible for developmental subtype specification may hinder iN maturation, we modulated *Ascl1SA6* expression and overexpressed *Mef2c*, a TF essential for Parvalbumin-positive interneuron specification and whose expression is maintained in mature neurons. Overexpression of *Mef2c* alongside *Ascl1SA6* generated iNs displaying markedly more complex morphologies and progressively decorating the soma and dendrites of nearby iNs with extensive arborizations, resembling endogenous Basket cells. Crucially, however, while *Ascl1SA6* was necessary for efficient reprogramming, its expression at high levels interfered with the acquisition of *Mef2c*-induced features. To test if reducing *Ascl1SA6* expression following reprogramming initiation, alone or in combination with *Mef2c* overexpression, might suffice to promote iN maturation, we employed a published microRNA-based self-regulating system, thus negatively coupling acquisition of neuronal identity and *Ascl1SA6* expression. In vitro experiments show *Ascl1SA6* expression to be significantly and selectively dampened among iNs, with a concomitant long-term increase in iN survival. Ongoing experiments are assessing if and how these effects may be compounded by concomitant *Mef2c* overexpression.

Reprogramming astroglia into neurons with hallmarks of fast-spiking parvalbumin-positive interneurons by phospho-site-deficient Ascl1; 10.1126/sciadv.adl5935

Abstract No. P096**Mapping the marker landscape of early germ layer differentiation for stem cell quality control and experimental planning**

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*Presenting author

Human pluripotent stem cell quality control may be perceived as a necessary obstacle delaying visionary researchers from performing transformative experiments. However, when carefully designed and streamlined, it can provide valuable biological insight into stem cell identity, differentiation capacity, and experimental reproducibility. We recently reported the need to curate and update marker gene lists for gene expression-based quality control assays and extensively validated hiPSCore, a machine learning-based functional pluripotency classifier. In parallel, we established an expanded set of validated marker genes for early induced pluripotent stem cell quality control.

Building on these studies, we substantially expanded the hiPSCore database by incorporating additional cell lines, differentiation states, culture media, and matrix compositions, thereby enabling a broader and more practical assessment of pluripotency and early differentiation competence across diverse experimental conditions. We further demonstrate that hiPSCore can reduce time and costs in the development of advanced three-dimensional model systems for drug testing by identifying suboptimal differentiation trajectories before resource-intensive experiments are initiated. Finally, we show that hiPSCore is broadly applicable to quality control scenarios, including cell lines reported to fail conventional pluripotency assays.

Together, our findings highlight that stem cell quality control is not an end in itself, but a powerful strategy to improve experimental success, reduce unnecessary costs, and uncover biologically meaningful variation in pluripotent stem cell differentiation potential.

Dobner, J., Diecke, S., Krugmann, J. et al. Reassessment of marker genes in human induced pluripotent stem cells for enhanced quality control. Nat Commun 15, 8547 (2024). <https://doi.org/10.1038/s41467-024-52922-1>

Defining early human developmental identity: A curated, cross-platform marker framework Jochen Dobner, Alessandro Prigione, Andrea Rossi bioRxiv 2026.03.26.714482; doi: <https://doi.org/10.64898/2026.03.26.714482>

Abstract No. P097**Nuclear and chromatin architecture & mechanics in stem cell fate regulation**

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Cells continuously sense and respond to a dynamic combination of biochemical and mechanical cues from their microenvironment. These signals integrate at the nuclear periphery, where they regulate genome organization and influence cell state. The mechanisms by which mechanical and biochemical cues integrate to drive stem cell state transitions remain incompletely understood.

Here, I investigate how dimensionality and biochemical signals jointly regulate the exit from primed pluripotency in human induced pluripotent stem cells. I show that transitioning cells from a 2D, ECM-adherent culture to a 3D, ECM-free environment accelerates the exit from primed pluripotency and induces a symmetry-breaking event characterized by the emergence of nuclear YAP- and E-cadherin-high cell clusters. Furthermore, the transition to a 3D topology triggers a significant reduction in nuclear volume accompanied by sustained remodeling of chromatin architecture, ultimately promoting cell state specification. Remarkably, re-establishing 2D culture after 3D aggregation preserves both the symmetry-broken phenotype and the associated chromatin landscape, suggesting the establishment of a persistent mechanical memory.

Together, these findings demonstrate that topological and biochemical cues remodel nuclear architecture and chromatin organization to direct stem cell state dynamics.

Stephens, R. K., & Miroshnikova, Y. A. (2024). Nuclear periphery and its mechanical regulation in cell fate transitions. Current opinion in structural biology, 87, 102867. <https://doi.org/10.1016/j.sbi.2024.102867>
Pecori, F., & Torres-Padilla, M. E. (2023). Dynamics of nuclear architecture during early embryonic development and lessons from liveimaging. Developmental cell, 58(6), 435–449. <https://doi.org/10.1016/j.devcel.2023.02.018>

Abstract No. P098**Understanding the mechanisms controlling human pancreas organogenesis using a new in vitro platform***Daniella Fradin^{1,*}, Irene Talon¹, and Ludovic Vallier^{2,3}*¹ Max Planck Institute for Molecular Genetics² Berlin Institute of Health, BIH Centre for Regenerative Therapies, Berlin³ Max Planck Institute for Molecular Genetics, Berlin.

*Presenting author

Understanding early pancreas organogenesis is essential to reveal the mechanisms resulting into developmental disorders and to develop regenerative therapies. However, early pancreas organogenesis remains relatively unexplored, especially in humans, due to ethical and technical reasons. As a result, much of our current knowledge has been built on studies in mouse models, and the mechanisms by which signaling pathways and transcriptional networks coordinate to establish distinct lineages in humans remain to be fully uncovered. Here, we established a novel human induced pluripotent stem cell (hiPSC)-derived foregut culture system that recapitulates a critical window of early lineage specification, simultaneously giving rise to multiple progenitor populations with pancreatic (*SOX9*, *PDX1*, *NKX6.1*), biliary (*CK19*, *CK7*), and hepatic (*HNF4a*, *AFP*) identity. Using this platform, we investigated the effect of oxygen tension in early pancreatic organogenesis. By systematically modulating oxygen levels (hypoxia 5% O₂ vs. normoxia) we demonstrate that low oxygen tension inhibits early pancreatic lineage specification. To further validate these observations, we pharmacologically stabilized hypoxia-inducible factors (HIFs) with an HIF-prolyl hydroxylase inhibitor and confirm that specific progenitor fates can be selectively promoted or suppressed by manipulating signalling pathway downstream of hypoxia. These changes in cell identity are accompanied by a modification of cell size and number suggesting an involvement of cell cycle regulations and/or mechanosensing mechanisms. To confirm these hypotheses, we are currently performing single cell RNA analysis during pancreatic differentiation using different oxygen tension conditions. Finally, pancreatic progenitors generated in these culture conditions can form organoids which self-organized into branching structure resembling early pancreatic bud. Overall, our results provide a new hiPSC-derived differentiation system to investigate how oxygen levels and HIF-driven gene programs contribute to the network of signals that guide further pancreatic development. This platform opens new possibilities for understanding how the local environment can influence cell fate choice during early human organogenesis.

Abstract No. P099/T02**Mosaic gastruloids reveal a temporal restriction for developmental cell competition**

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The selective elimination of suboptimal cells is critical for maintaining developmental integrity during early mammalian embryogenesis. Toward this goal, cells undergo **Cell Competition**, a **non-autonomous quality control** in which “winner” cells outcompete viable but suboptimal “loser” cells based on relative fitness differences. Here, we use **mosaic 3D mouse gastruloids** to investigate cell competition dynamics. Introducing just two Trp53-deficient supercompetitor cells per gastruloid suffices to impair growth in neighboring wild-type cells through mitochondrial **apoptosis**. Competition is tightly restricted to a developmental transition-stage between primed pluripotency and early gastrulation and involves gene regulatory networks of pluripotency-exit. Heterochronic gastruloids from developmental stage-shifted cells, EpiGastruloids, and dynamic p53-degrons reveal that both winners and losers must reside within this permissive stage, during which acute relative p53 protein levels determine competitive outcomes. These findings advance our understanding of cell fitness evaluation and establish gastruloids as a powerful 3D-model for investigating developmental stage-specific cell competition in mammalian embryogenesis.

Abstract No. P100**Genetically engineered research tools based on extensively characterized control hiPSCs**

Christian Freund^{1,*}, Babet van der Vaart¹, Albert Blanch-Asensio², Christiaan Arendzen¹, Laura Blijleven¹, Emile Nühn¹, Herak Manjikian¹, Jacyra Duinkerken¹, Xenia Theodorou¹, Christine Mummery¹, Richard Davis¹, and Niels Geijsen¹

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The Leiden hiPSC Centre offers control hiPSC lines LUMC0099iCTRL and LUMC0065iCTRL generated from a healthy female and male donor with an updated informed consent including various research applications. In addition to the standard characterization (pluripotency status, functional pluripotency, cell identity, karyotype) these lines have undergone microbiology testing, transcriptome analysis, WGS and HLA typing, have been registered at hPSCreg (LUMCi004-A and LUMCi031-A, respectively) and have been used successfully in various research projects by multiple users.

Furthermore, we equipped both lines with STRAIGHT-IN landing pads in safe harbor loci AAVS1 and CLYBL allowing for efficient integration of large DNA payloads. Currently we use these STRAIGHT-IN hiPSCs for developing various research tools for a wide range of applications such as

- 1) hiPSCs with a Doxycycline-inducible transcriptional activator or repressor (CRISPRa/i): Preliminary data showed that targeting SOX2 in CRISPRi hiPSCs leads to a reduction of pluripotency markers Nanog and TRA-1-60 assessed by FACS after 3 days,
- 2) hiPSCs with Doxycycline-inducible transcription factors for forward programming: in a proof-of-concept doxycycline-induced overexpression of transcription factor MyoD1 was able to efficiently differentiate hiPSCs into myogenic progenitors based on morphology and expression of MHC and
- 3) hiPSCs equipped with the split-fluorescence system for efficient generation of reporter hiPSCs. In a first step, the sequence of the large fragment of mNeongreen (mNG2) was integrated into AAVS1 where it is constitutively expressed but non-fluorescent. We then targeted histone H2B with the short mNG2 fragment using a ssODN. Expression of H2B in undifferentiated cells led to a fully functional mNG2 resulting in hiPSCs with green, fluorescent nuclei. We are currently further validating the system. In addition, we are adding more colors and are generating hiPSCs with two colors which can be used for the generation of dual reporters.

Abstract No. P101**Human induced pluripotent stem cell differentiation for in vitro skin modelling**

Ronja Friede^{1,*}, Julia Hunkemöller², Elisabeth Rehbein-Bode², Nadine Brandes³, Sebastian Schröder², and Doris Heinrich⁴

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The skin, one of the most important organs of the human body, protects internal tissue from external influences. In case of disorders, personalized medicine can support efficient treatment. Patient-specific skin equivalents can be used for drug testing and the investigation of disease progression. However, skin equivalents from isolated primary cells lack complexity and are limited in number, due to the demanding maintenance and amplification of primary keratinocytes. Therefore, human induced pluripotent stem cells (hiPSC), as an abundant cell source, can be used to derive skin cells and to generate personalised skin equivalents.

Protocols for the differentiation of hiPSCs towards dermal fibroblasts and epidermal keratinocytes were established, followed by characterisation of the derived cells. Both, hiPSC-derived keratinocytes (hiPSC-dK) and hiPSC-derived fibroblasts (hiPSC-dF), were characterised by qPCR and immunofluorescence (IF) staining and compared to primary cells. Functionality was further evaluated in terminal differentiation of hiPSC-dK and in 3D-models. Terminal differentiation was induced by increasing the extracellular calcium levels, resulting in involucrin/keratin 1-positive cells, validated in qPCR and IF staining. Functional skin equivalents were generated using microporous scaffolds, promoting adhesion, proliferation, and the synthesis and secretion of extracellular matrix (ECM) proteins. hiPSC-dF were seeded on these scaffolds. After 14 days of culture, formation of a dermal equivalent was observed, and the secretion of ECM proteins was investigated via IF staining. hiPSC-dK were seeded onto dermal and stratified at air-liquid interface for 21 days. Stratification resulted in the formation of the epidermal layers stratum basale (keratin 5/14-positive cells) and stratum spinosum (involucrin/keratin 1-positive cells).

In conclusion, we developed an in vitro skin model that consists solely of hiPSC-derived skin cells and has the potential to be a patient-specific disease model.

This model provides the foundation for inclusion of early-stage hiPSC-dK, potential epidermal stem cells, leading to a more physiological skin model in the future.

Abstract No. P102**Foreign mitochondria, fragile stem cells: a hidden challenge for human naïve stem cell culture***Ana Lourdes Gama Manon^{1,*}*¹ Oakland University

*Presenting author

Human pluripotent stem cells (hPSCs) rely on tightly regulated mitochondrial function to maintain pluripotency, metabolic balance, and differentiation potential. Although feeder layers such as mitotically inactivated mouse embryonic fibroblasts (iMEFs) remain widely used, it has remained unclear whether xenogeneic mitochondria can transfer to hPSCs and influence their bioenergetic state. Here, we addressed this gap by investigating both direct and indirect mitochondrial transfer from iMEFs to hPSCs through imaging, flow cytometry, species specific PCR, nanoparticle tracking analysis, and metabolic flux assays. We discovered that iMEFs transfer mitochondrial material to hPSCs through contact-dependent tunneling nanotube-like structures, but mainly and through extracellular vesicle-associated mitochondria (EVM) enriched in conditioned medium. The transfer, of EVMs yielding a high frequency uptake of mouse mitochondria in recipient cells, which was and detectable by mouse COX2 mtDNA expression in recipient cells. Although the xenogeneic mtDNA is gradually lost over subsequent passages, its transient presence coincides with disrupted mitochondrial membrane potential, reduced oxidative ATP production, compensatory glycolysis, and selective changes in lineage associated gene expression. These findings establish xenogeneic mitochondrial transfer as a biologically meaningful modifier of stem cell function, revealing a previously underappreciated source of variability in feeder based systems. This work highlights and supports the importance of xenofree, defined culture conditions to preserve mitochondrial stability, minimize culture-derived variability, improve experimental reproducibility, and strengthen the translational robustness of hPSC based applications.

Abstract No. P103**Deciphering the role of NR2F1 in craniofacial and brain development: from locus-specific regulation to genome-wide impact**

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NR2F1 is a developmental transcription factor with essential roles across multiple tissues, including the brain, visual system, and craniofacial structures. Heterozygous mutations or deletions of *NR2F1* cause Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS), an autosomal dominant neurodevelopmental disorder characterised by developmental delay, visual system and craniofacial abnormalities.

The pleiotropic role of NR2F1 is well established; however, its molecular pathway in brain and neural crest tissues remains to be elucidated. To address this, we CRISPR/Cas9-engineered human induced pluripotent stem cell (hiPSC) lines carrying an *NR2F1* deletion, leading to reduced *NR2F1* expression. Time-resolved transcriptomic analysis of hiPSC-derived neural progenitor cells (NPCs) and early cortical neurons (CNs) to mimic brain development, as well as cranial neural crest cells (CNCCs) modelling craniofacial structures, identified key biological processes sensitive to *NR2F1* dosage and reflecting BBSOAS phenotypes/characteristics. These processes include genes that shape specific programs related to neurodevelopment (*SOX1*), craniofacial programs (*COL2A1*), as well as shared (*CDON*, *MSX2*) features, highlighting the relevance of *NR2F1* dosage in BBSOAS.

To further assess the role of *NR2F1* dosage, we investigated the role of cis-regulatory elements neighboring *NR2F1*, including the adjacent to *NR2F1* divergently transcribed long non-coding RNA (lncRNA) *lncNR2F1*. CRISPR/Cas9 deletion of *lncNR2F1* resulted in altered *NR2F1* expression across NPCs/CN and CNCCs, suggesting the presence of a cis-regulatory element located within the *lncNR2F1* locus. Transcriptomic analysis revealed both *lncNR2F1*-specific and some overlapping features, with those affected by *NR2F1* loss. To dissect NR2F1 targets affected by *NR2F1* deletions and *lncNR2F1* deletions, we are performing time-resolved ChIP-seq from NPC/CN and CNCC. We aim to determine *NR2F1* dosage-dependent regulatory networks across brain and craniofacial tissue types. Our data will provide novel insights into BBSOAS pathophysiology that may contribute to improved molecular diagnosis and facilitate the development of novel therapeutic approaches.

Abstract No. P104**Establishment of human immune-competent induced pluripotent stem cell-derived lung organoids to model alveolar macrophage niche during development and disease**

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In the last decade, human organoids have become useful models for lung development, physiology, and disease modeling. However, these systems often lack the tissue-specific immune cell compartment. The crosstalk between the lung epithelium and alveolar macrophages is crucial for proper lung function during homeostasis and disease. This study aims to establish a reliable protocol to generate human induced pluripotent stem cell (hiPSC)-derived immune-competent lung organoids for disease modeling. For this purpose, hiPSCs were differentiated into definitive endoderm, verified by the expression of C-Kit, CXCR4, FOXA2, and SOX17. Endodermal cells were further differentiated into anterior foregut endoderm and subsequently into NKX2.1-expressing lung progenitors. NKX2.1+ progenitors were purified via cell sorting of NKX2.1GFP reporter or CD26lowCD47high cells. To generate hiPSC-derived precursor macrophages (pre-Macs), embryoid bodies were formed, differentiated into mesoderm, and subsequently into hemogenic endothelium. The resulting myeloid-cell-forming complexes generated CD45, CD14, CD11b, and CD163 expressing pre-Macs as shown by flow cytometry. Lung bud tip organoids or alveolospheres were generated by culture of NKX2.1+ lung progenitors in the presence or absence of pre Macs. Immunofluorescence staining of the co-cultures revealed that the pre-Macs engrafted into the lung bud tip organoids. Moreover, co-cultures of lung progenitors and pre-Macs resulted in higher organoid formation efficiency compared to lung progenitors alone. These data indicate that the crosstalk between epithelial cells and macrophages is beneficial for organoid growth and survival. Our ultimate goal is to utilize the immune-competent organoids to investigate the interplay between lung epithelial cells and alveolar macrophages within lung development and disease.

Abstract No. P105**GMP-compliant iPSC banking and robust cardiac differentiation platform for clinical translation**

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Background: iPSC-based cardiac therapies require genomically stable GMP starting materials and robust, scalable differentiation protocols. We have recently validated a new single-cell passaging-based banking process and refined our cardiac platform toward clinical readiness.

Methods: Single-cell passaging with Rho kinase inhibition (ROCKi) generated GMP master banks from cord blood (R34 EU; R35/R36 US; R38 pipeline). QC included karyotyping, ddPCR, WGS, and cancer-gene sequencing. The cardiac platform features WNT/BMP/FGF/TGF β co-stimulation, optimized media changes, 3D compatibility, single-cell dissociation/re-aggregation, GMP reagents, and EHT contractility assays. Cryopreservation efficiency was assessed post-dissociation.

Results: The new GMP iPSC lines met all release criteria with acquired pluripotency, normal karyotypes, no critical cancer mutations, and low overall mutation burden. Interestingly, ROCKi served to mitigate - not enhance - selective pressure on the cells, thereby contributing to preserved genomic integrity. This conclusion was based on competition experiments between wild-type iPSCs and mutant clones with duplicated chr20q11, thereby providing formal evidence for the claim.

In the cardiac induction protocol, all lines displayed >90% cardiomyocyte (CM) purity indicating *bona fide* cell line-independence. The weekend-free platform further showed high batch-to-batch robustness and scalability. Recent advancements include the incorporation of a cryopreservation step following single-cell dissociation of the primary CM aggregates, and successful transition to GMP reagents, and development of contraction force-based potency assay.

Conclusions: Validated GMP iPSC banking plus the optimized cardiac platform deliver genetically stable, high-purity, functional cardiomyocytes with GMP compatibility and cryopreservation readiness—providing a streamlined path to clinical cardiac cell therapy.

Abstract No. P106**Characterizing COQ4-associated neuronal phenotypes using CRISPR-edited hiPSC derived neurons**

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Mitochondrial dysfunction is increasingly recognized as a major contributor to both neurodevelopmental and neurodegenerative disorders. Coenzyme Q10 (CoQ10) is a central component of the mitochondrial electron transport chain and a key determinant of cellular ATP production, redox balance, and mitochondrial homeostasis.

Pathogenic variants in COQ4, a gene encoding an essential enzyme in the endogenous CoQ10 biosynthesis pathway, cause primary coenzyme Q10 deficiency-- 7 (OMIM #616276) and have been associated with severe neurological phenotypes. However, the precise effects of COQ4 mutations on human neuronal development, maturation and network function remain incompletely understood, and clinical benefit from CoQ10 supplementation is often limited, underscoring the need for more informative human cellular models.

Here, we apply CRISPR/Cas9-engineered isogenic human iPSC lines carrying homozygous pathogenic COQ4 variants alongside matched controls to examine neuronal differentiation, mitochondrial integrity and functional network behavior in a human-relevant system. Using a multimodal approach, we aim to evaluate how COQ4 dysfunction shapes cellular phenotype and emergent network activity during neuronal maturation. That approach includes morphological and immunofluorescent assessment of neural progenitor and neuronal markers, quantitative RT PCR profiling of neuronal and synaptic genes, mitochondrial membrane potential measurements, and high-density microelectrode array recordings. Beyond characterizing disease-relevant phenotypes, this platform is designed to support translational follow-up: it can be used to screen pharmacological interventions and test candidate compounds for their capacity to rescue mitochondrial and electrophysiological deficits.

Collectively, these studies seek to facilitate mechanistic insight and preclinical evaluation to further potential therapeutic avenues for COQ4-associated neurological disease.

Abstract No. P107**Beyond viability: evaluating post-thaw recovery in iPSC-derived and 3D cell culture workflows***Mariya Hrynychak*

NIPPON Genetics EUROPE, Düren

While immediate post-thaw viability is an important quality indicator, the practical success of cryopreservation in induced pluripotent stem cell (iPSC), stem cell-derived, progenitor cell, cardiomyocyte, and 3D culture workflows is often determined by whether usable cultures can be re-established after thawing. Relevant indicators include attachment, morphology, recovery behavior, expansion, and, where applicable, functional readouts.

Across stem-cell-related cryopreservation settings, Bambanker™, an FBS-free, ready-to-use cryopreservation medium, has been used with undifferentiated human iPSCs, iPSC-derived dopaminergic neurospheres, neural progenitor cell workflows, hiPSC-derived cardiomyocytes, and 3D organoid models.

In iPSC-derived dopaminergic neurospheres, Bambanker™ cryopreserved cells retained dopaminergic marker expression, dopamine secretion, and electrophysiological activity. Following transplantation into 6-OHDA-lesioned rats, cryopreserved cells survived, differentiated into mature dopaminergic neurons, and improved abnormal rotational behavior. Other stem-cell-related workflows highlight additional post-thaw assessment needs, including morphology and lineage marker expression in progenitor cell models, contractility and electrophysiological profiling in cardiomyocyte models, and growth recovery and structural integrity in 3D organoid models.

Together, these examples highlight the relevance of evaluating cryopreservation outcomes beyond immediate viability alone, particularly when working with valuable and sensitive stem-cell-related models. This poster shows application examples for Bambanker™ in iPSC-derived, progenitor, cardiomyocyte, and 3D cell culture cryopreservation workflows, with the aim of supporting practical medium selection for post-thaw culture recovery and downstream usability.

Abstract No. P108/T43**CTCF insulates gene expression to safeguard migratory myogenic progenitor cells**

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During embryogenesis, multipotent progenitors present in the dermomyotome compartment of the somite generate multiple lineages including skeletal muscle, dermis and endothelial cells of the vasculature. The transcriptional programs governing these fate choices, coordinated by paired box transcription factor PAX3, are well established. How genome organization contributes to lineage specification remains poorly understood. CTCF, a key architectural protein that maintains chromatin domain boundaries to insulate gene expression, also cooperates with myogenic determination factor MYOD in muscle differentiation. However, the functional requirement for CTCF during cell fate specification *in vivo* has not been defined. Here we show that genetic inactivation of *Ctcf* in *Pax3*-expressing multipotent progenitors disrupts the specification of migratory myogenic progenitors, while closely related lineages, such as non-migratory myogenic progenitors and vascular endothelial cells, are spared. Our findings demonstrate a cell-autonomous requirement for CTCF's insulator function in safeguarding migratory myogenic progenitors from inappropriate enhancer–promoter interactions and aberrant gene expression. This model is illustrated by aberrant activation of *Ikaros* in the somitic mesoderm, through inappropriate usage of neighbouring enhancers associated with somite expression, resulting in impaired myogenic differentiation. Our findings define CTCF as a context-dependent regulator of cell fate specification, ensuring the emergence of the migratory myogenic progenitors of the *Pax3* lineage.

Abstract No. P109**Synthetic peptide growth factors enable defined 3D culture of human iPSCs in hydrogel systems***Bärbel Icheln^{1,*}, Robert Brownlee², and Kazuki Shigematsu³*¹Active Bioscience²Opes Diagnostics³Peptigrowth Inc.

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Growth factors derived from conventional sources (e.g., serum or recombinant expression) have supported advances in regenerative medicine and cell therapy, but their instability, batch variability, and animal-derived components limit reproducibility and scalability. Chemically defined alternatives that can recapitulate essential signaling pathways are therefore needed to establish more controllable stem cell culture systems.

Synthetic peptide growth factor mimetics represent a promising approach to replacing conventional biologics. These peptides are designed to activate native receptor signaling while offering advantages in stability, chemical definition, and manufacturing consistency.

Here, we evaluated a synthetic FGF2 alternative peptide (FGFR1c agonist) for human induced pluripotent stem cell (iPSC) culture in both two-dimensional and three-dimensional hydrogel environments. iPSCs were expanded in xeno-free medium supplemented with the peptide and subsequently cultured within a synthetic hydrogel matrix to establish 3D cultures. Under these conditions, the peptide supported robust cell proliferation while maintaining pluripotency markers, including OCT4 and LIN28.

In 3D hydrogel culture, iPSCs grown with the synthetic peptide exhibited enhanced growth compared with cultures using heat-stable recombinant FGF2, despite the peptide being used at lower concentrations. The hydrogel-based system enabled high-density expansion and uniform cell morphology while preserving stem cell characteristics, demonstrating compatibility between synthetic peptide signaling and defined extracellular matrices. These findings indicate that synthetic peptide growth factors can function effectively in complex 3D environments, not only in conventional 2D culture systems.

This study highlights the potential of combining chemically defined peptide growth factors with synthetic hydrogels to establish scalable and reproducible platforms for pluripotent stem cell expansion. Such defined culture systems may facilitate the development of standardized manufacturing processes for regenerative medicine and cell therapy applications.

Abstract No. P110**A high-throughput disease modeling system offers large-scale investigation of rare genetic diseases**

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*Presenting author

Rare genetic diseases cause 72% of all rare diseases known worldwide. However, individual disease modeling remains a challenge due to limited scalability and non-consistent reproducibility.

Here, we introduce you to a patient-specific, large-scale disease modeling system based on the generation of high-quantity human brain organoids (Hi-Q organoids). This established modeling platform is used to investigate alterations in the brain, as presented in rare neurodevelopmental disorders. Therefore, we achieved to collect more than 20 patient-derived cell lines as a base for our Hi-Q organoids. The genetically manifested phenotypes in our affected individuals range from normal brain imaging but present intellectual disability, to brain malformations and primary microcephaly. By applying our disease modeling system, we can provide a robust, large-scale palette of brain-like tissue, by which we enable functional analysis across different genetic backgrounds, as well as the identification of common biological mechanisms and potential corresponding therapeutic targets.

Abstract No. P111**Improving cross-species comparability of human and cynomolgus macaque iPSCs**

Antonia Keßler*, Felix Pförtner, Ines Hellmann, and Wolfgang Enard

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Cross-species comparisons are essential for understanding human development and the evolution of gene regulatory networks, yet, they require orthologous cell types across species that are largely inaccessible *in vivo* due to ethical and practical constraints. Somatic cell reprogramming into induced pluripotent stem cells (iPSCs) enables the generation and experimental manipulation of these cell types from diverse species *in vitro* and has revolutionized cross-species comparisons. The cynomolgus macaque, a well-established non-human primate model for early development, represents an ideal system for investigating the evolution of gene regulatory networks due to its phylogenetic distance from humans. However, limited numbers of cell lines and substantial variability among iPSC lines currently constrain cross-species comparability.

The defining feature of any iPSC line is pluripotency, its ability to differentiate into all three germ layers. Yet, despite the fact that iPSC lines typically express the appropriate marker genes and display the expected colony morphology, they often differ substantially in their differentiation potential. This heterogeneity is known to be influenced by technical factors like cell type of origin, reprogramming method and culture conditions. In particular, part of the variability in the differentiation potential among iPSC lines is thought to be due to an incomplete reprogramming of the epigenetic state. Such factors may also exacerbate species differences, reducing the comparability of iPSCs across species.

To systematically address these challenges, we generated a panel of iPSC lines from both human and cynomolgus macaque cells using fibroblasts or urine-derived stem cells as primary cell source. Through comprehensive characterization of clonal lines, controlled comparison of culture conditions, and increased number of cell lines, we aim to identify conditions that maximize similarity in gene expression profiles between species. By minimising technical and epigenetic variability, this work seeks to enhance the power of cross-species comparisons.

Abstract No. P112**Generation of functional oligodendrocyte progenitor cells through serial replating of iPSC-derived NPC spheres***Junmyeong Park¹, Johnny Kim², Steven Goldman³, and Kee-Pyo Kim^{1,*}*

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Oligodendrocytes (OLs) are essential for myelin formation in the central nervous system, and their loss or dysfunction is a hallmark of various demyelinating and neurodegenerative disorders. Although oligodendrocyte precursor cells (OPCs) represent a promising cell source for remyelination therapies, existing protocols for generating OPCs from human-induced pluripotent stem cells (iPSCs) are often limited by prolonged culture duration, low efficiency, and cellular heterogeneity. Here, we report an efficient and reproducible platform for generating OPCs from iPSC-derived neural progenitor cells (iNPCs) through stage-specific modulation of developmental signaling pathways. Directed differentiation of iNPCs recapitulated key developmental transitions, progressing through OLIG2+/NKX2.2+ progenitors to CD140a+/O4+ OPCs within a significantly shortened timeframe compared to conventional approaches. Notably, iNPC-derived spheres functioned as a progenitor-like niche, enabling sustained OPC production through serial replating. Purified OPCs could differentiate into MBP+ oligodendrocytes and demonstrated myelination capacity both in vitro, via nanofiber ensheathment and in vivo following transplantation into shiverer (shi/shi) mice, where they formed myelin sheaths around host axons. Despite these advances, OPC differentiation and maturation efficiencies remained suboptimal, highlighting the need for further optimization. Collectively, our findings establish a scalable and time-efficient strategy for iPSC-derived OPC generation and underscore their potential for disease modeling and cell-based remyelination therapies.

Abstract No. P113**Electroporation of cortical organoids for functional investigation of neurogenesis**

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Cortical organoids have become important tools for studying mammalian brain development, neurodevelopmental disorders, and brain evolution. Thus far, studies analysing gene function by overexpression or knockout have been instrumental in animal models to provide mechanistic insights into the regulation of neocortex development. Here, we present a detailed workflow for CRISPR/Cas9-mediated acute gene knockout by electroporation of sliced human cortical organoids (hCOs). The slicing of cortical organoids aids the identification of ventricle-like structures for injection and subsequent electroporation, making this a particularly well-suited model for acute genetic manipulation during neocortex development. We describe the design of guide RNAs and the validation of targeting efficiency *in vitro* and in cortical organoids. Electroporation is performed at mid-neurogenic stages, enabling the targeting of most major cell classes in the developing neocortex, including apical radial glia, basal progenitor cells, and neurons. The approach has been applied, for example, to explore the role of secreted proteins such as EPIREGULIN on neural development. Additionally, we show that this method can be utilised in primate cerebral organoids to study brain evolution. Taken together, the electroporation of cortical organoids represents a powerful technique to investigate gene function, gene regulation, and cell morphology during brain development.

Kolodziejczyk, A., et al. (2024). *Electroporation of Sliced Human Cortical Organoids for Studies of Gene Function*. *J. Vis. Exp.* DOI: 10.3791/67598.

Abstract No. P114**Differentiation of human pluripotent stem cells to steroid-producing adrenal cells**

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Adrenal glands are the central stress organ in the human body producing hormones such as cortisol, aldosterone and adrenaline, thereby regulating stress responses, metabolism, blood pressure and fluid balance. Adrenal insufficiency is a life-threatening condition. It is associated with weight loss, low blood pressure, fatigue, seizures and metabolic disturbances. Current treatment approaches are based on hormone replacement therapies which, however, have significant limitations. Differentiation of human pluripotent stem cells (hPSCs) into steroid-producing cells could represent a therapeutic option for the treatment of adrenal insufficiency. The goal was therefore to generate human induced steroidogenic cells (hiSCs) derived from hPSCs.

We optimized a protocol for the directed differentiation into adrenal cells using activators of the wntless and int-1 (WNT), retinoic acid (RA), and bone morphogenic protein (BMP) signaling pathways. Lentiviruses were produced and utilized to introduce genes encoding steroidogenic factor 1 (SF-1), adrenocorticotrophic hormone receptor (ACTH receptor; MC2R) – and its accessory protein (MRAP). To determine whether the differentiated cells are indeed steroid-producing adrenal-like cells, the expression of genes for pluripotency (*Oct4*), for early stage of adrenal development (*DAX1*, *GATA4*, *GATA6*, *WT1*, *DLK*, *OSR2*), and for adrenal gland (*NR5A1*, *MC2R*, *MRAP*, and *CYP11B1*) were analyzed. We pursued a strategy to achieve optimal outcome by generating a score based on both the expression of adrenal-compatible markers and negative influencing factors. We were able to demonstrate that hiSCs exhibit characteristics of adrenal cells. The induction of SF-1, MC2R and MRAP expression results in dose-dependent ACTH reactivity; however, this responsiveness could be further improved.

In conclusion, cell-based therapy could offer a physiologically relevant approach for improved treatment of adrenal insufficiency. HiSCs exhibit the ability to release adrenal hormones when stimulated by steroidogenic inducers. Further optimization is required to increase the proportion of adrenal cells within the population of differentiated cells and to boost steroid production.

Abstract No. P115**Effect of microfilaments on embryoid body morphogenesis and early neuronal induction**

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Generating cerebral organoids with high structural complexity while maintaining experimental reproducibility remains a major challenge. Unguided differentiation protocols preserve important aspects of spontaneous brain-like self-organization but frequently exhibit substantial variability in embryoid body morphology, neuroectoderm formation and subsequent tissue organization. In contrast, strongly guided differentiation protocols improve standardization but may restrict developmental diversity. Microfilament-mediated elongation of embryoid bodies has previously been shown to influence cerebral organoid development, although its implementation remains technically demanding and difficult to scale.

Here, we present the MatriFilamentSystem® (MFS), a scaffold-based culture platform designed to provide physical guidance during early cerebral organoid development. The system combines integrated microfilaments with a thermoformed culture architecture, enabling controlled embryoid body formation, simplified handling and parallel organoid production without extensive biochemical patterning.

Early embryoid body morphology and neural induction were assessed by daily brightfield imaging, immunofluorescence staining and gene expression analysis. Compared with conventionally generated unguided organoids, MFS-derived embryoid bodies exhibited distinct changes in geometry and early tissue organization. During neural induction, scaffold-guided aggregates showed pronounced peripheral tissue organization and differences in the appearance of neuroepithelial regions.

Overall, the MFS provides an adaptable approach for improving experimental control in unguided cerebral organoid culture while preserving spontaneous self-organization. The platform may facilitate the systematic investigation of how early aggregate geometry, mechanotransduction and cell–cell adhesion influence human neural development in vitro.

Abstract No. P116**Modelling the molecular basis of human speech evolution**

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Heterozygous loss-of-function mutations in the transcription factor FOXP2 lead to speech and language disorder. Two human-specific amino acid substitutions (T302N and N324S) occurred in the conserved FOXP2 protein after the human-chimpanzee split, suggesting a role of FOXP2 in the evolution of speech. Mouse models (Foxp2hum) with these human-specific substitutions showed enhanced synaptic plasticity in the form of stronger long-term depression (LTD) in the medium spiny neurons (MSN) of the striatum, decreased tissue dopamine levels, enhanced performance during certain learning tasks, and altered gene expression of striatal neuronal pathways during learning. To understand the molecular basis of speech development and evolution, we use homology-directed CRISPR-cas9 genome editing to create the two amino acid substitutions, which generates a “chimpanized” version of FOXP2 in human iPSC cell line. Additionally, we generate mouse iPSCs from Foxp2hum and WT mice. We will differentiate these cells into MSNs and subsequently perform RNA-seq to investigate to what extent human and mouse in vitro MSNs can recapitulate the differential expression found in the striatum of Foxp2hum mouse model.

Abstract No. P117**Characterization of NMDA receptor function in human NGN2 induced neurons***Yi Lien*, and Christian Rosenmund*

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Human induced pluripotent stem cell (hiPSC)-derived neurons provide a platform to study the function of human neurons. Many differentiation protocols have been developed to generate glutamatergic induced neurons (iNs), yet a key functional feature of mature glutamatergic neurons, NMDA receptor (NMDAR) function, has remained difficult to establish in these models, despite being well characterized in rodent systems. In particular, neurogenin-2 (NGN2)-driven iNs, in which the neural progenitor cell stage is skipped, show little to no NMDAR current (Nehme et al., 2018), in contrast to their robust AMPA receptor-mediated current. We have recently developed an NGN2 induction protocol (Dimitrov et al., 2025) to generate human glutamatergic iNs and observed substantial NMDA-evoked currents, indicating a more mature neuronal phenotype than previously reported. We hypothesized that these iNs would show a GluN2B-to-GluN2A switch, a developmental process required for proper synaptic maturation as shown in vivo (Monyer et al., 1994). We performed electrophysiology to assess NMDAR subunit composition and function across differentiation timepoints. These results support the use of iNs generated with this NGN2 protocol as a model for studying NMDAR function in human neurons.

Dimitrov et al., Proteomics-based receptor-ligand matching enhances differentiation maturity of human-stem-cell-derived neurons, Stem Cell Reports (2025), <https://doi.org/10.1016/j.stemcr.2025.102604>

Abstract No. P118**A hiPSC-derived endothelial cell model with kallikrein activity for hereditary angioedema**

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Introduction

Hereditary angioedema (HAE) is a rare genetic disorder characterized by recurrent episodes of swelling caused by excessive bradykinin (BK) production due to dysregulation of the kallikrein-kinin system (KKS). Human endothelial cell-expressed bradykinin receptor 2 (B2R) is the primary target of bradykinin-mediated vascular leakage; however, physiologically relevant human models for studying endothelial responses within the KKS remain limited. We aimed to establish and characterize a human induced pluripotent stem cell-derived endothelial cell (hiPSC-EC) model suitable for investigating HAE-associated vascular dysfunction.

Methods

We established a protocol to differentiate human induced pluripotent stem cells (hiPSCs) into a pure population of endothelial cells (hiPSC-ECs). The resulting hiPSC-ECs were characterized by flow cytometry, immunofluorescence staining, and functional enzymatic assays to assess endothelial identity and KKS-related properties.

Results

The differentiation protocol consistently yielded a highly pure population of cells exhibiting classic cobblestone endothelial morphology. Characterization confirmed that the hiPSC-EC model expresses canonical endothelial markers, including CD31, CD144 (VE-Cadherin), and VEGFR2 (KDR), indicating a mature endothelial phenotype. IF staining revealed correct localization of VE-Cadherin at cell-cell junctions. Furthermore, hiPSC-ECs demonstrated significant expression of the bradykinin receptors B1R and B2R, key components of the KKS and critical for bradykinin function. Importantly, functional analysis confirmed that the hiPSC-EC model possesses measurable kallikrein activity.

Conclusions

We successfully established a hiPSC-derived endothelial cell model that recapitulates key endothelial and kallikrein-kinin system features relevant to HAE. This platform provides a promising tool for studying disease mechanisms and evaluating therapeutic strategies targeting bradykinin-mediated vascular dysfunction.

Abstract No. P119**The impact of cohesin on human head and face development**

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Cohesin is a highly conserved multiprotein complex composed of the core ring components Structural Maintenance of Chromosomes (SMC1A, SMC3) and auxiliary proteins. Beyond the maintenance of genome integrity through sister chromatid cohesion and DNA damage repair, cohesin has an essential role in gene expression regulation by organizing 3D chromatin architecture, facilitating enhancer-promoter communication. Disruption of the transcriptional regulation caused by mutations affecting cohesin or cohesin-related proteins leads to Cornelia de Lange syndrome (CdLS), a congenital multisystem neurodevelopmental disorder characterized by heart defects and cognitive deficits, limb and craniofacial (head and face) malformations. Although the craniofacial abnormalities are essential aspects of the CdLS diagnosis, the relationship between the cohesin mutations and human craniofacial development has not been investigated. To address this, we aim to determine 3D chromatin and transcriptional programs in human induced pluripotent stem cells (hiPSC) and hiPSC-derived cranial neural crest cells (CNCC), which substantially contribute to craniofacial structures. Our previous time-resolved inference of gene regulatory networks (GRNs) underlying CNCC development using transcriptomic and chromatin accessibility mapped novel regulatory relationships, linking non-coding genetic variants associated with orofacial clefting to both known and novel putative target genes, otherwise missed in analyses based on a single timepoint. To elucidate how cohesin mutations alter GRNs in hiPSC and hiPSC-derived CNCC, we have generated CRISPR/Cas9-engineered hiPSC with SMC1A and SMC3 mutations. Subsequent transcriptomic, epigenomic, and chromatin conformation assays combined with bioinformatics analyses will allow us to investigate if mutations or other alterations of the identified GRNs (1) are linked to undiagnosed cases of individuals with craniofacial deformities, (2) are risk factors for multifactorial diseases, (3) contribute to the variability of a phenotype. Thus, insights gained from the rare disease CdLS may be translated to more common disorders, opening new opportunities for diagnosis and therapy.

Abstract No. P120**An insight into gorilla (*Gorilla gorilla*) induced pluripotent stem cells**

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Humans and gorillas (*Gorilla gorilla*) share genetic, molecular, and developmental traits. Therefore, gorilla induced pluripotent stem cells (iPSCs), provide a powerful platform for disease modeling, regenerative medicine, evolutionary biology, and comparative biomedical studies. In this study, we generated three iPSCs lines from one male and two female gorillas [MPH-GorGor-C00001 (G1), MPH-GorGor-C00002 (G2) and MPH-GorGor-C00003 (G3)].

To do so, skin fibroblasts were subjected to Sendai virus-reprogramming. Treatment with and without enhancers (EPZ5676 and CBP30), feeder and feeder-free systems, as well as medium change with mouse embryonic fibroblasts-conditioned medium (MEFs-conditioned medium) before gradual transition to pluripotency medium (mTeSR or StemMACS) were evaluated. TRA-1-60 sorting was repeatedly performed and pluripotency was assessed by immunostaining and tri-lineage differentiation.

Overall, feeder systems and enhancer treatment improved reprogramming efficiency. MEFs-conditioned medium supported early pluripotency induction but caused colony deterioration after prolonged use, requiring gradual transition to pluripotency medium. In G2 and G3, StemMACS outperformed mTeSR. Repeated TRA-1-60 sorting improved stability of lines. Generated lines have normal karyotypes, express pluripotency markers and differentiate into cells of the three germ layers.

In conclusion, gorilla iPSCs establishment remains technically challenging, particularly during early reprogramming and pluripotency stabilization. Short treatment with MEFs-conditioned medium before transitioning to pluripotency medium, feeder systems, and enhancer treatment improved culture stability and reprogramming efficiency. For iPSCs growth, StemMACS outperformed mTeSR under our conditions. These findings support the successful derivation of stable gorilla iPSCs lines and contribute to the optimization of species-specific reprogramming protocols for translational and comparative biomedical research.

Abstract No. P121**Genetic Engineered-hypoimmunogenic human induced Pluripotent Stem Cells evade surveillance of the immune system in a murine xenotransplantation model**

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Immunoreaction after transplantation, mainly caused by the interaction of cell surface proteins of the graft with receptors of immune cells of the host, is one of the major hurdles to overcome for stem cell-based therapeutic approaches. Xenotransplantation experiments and approaches play an important role in providing graft materials for transplantation and for basic transplantation research.

Deuse et al. (2019) showed that deleting the Major Histocompatibility Complex (MHC I and II) while overexpressing CD47 could effectively render grafts undetected by the allogeneic recipient's immune system.

We generated a hypoimmunogenic human iPSC line designed to evade immune recognition in a murine host. Using CRISPR/Cas9, MHC class I and II complexes were deleted, and murine CD47 was transgenically expressed via a piggyBac system. Hypoimmunogenic hiPSCs were transplanted into immunocompetent mice, and immune responses were assessed in vivo. Preliminary data indicate that hypoimmunogenic hiPSCs evade host immune surveillance and persist long-term, resulting in teratoma formation. Comparable graft persistence is observed in wild-type hiPSC transplants only under systemic immunosuppressive treatment, whereas wild-type hiPSCs transplanted without immunosuppression are rapidly rejected, preventing long-term follow-up.

Deuse, T., et al., Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. Nature Biotechnology, 2019. Mar; 37

Abstract No. P122**Analyses of the impaired endothelial blood-brain barrier and senescence-associated molecular phenotypes in Rett syndrome**

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Background and Research Question:

Rett Syndrome (RTT) is a rare, X-Chromosome linked neurodevelopmental disorder caused by mutations in the MECP2 gene and affects almost exclusively girls. It is characterized by an initial period of normal development, followed by a regression phase between 6 and 18 months of age, where the child loses acquired skills, particularly in speech, hand function, and social interaction.

Although alterations in several components of the neurovascular unit, including neurons and astrocytes, have been described in RTT, the role of endothelial cells remains poorly understood. To address this gap, this study investigates blood–brain barrier (BBB) function using induced pluripotent stem cell (iPSC)-derived endothelial cells. RTT cells were compared with healthy cells and CRISPR/Cas-corrected isogenic cells.

Method:

Barrier integrity of BBB endothelial cells was assessed by transendothelial electrical resistance (TEER) measurements and sodium fluorescein permeability assays. Senescence-associated and proliferative phenotypes were evaluated by immunofluorescence staining and flow cytometric analysis of the proliferation markers PCNA and Ki-67. In addition, the expression of cell cycle- and senescence-related markers, including p21, γH2AX, RB, and p53, were evaluated using immunostaining, flow cytometry, and western blot analysis. Cell cycle distribution was further examined by propidium iodide-based flow cytometry.

Results:

The results indicate impaired BBB function in RTT-derived endothelial cells, accompanied by altered cell cycle regulation and a senescence-associated phenotype. Taken together, these findings provide novel insight into the vascular contribution to RTT pathogenesis and suggest that endothelial dysfunction may represent an important, previously underappreciated aspect of the disorder.

Abstract No. P123**UV-micropatterning for high-throughput generation of embryoid bodies**

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Embryoid bodies (EBs) are three dimensional aggregates of pluripotent stem cells which self organize and exhibit multilineage differentiation potential, making them indispensable tools for drug screening, disease modeling, and regenerative medicine. Conventional EB production, however, relies on dissociating cells into single cell suspensions followed by centrifugation, forcing the cells to re-aggregate. We previously demonstrated that geometrically confined colonies of induced pluripotent stem cells (iPSCs) on micro-contact-printed vitronectin circles detached upon confluency to form size-controlled aggregates. Building on this concept, we developed an ultraviolet micropatterning approach (UV μ -pattern) that further improves the efficiency, uniformity, and scalability of EB generation. For this approach, glass slide inserts were initially coated with a protein-repellent layer, then locally exposed to UV light to degrade the repellent layer and to define patterns. Subsequently, the irradiated regions were coated with vitronectin, allowing localized iPSC attachment and after five to six days, reliable self-detachment as EBs. Compared with conventional U-bottom wells and the AggreWell™ system, UV μ -pattern EBs showed comparable morphology, maintenance of pluripotency, and robust multilineage differentiation towards endoderm, mesoderm and ectoderm. Gene expression profiles supported the notion that UV μ -pattern EBs have similar differentiation potential, with less evidence for hypoxia than observed for the AggreWell system. Furthermore, UV μ -pattern EBs were successfully used for differentiation towards cardiac lineage. Finally, we could demonstrate that this new method for EB generation could be implemented on an automated cell culture system, to facilitate gentle EB generation without the need for additional centrifugation steps. Overall, our new approach provides a cost-effective, scalable, and automation-friendly method for standardized EB generation.

Abstract No. P124**Membrane-stirrer bioreactor platform enables record-breaking human iPSC expansion at clinically relevant scales**

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Human induced pluripotent stem cell (hiPSC) manufacturing remains a critical bottleneck limiting the clinical and industrial translation of stem cell-based therapies. Here, we present a novel membrane-stirrer bioreactor platform that achieves unprecedented hiPSC expansion under suspension culture conditions, demonstrated at both 300 mL and 2 L scales.

Process development began with systematic identification of key bioprocess limitations. Contrary to initial expectations, oxygen transfer was not the primary constraint; instead, medium composition emerged as the dominant limiting factor, with enriched formulations enabling substantially higher cell densities even under fed-batch conditions. This finding redirected process optimization toward intensified perfusion cultivation integrating real-time dissolved oxygen (DO) control, pH monitoring, controlled perfusion, and optimized nutrient feeding strategies.

The resulting intensified bioprocess effectively minimized nutrient depletion and metabolite accumulation while ensuring adequate oxygen transfer — enabled by the membrane-stirrer's exceptional mass transfer performance, as reflected in its high kLa values. Under fully controlled conditions, iPSC aggregates were expanded over 8-day (300 mL) and 5-day (2 L) cultivation runs, maintaining high viability, robust growth kinetics, and pluripotency-associated morphology throughout.

The platform achieved record viable cell densities of 44×10^6 hiPSCs/mL at 300 mL and 11×10^6 hiPSCs/mL at 2 L scale — the highest reported for hiPSC suspension expansion to date. Critically, scale-up from 300 mL to 2 L was accomplished without compromising process stability or culture performance. Harvested cells retained pluripotency marker expression above 90% and displayed homogeneous, highly circular aggregate morphology, confirming maintenance of cell quality at scale.

These results establish intensified perfusion-based bioprocessing as a robust and scalable strategy for clinically relevant hiPSC manufacturing, with the membrane-stirrer design as a key enabling technology for next-generation cell therapy production.

Abstract No. P125**PSC suspension culture eases the transition to 3D differentiation workflows**

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Pluripotent stem cells (PSCs) have unlimited expansion capacity and can be differentiated into any cell type, making them powerful tools to study development and disease. The emergence of PSC-derived organoid cultures has further improved the physiological relevancy of these cell models. However, conventional PSC culture is based on adherent culture techniques that have limited scalability potential and require the dissociation and reaggregation of PSCs for many organoid differentiation methods. To address these workflow limitations, StemScale PSC suspension medium was developed to support PSC growth in suspension which can reduce hands-on time and enable rapid scale-up from small culture vessels to bioreactors while maintaining key stem cell attributes. Indeed, PSCs cultured for >30 passages maintained stemness marker expression, trilineage differentiation potential and a stable karyotype. Moreover, RNAseq indicated the global transcriptome of PSCs cultured in StemScale was highly concordant with conventional adherent medium PSC cultures. A cohort of 230 differentially enriched genes was detected in adherent cultures that were primarily associated with ECM and focal adhesin KEGG pathways. Suspension culture enriched genes were not associated with any KEGG pathway and principal component analysis suggested transcriptome differences were driven less by the culture medium and more PSC-line specific. Furthermore, we discuss how PSC suspension culture may ease the transition into 3D differentiation protocols leading to lineage dependent and lineage agnostic benefits. Differentiating PSCs in suspension may shorten the time to produce dopaminergic neurons from 35 to 25 days and electrophysiology suggested these cells also achieve a higher maturation level than their matched 2D controls. Likewise, PSCs could be differentiated into cytotoxic CD56+CD3-CD16+ natural killer cells, capable of killing patient derived tumoroids, in a more scalable manner. Overall, this data suggests PSC suspension culture is well-suited for scalable PSC workflows including 3D differentiation and for generating more reliable, reproducible, and human-relevant cell models.

Abstract No. P126**Embryonic macrophages direct hepatocyte expansion in liver development**

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In liver development, hepatoblasts are epithelial progenitors that differentiate into hepatocytes or biliary epithelial cells (BECs). Despite their bipotential nature, hepatocytes vastly outnumber BECs, comprising approximately 80% of the adult liver. Embryonic macrophages populate the developing liver and become tissue-resident. However, their role in orchestrating epithelial output remains incompletely understood.

Here, we identify embryonic macrophages as promoters of epithelial progenitor expansion and hepatocyte development using a human fetal liver cell atlas, human immune-competent fetal liver organoids (FLOs), and complementary mouse models. Within the human fetal liver atlas, macrophage-to-hepatoblast interactions were most prominent during early development. In FLOs, HepPar1+ hepatocyte output positively correlated with IBA+ macrophage frequency. Pharmacological and genetic macrophage depletion through targeting of colony-stimulating factor 1 receptor (CSF1R) reduced cycling HNF4a+ hepatic and SOX9+ BEC progenitor populations. In addition, macrophage depletion reduced ALB+ PanCK- hepatocyte output and epithelial cluster expansion.

In vivo, Tnfrs11αCre/+ Csf1rfl/fl and Tnfrs11αCre/+ Spi1fl/fl mouse models deplete embryonic macrophages at embryonic day (E)12.5 and E14.5, respectively. Macrophage depletion at E12.5 but not at E14.5 decreased postnatal hepatocytes and periportal BECs, indicating that embryonic macrophages support progenitor cell expansion during early development. Despite reduced abundance, BEC structures were retained in vivo and in FLOs. Altogether, these findings highlight that fetal macrophages maintain epithelial progenitors and support hepatocyte development.

Mechanistically, ligand-receptor analysis identified TNF and LGALS9 as candidate mediators of macrophage-to-hepatoblast crosstalk. Surprisingly, combined TNF and LGALS9 exposure suppressed hepatocyte output in CSF1R KO FLOs, contrasting the pro-proliferative effects reported for TNFα in adult liver regeneration. These findings suggest that inflammatory signals may exert distinct functions during liver development. In summary, our findings identify embryonic macrophages as contributors to hepatocyte-dominant epithelial output during liver development.

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Abstract No. P127**Effective cryopreservation of retinal organoids via vitrification preserves retinal identity and developmental potential across differentiation stages**

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Human pluripotent stem cell (hPSC)-derived retinal organoids are a powerful platform for disease modeling, drug screening, and gene therapy. However, their utility is limited by continuous culture demands and the lack of reliable biobanking strategies. Cryopreservation offers a promising solution, yet its impact on retinal organoid integrity remains poorly characterized.

We applied a vitrification protocol to retinal organoids at day 25 and day 65 of differentiation. D25-vitrified organoids assessed at day 65 showed well-preserved morphology, including retinal layer lamination and a bright neuroepithelium, comparable to age-matched controls. Immunohistochemical analysis confirmed retention of retinal progenitor identity, with expression of VSX2, KI67, and BRN3, consistent with age-matched non-vitrified controls, indicating that vitrification does not disrupt progenitor expression, architecture, or proliferation.

D65-vitrified organoids maintained until day 100 demonstrated continued growth, with typical retinal neuroepithelial layers and increased organoid diameter comparable to controls. Immunohistochemical characterization is ongoing to confirm retinal identity recovery post-thaw.

In parallel, MEDY-based (methylcellulose, ethylene glycol, DMSO, Y-27632) cryopreservation was applied at day 20 and assessed at day 65. Immunohistochemical analysis revealed partial preservation of retinal progenitor markers, with reduced VSX2 and KI67 expression compared to controls, while BRN3 expression was comparable to both vitrified and non-vitrified groups. These findings provide a comparative insight into the performance of two distinct cryopreservation approaches at early retinal organoid stages.

These results suggest vitrification as an effective biobanking strategy across retinal organoid differentiation stages, opening the door to large-scale production and storage for disease modeling, drug screening, and gene therapy applications.

Mojica-Perez S, Stokes K, Jacobs S, et al. Cryopreservation of human cortical organoids using vitrification. bioRxiv. 2025. doi:10.1101/2025.04.08.647634

Xue W, Li H, Xu J, et al. Effective cryopreservation of human brain tissue and neural organoids. Cell Reports Methods. 2024;4:100777.

Abstract No. P128**FLT3L and SR1 regulate development and immunostimulatory function of human PSC-derived dendritic cells**

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Dendritic cells (DCs) are central regulators of adaptive immunity, and while their developmental pathways are well established, the mechanisms governing their functional specialization remain incompletely understood in humans. To address this, the field has increasingly shifted toward the development of human in vitro systems to better investigate the mechanisms regulating immune cell development and function. Here, we establish a feeder-free, fully defined human pluripotent stem cell-derived hematopoietic organoid system that enables sustained production of myeloid iDC subsets. Within this platform, we demonstrate that FLT3L signaling supports iDC development, while aryl hydrocarbon receptor modulation using SR1 promotes an activation-associated DC state. Supplementation of FLT3L alone or in combination with SR1 yielded comparable iDC subset composition, however SR1-conditioned iDCs displayed enhanced antigen uptake and processing capacity, consistent with an activation-associated transcriptional profile. To define the role of FLT3L signalling, we leveraged iPSCs carrying a loss-of-function FLT3L (FLT3LLOF) mutation. FLT3LLOF abrogated DC generation and impaired early hematopoietic specification, marked by loss of KDR⁺CD235a⁺ progenitors, identifying a developmental bottleneck upstream of iDC commitment. Functionally, SR1-derived iDCs induced robust activation of autologous T cells in response to cytomegalovirus (CMV) antigens, with sustained CD8⁺ T cell proliferation, increased acquisition of memory phenotypes, and elevated secretion of pro-inflammatory cytokines, including MCP-1, IL-8, IL-6, and IL-18. Together, these findings demonstrate that FLT3L signaling is essential for early human hematopoiesis and DC development, while SR1 modulates their functional programming, and establish a platform for the generation of antigen-presenting DCs.

Abstract No. P129**Investigating the impact of endocrine chemical disruptors on liver development using hiPSCs**

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Endocrine chemical disruptors (EDCs) are substances found in common products such as pesticides, cosmetics, plastics. EDCs can interfere with the endocrine system and the normal metabolic functions of the human body. In the liver, they are suspected to influence lipid metabolism which leads to an enhanced lipid accumulation and metabolic dysfunction-associated steatotic liver disease (MASLD). However, the impact of EDCs on the human liver development and the link to MASLD occurring later in life are still poorly understood. To study the impact of EDCs on liver development, we propose to use the HepBud platform which is based on the differentiation of human induced pluripotent Stem cells (hiPSCs) into self assembling liver buds. This approach models stages of liver development including liver bud formation, hepatoblast generation and then their differentiation into hepatocytes and cholangiocytes. We will differentiate hiPSCs in fetal and adult liver cell types (hepatoblast, hepatocytes and cholangiocytes) in the presence and in the absence of EDCs. In first results, live dead assay has shown that acute exposure of hepatoblast organoids to high dose of Bisphenol A (BPA) lead to rapid cell death thereby confirming the hepatotoxicity of EDC. However, short-term treatment to PFOA did not display any toxicity thereby suggesting that the impact of EDCs could be more subtle and affect other aspect of hepatocyte biology. Accordingly, we are planning to evaluate the functional activity of the resulting liver cells after chronic exposure of low dose EDCs. We will especially focus on their capacity to metabolise lipids and to resist to toxic stress.

Overall, we hypothesis that EDCs affect liver development resulting in the production of cells which are less resistant to lipotoxicity and more susceptible to MASLD. Our experiments also establish hiPSCs as a replacement to animal models for studying the impact of pollutant on human fetal health.

Abstract No. P130**Full-length laminin-521 recreates the native stem cell niche to support human pluripotent stem cell survival, migration, and expansion**

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Human pluripotent stem cells (hPSCs) require an extracellular matrix (ECM) for maintenance, proliferation, and differentiation. Laminins are a family of 16 distinct ECM proteins and represent a key component of the stem cell niche. Different laminin isoforms are expressed throughout development and are differentially expressed in across tissues, with specific isoforms either supporting stem cell maintenance or contribute to driving tissue-specific differentiation.

Laminins exert this influence by binding directly to both integrin and non-integrin receptors, as well as by sequestering and modulating the release of growth factors. Through these mechanisms, laminins direct intracellular signaling pathways and contribute to the local stem cell niche. In contrast, fragmented laminins lack many of these properties and are not naturally produced in healthy tissues.

Full-length laminin-521 has been shown to significantly enhance PSC survival and proliferation, all while enabling high-efficiency single-cell seeding with or without the addition of ROCK inhibitors. Previous studies have shown that hPSCs with a Laminin Alpha Chain 5 (LAMA5) knockout cannot survive and expand without exogenous supplementation of full-length laminin-521. In addition, we have demonstrated that PSCs cultured on full-length laminin-521 exhibit enhanced migratory capacity, effectively spreading to cover the entire culture surface, improving stability at high densities, and improving yields. In migration/gap-closure assays, PSCs cultured on laminin-521 spread evenly to cover nearly 100% of the culture surface, whereas fragmented laminins achieve only 29–44% coverage under the same conditions.

Importantly, these findings highlight the ability of full-length laminin-521 to recreate a more physiologically relevant cellular microenvironment. This capability is critical for advancing PSC culture techniques, improving differentiation protocols, refining disease modeling, and supporting the manufacture of groundbreaking cell therapies.

Abstract No. P131**Engineering the CDX2/PDX1 locus in human stem cells to study lineage-specific gene regulation**

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Precise control of gene expression is crucial for lineage specification during development. The transcription factors CDX2 and PDX1 are master regulators of intestinal and pancreatic lineage commitment, respectively, and are expressed in a mutually exclusive pattern, which is crucial for correct lineage specification. Despite their distinct expression patterns, the genes reside at the same genomic locus and share a gene regulatory landscape. How this landscape ensures the selective activation of both genes remains poorly understood. To dissect how the cis-regulatory landscape controls lineage-specific gene expression, we combine state-of-the-art genetic engineering of hiPSCs with directed differentiations. We apply a synthetic genomics approach to insert synthetic wildtype and variant versions of the ~100kb CDX2/PDX1 locus at a safe-harbour site. Differentiation of engineered hiPSCs into CDX2 and PDX1-expressing cell types allows us to compare expression from endogenous and synthetic alleles simultaneously and identify the changes driven by variant synthetic loci. Using this strategy, we aim to dissect whether an intervening lncRNA subdivides the locus or whether enhancer-promoter specificity allows differential CDX2/PDX1 expression. In addition, we use our platform to rebuild the mouse Cdx2/Pdx1 locus in hiPSCs and examine whether the regulatory logic is conserved between species. Taken together, our approach establishes a framework for stem cell engineering to dissect the genetic control of cell fate decisions during lineage-specification.

Abstract No. P132**Benchmarking the matrix transition: a standardized screening and validation framework for cerebral organoid cultures***Lisa Wolff^{*}, and Sven Hendrix*

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**Presenting author*

Although Matrigel remains the conventional gold standard for extracellular matrices due to its versatility and high complexity, it still has serious drawbacks. Its murine tumor origin, batch-to-batch variability, and undefined composition increasingly drive the biomedical field to seek more reproducible alternatives. However, shifting away from Matrigel entails scientific and technical hurdles that hinder broader adoption.

To streamline this transition, we identified these challenges and, as a result, designed a standardized framework for matrix selection and evaluation. We introduce a matrix selection checklist to align model-specific needs with available resources. In parallel, we designed OSCAR, the organoid scaffold assessment radar, as a multi-parametric screening tool designed to systematically evaluate matrix properties. We validated this combined approach within cerebral organoid cultures by quantitatively tracking embedding efficiency, structural integrity, and general biocompatibility.

This systematic pipeline successfully narrowed down a selection of viable matrix alternatives, which we then further benchmarked using RNA sequencing and histological validation. Our findings confirm that this framework effectively guides researchers toward optimized, model-specific options. While demonstrated here in cerebral organoids, this modular strategy can be readily adapted across diverse 3D cell culture models to improve reproducibility and translational impact.

Abstract No. P133**Human oligodendrocyte differentiation from induced neural plate border stem cells in 30 days**

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Human oligodendrocytes (OLs) myelinate axons and provide the metabolic support essential to central nervous system function. Their loss or dysfunction is central a wide range of demyelinating diseases such as multiple sclerosis. However, current hiPSC-based approaches for generating oligodendroglial cells for developmental & disease modelling is limited by low yields and long differentiation timelines of 75-100 days.

Here, we present a rapid oligodendroglial differentiation strategy based on induced neural plate border stem cells (iNBSCs), a directly reprogrammed human neural progenitor population. Patterning of iNBSCs using small molecules generated OLIG2⁺/SOX10⁺ oligodendrocyte progenitor cells (OPCs) within 3 weeks, and pre-myelinating O4⁺/MBP⁺ OLs within 30 days. The protocol was transferable to other neural progenitor lines, including iPSC-derived iNBSCs, demonstrating that its applicability was not restricted to directly reprogrammed iNBSCs.

Further, the selective use of 3D or 2D culture systems allow for selective enrichment of either mixed neuron/glia or pure oligodendroglial cultures, respectively. During differentiation, the resulting glial progenitors recapitulated surface-marker transitions observed in primary human tissue. Various growth factors drive commitment and differentiation during oligodendrogenesis. We examined how different receptor tyrosine kinase ligands influence the induction and expansion of early OPCs, and found that bFGF alone is sufficient, and more efficient than PDGF, at inducing an OPC population in our context.

Together, we show that rapid oligodendroglial specification is possible within 30 days, and that RTK ligand choice is a key determinant of OPC induction and expansion. Future studies will determine the transcriptomic & chromatin transitions during the gliogenic switch and oligodendroglial commitment, as well as functional maturation and myelination capacity of the resulting cells.

Abstract No. P134**Impact of LRP2 deficiency on human neuronal cell specification and forebrain patterning through SHH signaling pathway**

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Pathogenic variants affecting developmental signaling pathways are a major cause of congenital brain disorders. Disruption of the Sonic Hedgehog (SHH) signaling pathway is a leading cause of holoprosencephaly (HPE), an incomplete separation of the cerebral hemispheres with marked phenotypic variability. Although the mechanisms underlying this variability remain unclear, modifier genes are thought to influence disease susceptibility and resilience.

Our previous studies identified LDL receptor-related protein 2 (LRP2), a primary cilium-localized co-receptor and modulator of SHH signaling, as a critical determinant of forebrain development. *Lrp2* deficiency caused a fully penetrant HPE phenotype in C57BL/6N mice but was completely rescued on the FVB/N background, revealing a strong genetic modifier effect. We further identified candidate modifier genes such as *Unc-51 Like Kinase 4 (ULK4)* and *Pituitary Tumor-Transforming Gene 1 (PTTG1)* that enhance SHH signaling [1].

To elucidate the role of LRP2 in SHH-dependent forebrain patterning and disease mechanisms during human brain development, we differentiated *LRP2*^{-/-} pathogenic human induced pluripotent stem cells (hiPSCs) and isogenic controls into dorsal and ventral neuronal precursor cells (NPCs) and forebrain organoids. LRP2-deficient NPCs exhibited aberrant cell specification and cellular morphology. LRP2-deficient forebrain organoids displayed patterning defects and impaired rosette formation compared to isogenic controls. Transcriptomic and immunocytochemical analyses of human neuronal cultures further revealed alterations in primary cilia morphology and dynamics following LRP2 loss.

In summary, our data establish LRP2 as a key regulator of early neuroectodermal patterning by controlling primary cilium-dependent SHH signaling, cellular polarity, and cell fate specification. Disruption of LRP2 alters ciliary structure, microtubule organization, and early brain patterning. The identification of SHH signaling modulators and microtubule-associated factors further supports a model in which ciliary components act as genetic modifiers, ameliorating or exacerbating developmental defects. Together, these findings will provide mechanistic insight into how cilium-associated regulators contribute to phenotypic variability in neurodevelopmental disorders.

[1] <https://doi.org/10.1242/dev.199307>

Abstract No. P135**Biobanking and stem cell technologies across species for conservation and basic research**

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Biodiversity is declining at an alarming rate. Without targeted protection of the remaining genetic diversity, it is unlikely that species extinction can be halted in time. It is therefore increasingly important to preserve living cellular material from numerous species and individuals before they disappear entirely or the genetic pool required for the long-term survival of a species becomes critically depleted.

Since the 1990s, the Leibniz Institute for Zoo and Wildlife Research (Leibniz-IZW) has been developing a continuously expanding wildlife biobank. The biobank cryopreserves tissues, gametes, embryos, cell cultures, and pluripotent stem cells (PSCs) from more than 500 animal species, including mammals, birds, reptiles, amphibians, and fish. These biological resources are made available to researchers worldwide for conservation as well as basic and biomedical research.

Of particular importance are our PSC lines. Currently, the biobank contains two embryonic stem cell lines derived from the southern white rhinoceros and a total of 28 induced PSC lines (iPSCs), including multiple individuals and clones from seven different species, among them five non-human primates and three rhinoceros species.

Taken together, living cellular material represents a dynamic, functional, and shareable resource enabling a wide range of applications. Whereas fibroblasts can be directly applied in, among other fields, -omics analyses, aging research, and disease modeling, PSCs provide new opportunities to investigate embryogenesis and organogenesis, including developmental timing, comparative biology, and evolution, in line with the 3R principles.

While the primary aim of this biobank is to support conservation efforts and genetic rescue strategies, it also constitutes a valuable and versatile resource for a broad spectrum of fundamental biological research.

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Abstract No. P136**Modeling liver aging with tissue-derived organoids***Anna Dowbaj*

Center for Organoid Systems (COS), Technical University of Munich (TUM)

Aging is conventionally characterized as a progressive, time-dependent decline in physiological function but, more broadly, it represents a loss of regenerative capacity. The liver, the largest internal solid organ in mammals, is notable for its robust regenerative response to injury compared to other organs. Interestingly, the liver's regenerative ability also decreases with age. Traditional tissue-derived organoid models have been limited by their inability to fully replicate the liver's intricate cellular composition and architecture, restricting their utility for modeling liver homeostasis and disease in vitro. In my previous work, I have developed advanced organoid systems comprised of adult cholangiocytes and mesenchymal cells—and subsequently adult hepatocytes, cholangiocytes, and portal fibroblasts—to reconstruct the liver's periportal region and simulate key aspects of regeneration and fibrosis in a dish. These proof-of-concept studies paved the way for more sophisticated tissue-derived organoid models that better mimic hepatic tissue, enabling investigation of aging-related disruptions in stem cell–niche cell-cell interactions. Recently, I have established organoid (hepatocyte, cholangiocyte) and primary mesenchymal niche cell lines from young and aged mice. I am now employing complex organoid models composed of epithelium and mesenchyme to study aging-related phenotypes. My studies facilitate the identification of age-related signals that are diminished during aging, resulting in stem cell dysfunction—as evidenced by the reduced organoid formation efficiency observed in organoids derived from aged mice. The overarching aim of this research is to discover therapeutic targets that restore regenerative function in aged livers.

Abstract No. P137/T22**A human brain organoid model of ageing identifies chromatin-mediated control of transposable elements during neural ageing**

Elisa Gabassi^{1,*}, Sara Campagnoli¹, Lisa Fellner^{1, 2}, Stefan Salzmann¹, Julia-Anna Ulz¹, Nicole Amberg³, Theresa Lindlbauer^{1, 2}, Katharina Günther¹, Nina Grill^{1, 2}, Nikita Arnst¹, Samuel Aguirre⁴, Marian Martínez-Balbás⁴, Romana Höftberger³, Ahmad Salti⁵, Christopher Esk¹, and Frank Edenhofer¹

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Studies of human brain ageing have been constrained by limited access to patient-derived tissue and by the inability of induced pluripotent stem cell (iPSC) models to retain age-associated signatures. To address this limitation, we developed an inducible 3D human brain organoid model based on progerin overexpression aimed to recapitulate key hallmarks of ageing and to identify novel ageing regulators. Induced ageing in organoids resulted in epigenetic instability, characterised by a 40% reduction in H3K9me3-enriched heterochromatin, a two-fold increase in DNA damage, mitochondrial dysfunction, lipofuscin accumulation, and increased cellular senescence. Notably, ageing-associated phenotypes were partially reversible following pharmacological intervention.

To explore the molecular basis of these changes, transcriptomic profiling identified a distinct age-associated gene expression programme that closely mirrored profiles observed in aged human *post-mortem* cortical tissue, including downregulation of synaptic genes such as *SYN1* and *CALM2*, and known ageing regulators, including *SIRT1* and *HSPA8*. Among dysregulated genes, a histone demethylase was consistently reduced in both aged organoids and human cortical samples. ChIP-seq analysis identified enrichment of genomic regions containing motifs recognised by the KRAB zinc-finger protein ZNF93, a regulator of transposable element repression. Consistent with disruption of this regulatory pathway, aged organoids exhibited a 20% increase in transposable element expression, accompanied by re-localisation of ZNF93 from the nucleus to the cytoplasm, suggesting impaired chromatin recruitment downstream of reduced histone demethylase expression.

Together, these findings establish inducible ageing organoids as a human-specific model for investigating epigenetic instability and transposable element dysregulation during brain ageing, with implications for understanding neurodegenerative disease mechanisms and evaluating therapeutic interventions.

Abstract No. P138**A novel human stem cell culture model for severe traumatic brain injury reflecting sexual dimorphism in heterotopic ossification**

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Traumatic brain injury (TBI), which occurs more frequently in males than females, can cause critical primary and secondary injuries. In particular, severe TBI can induce heterotopic ossification (HO), the formation of ectopic bone in soft tissue outside of the skeletal system. The human mechanisms linking TBI to ectopic bone formation remain unclear. We therefore established an in vitro model using human adult stem cells to identify osteoinductive factors in sera of TBI patients. Supplementing osteoinductive medium with TBI serum led to highly increased osteogenic differentiation levels, with the most pronounced effect in male skeletal stem cells (SSCs). TGF- β 1 emerged as an important factor contributing to these osteogenic processes, with significantly higher serum levels in male than in female TBI patients 14 days post-injury. Neutralizing TGF- β 1 revealed a sexual dimorphism, markedly reducing osteogenic differentiation in male SSCs while no statistically relevant effect was detectable in female SSCs. These findings indicate that TGF- β 1 may play a central role in the development of HO in males, whereas alternative signaling mechanisms may be relevant in female patients.

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Abstract No. P139**Understanding the role of H3K36 methylation in epigenetic aging**

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Aging is associated with robust and reproducible changes in DNA methylation (DNAm), however the upstream chromatin mechanisms governing these methylation patterns are poorly understood. Histone 3 lysine 36 (H3K36) methylation, specifically H3K36me2/me3, recruit DNA methyltransferases, DNMT3A/B, to target regions. Therefore, we aimed to elucidate whether H3K36 methylation patterns change with aging and if this contributes to age-associated DNAm. H3K36me2 and H3K36me3 profiles were investigated in peripheral blood derived mononuclear cells of 5 old and 5 young donors. We observed significant changes in both marks, with particularly loss of H3K36me2 and gain of H3K36me3. Notably, these genomic regions were enriched in genes associated with leukocyte and immune function. We observed that subsets of both patterns overlap with each other, suggesting a shift toward H3K36me3 in aging. However, the changes in H3K36 marks did not reveal clear association with genomic regions with age-associated DNAm. To better understand how the H3K36 marks interact with DNAm, we generated CRISPR-Cas9 knockout of the histone methyltransferases, NSD1 and SETD2, which are responsible for depositing H3K36me2 and H3K36me3, respectively, in induced human pluripotent stem cells. In fact, NSD1 and SETD2 knockout clones showed corresponding decrease in either H3K36me2 or H3K36me3 patterns. NSD1 knockout resulted in a significant hypomethylation, but it was not considerably impacting age-associated DNAm. On the other hand, we did not see any evident DNAm changes in SETD2 knockout lines. Collectively, our work demonstrates an age-associated shift from H3K36me2 to H3K36me3, but these changes appear to be independent from age-associated DNAm.

Abstract No. P140**Divergent NF- κ B responses distinguish TNF- α -sensitive and -resistant human cancer stem cells**

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Cancer stem cells (CSCs) contribute to tumor initiation, metastasis, therapy resistance, and recurrence. We isolated and characterized primary human CSC populations from several solid tumor entities, including non-small-cell lung cancer (NSCLC), prostate cancer, and glioblastoma. Although the CSC populations clustered independently of tissue origin, full-length cDNA nanopore sequencing revealed significant enrichment of the GO term “NF- κ B binding” across all investigated populations. Moreover, the NF- κ B family member RELA was expressed in every population, suggesting a shared involvement of NF- κ B signaling in CSC biology.

Immunocytochemical analyses confirmed broad RelA expression but revealed marked differences in basal nuclear localization, expression of RelB and c-Rel, as well as TNF- α -induced nuclear translocation. These molecular differences were accompanied by divergent functional responses. TNF- α reduced survival of prostate CSCs, particularly in populations with impaired RelA translocation. In contrast, TNF- α enhanced survival of glioblastoma and NSCLC CSCs. Treatment with PDTTC reduced CSC viability and counteracted the cytoprotective effect of TNF- α , supporting a contribution of NF- κ B signaling to CSC survival. However, non-malignant human cardiac stem cells were more sensitive to PDTTC than the investigated CSCs, indicating limited tumor selectivity of untargeted pharmacological inhibition.

Our findings show that NF- κ B does not constitute a uniform survival program across human CSCs. Instead, basal and inducible NF- κ B states are associated with context-dependent TNF- α responses ranging from cytoprotection to apoptosis. Functional stratification of these response states may guide the development of cancer-specific strategies targeting NF- κ B signaling while limiting adverse effects on non-malignant stem cells.

Abstract No. P141**Analysis of senescence in blood brain barrier endothelial cells derived from Alzheimer specific induced pluripotent stem cells**

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Alzheimer's disease (AD) is the most common cause of dementia. Novel monoclonal anti-amyloid- β antibodies have so far only demonstrated therapeutic benefits in the early stages of AD and are associated with severe adverse effects such as cerebral edema. This highlights the need for additional therapeutic approaches. A pathological characteristic of AD is an alteration in the functionality of the blood-brain barrier (BBB), resulting in dysregulated amyloid- β transport. Furthermore, cellular senescence is increasingly discussed as a prodromal factor in the pathogenesis of AD. This raises the question of whether senescence in BBB endothelial cells represents a key pathogenetic mechanism in AD and whether senotherapeutic interventions, such as metformin and dasatinib/quercetin, may offer a potential therapeutic strategy.

To investigate senescence in AD, BBB endothelial cells were generated from induced pluripotent stem cells (iPSCs) derived from somatic cells of AD patients carrying an APOE4/4 genotype. The senescence-associated phenotype (SAP) and experimentally induced senescence with neocarcinostatin were compared between AD-derived and healthy control cell lines. Barrier integrity was assessed by measuring transendothelial electrical resistance (TEER) and permeability to sodium fluorescein. The SAP was characterized by immunofluorescence staining and flow cytometry, with analysis of proliferation markers, cell cycle regulators, and general senescence markers. In addition, a novel panel of 17 markers was established to analyze the senescence-associated secretory phenotype (SASP), enabling evaluation of paracrine, autocrine, and endocrine signaling effects.

The results demonstrate a pronounced SAP, including alterations in proliferation, cell cycle regulation, and general senescence markers, accompanied by a characteristic SASP signature.

This study underlines the importance of senescence in BBB endothelial cells with an APOE4/4 genotype. Targeting senescence, including the associated BBB alterations, by the use of senotherapeutic agents represents a promising scientific perspective and a potential therapeutic approach.

Abstract No. P142/T23**Extreme heterogeneity in hematologic aging is predominantly driven by stochastic variation, not by environmental nor genetic variation**

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Organismal aging is thought to arise from cumulative genetic and environmental interactions, complicating mechanistic dissection. However, the degree of heterogeneity has been largely unexplored.

Here, we generated a multi-layered hematopoietic dataset using a large cohort (>100 individuals) of young (2 months), middle aged (18 months) and old (>24 months) inbred female C57BL/6 mice housed under specified pathogen free conditions, to gain a comprehensive characterization of the aging process across blood, bone marrow, spleen, and transplantation assays.

While we recapitulate the expected age-related hematopoietic traits across the cohort, aged mice showed pronounced phenotypic heterogeneity: with some 24-month-old mice displaying parameters comparable to young controls, while others showed extreme aging outcomes. Surprisingly, a cohort-wide correlation analysis of the aged individuals discounted the previously accepted cause-effect relationships between hematopoietic stem cell (HSC) functionality (measured by transplantation output), HSC expansion, myeloid bias and anemia, with these outcomes being spread across different individual mice, suggesting that hematopoietic aging does not follow a single trajectory.

To investigate the molecular basis of this variability, we derived cell type-specific aging scores from bone marrow scRNA-seq data in a subset of the cohort, capturing transcriptomic dysregulation in hematopoietic and niche populations. Aging scores varied widely among aged individuals. To link aging traits with molecular data we performed intercellular communication analysis and identified distinct inflammatory signaling axes in the bone marrow niche associated with specific aging phenotypes. Elevated neutrophil-derived Oncostatin M signaling in a subset of mice seemed to remodel the AdipoCAR-HSC niche axis and drives myeloid bias, while in another subset of mice IL1 signaling from neutrophil to AdipoCAR correlated with anemia.

Our findings challenge the notion of a linear ageing process and rather indicate that hematopoietic aging proceeds through multiple independent routes, shaped by distinct inflammatory cell-cell communication axes within the bone marrow.

Abstract No. X1**Interplay between genetic and epigenetic alterations in liver cancer initiation**

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Loss of epigenetic patterns and gain of somatic mutations are known to play a key role during the acquisition of cellular plasticity leading to epithelial carcinogenesis (Hannahan, 2022). Despite the importance of these mechanisms, their interplay remains to be fully uncovered. Interestingly, our group has shown that liver cells become more plastic during the progression of chronic liver disease. This process results in the production of bi-phenotypic cells sharing expression of hepatocyte and cholangiocyte markers (Gribben et al., 2024). More recently, the same cells have been identified in hepatocellular carcinoma and cholangiocarcinoma, suggesting a role for this cellular plasticity in the progression to liver cancer.

To investigate cooperation between epigenetic remodelling and acquisition of oncogenic mutations in liver cancer initiation, we are taking advantage of Intrahepatic Cholangiocyte (ICOs) and Hepatocyte Organoids (HepOs) derived from primary tissues. These organoids are genetically modified to carry cancer driver mutations using RNP-based CRISPR. We achieved knockout efficiencies of 85-95% in three different ICOs lines carrying mutations in tumour suppressor genes. In parallel, we are treating the same organoids with small molecules affecting the epigenetic machinery. Cellular plasticity in these organoid lines is currently being assessed, with preliminary results suggesting that P53 deletion could increase the transdifferentiation capacity of ICOs.

Overall, this study will shed light on the molecular mechanisms that connect cellular plasticity with liver carcinogenesis. Such knowledge could help to develop new therapies promoting tissue regeneration while limiting tumorigenesis.

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Abstract No. P143/T40**Liver regeneration during chronic liver disease: SOX4 and KLF6 play a role in liver cell plasticity**

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Liver regeneration in the context of acute liver injury has been extensively studied. However, the regenerative processes occurring during chronic liver diseases are not fully understood yet. Previous investigations performed by our group have revealed that hepatocytes and cholangiocytes can transdifferentiate into one another during chronic injury. This process is marked by the production of biphenotypic cells expressing markers specific for the two cell types. The aim of this study is to identify the main factors that drive such cellular transdifferentiation during chronic disease progression.

We performed single nuclei RNA sequencing on liver biopsies of patients at different stages of the disease. That allowed us to identify a set of transcription factors that potentially play a role in the transdifferentiation process between hepatocytes and cholangiocytes. We identified 2 transcription factors, SOX4 and KLF6, as potentially crucial for acquiring the plasticity necessary for such cellular transdifferentiation. The expression of SOX4 and KLF6 in transdifferentiating cholangiocytes/hepatocytes was confirmed *in vivo* by immunostaining of human tissue slides. We then established two *in vitro* approaches to confirm the functional importance of these factors in transdifferentiation using patients'-derived intrahepatic cholangiocyte organoids (ICOs) and primary cells-derived hepatocyte organoids (HepOs). Both cell types can be transdifferentiated *in vitro* into biphenotypic cells, recapitulating the process observed *in vivo*. Through loss of function experiments conducted on ICOs, we showed the importance of SOX4 and KLF6 for the maintenance of cholangiocyte identity. When these 2 transcription factors are silenced, cholangiocytes are more prone to express hepatocyte markers upon transdifferentiation.

Overall, our experiments have identified two plasticity factors, SOX4 and KLF6, which could play an important role in regenerative processes occurring during MASLD progression.

Abstract No. P144**Impact of developmental programming by maternal obesity on alveolar macrophage-epithelial cell interactions in lung development and function**

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Lung development and function depend on coordinated interactions between alveolar epithelial type II cells (AECII) and tissue-resident alveolar macrophages (AMs). Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a well-established mediator of epithelial regeneration as well as AM survival and function. Maternal obesity alters key metabolic pathways, including glucose and lipid metabolism, and may thereby disrupt AECII–AM crosstalk. However, the molecular mechanisms underlying this disruption remain poorly understood. To elucidate these mechanisms, we established a 3D murine bronchioalveolar lung organoid (BALO) model by co-culturing FACS-sorted bronchioalveolar stem cells (BASCs) with lung-resident mesenchymal cells (rMCs). AMs were microinjected into BALOs to study AECII–AM crosstalk in an alveolar-like environment. Using this *ex vivo* immunocompetent model, we demonstrated functional AM–AECII interactions following influenza A virus (IAV) infection, as evidenced by the significant upregulation of IL-1 β , IL-6, and TNF- α at 48 hours post-infection in the presence of AMs. To investigate the effects of maternal obesity on lung regeneration, adult offspring of high-fat diet-fed mothers (HFDm) and control diet-fed mothers (CDm) were infected with IAV. During the course of infection, HFDm mice exhibited reduced survival, increased weight loss, and depletion of epithelial and airway cell populations compared with CDm mice. Furthermore, AECII-derived alveolospheres generated from IAV-infected HFDm mice exhibited significantly reduced organoid-forming efficiency compared with those generated from CDm mice, indicating impaired AECII regenerative capacity. By combining this *in vivo* model of maternal obesity with *ex vivo* BALOs generated from adult offspring, we aim to elucidate the mechanisms underlying these defects and gain insight into how maternal obesity influences long-term respiratory health and susceptibility to chronic lung disease in adult offspring.

Abstract No. P145**Modeling spinal and bulbar muscular atrophy using patient-derived neuromuscular organoids**

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Spinal and bulbar muscular atrophy (SBMA) is a rare X-linked neuromuscular disorder caused by a CAG expansion in the androgen receptor gene. Although traditionally considered a motor neuron disease, increasing evidence suggests that skeletal muscle pathology may contribute to motor neuron degeneration, potentially involving neuromuscular junctions (NMJs). However, whether early NMJ dysfunction contributes to non-cell autonomous motor neuron impairment in human SBMA remains unclear. Moreover, currently available human in vitro models lack the three-dimensional architecture required to preserve functional NMJs and muscle contractility, limiting their ability to capture key disease mechanisms.

To overcome these limitations, we established a patient-derived neuromuscular organoid (NMO) model of SBMA that recapitulates the essential cellular components of the NMJs, including spinal motor neurons, skeletal muscle fibers, and Schwann cells. NMOs self-organize in three dimensions and form functional NMJs that mature over time and drive skeletal muscle contraction. NMOs were generated from induced pluripotent stem cells derived from two brothers, one affected by SBMA and one unaffected.

Using this system, we observed an early impairment in spontaneous muscle contractility in SBMA-NMOs. Longitudinal analyses revealed progressive NMJ instability, indicating impaired maintenance of muscle-motor neuron connectivity. This neuromuscular dysfunction was associated with mitochondrial stress, reflected by reduced mitochondrial membrane potential and increased oxidative stress in both neuronal and muscle compartments. In addition, mitochondria exhibited increased fragmentation, consistent with reduced network connectivity and altered metabolic efficiency. Metabolic imaging using multiphoton fluorescence lifetime microscopy further indicated a shift of SBMA muscle fibers toward glycolytic, anaerobic metabolism.

Together, these findings demonstrate that patient-derived NMOs recapitulate early neuromuscular and metabolic alterations associated with SBMA in a human-relevant three-dimensional system, providing a valuable platform to dissect disease mechanisms and evaluate therapeutic strategies.

La Spada et al, Nature (1991), 352: 77-79

Faustino Martins et al, Cell Stem Cell (2020), 26: 172-186

Abstract No. P146**ECM-sensing modulates hepatocyte polyploidy in health and disease***Tom Deshayes^{1,*}, Carla Frau¹, and Ludovic Vallier^{1, 2}*¹ Berlin Institute of Health at Charité, Berlin Center for Regenerative Therapies, Berlin² Max Planck Institute for Molecular Genetics, Berlin

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Hepatocytes are the main cell type of the liver and one of the very few cells that naturally sustain polyploidy. The human liver contains 40% of polyploid hepatocytes and this proportion increases with age and in diseases such as MASLD/MASH and liver fibrosis. Hepatocyte polyploidization, or whole-genome duplication, can occur through different routes. Failed cytokinesis gives rise to hepatocytes with multiple nuclei (cellular polyploidy) while endocycling and failed chromosome segregation result in hepatocytes with nuclei containing multiple copies of the genome (nuclear polyploidy). These mechanisms are regulated by the E2F family proteins and the first wave of polyploidization occurs through the insulin pathway. Having multiple copies of the genome could act as a protective mechanism against mutation and cancer initiation, increase the metabolic potential of hepatocytes or “prime” them to quickly produce new cells in case of an injury. However, how polyploidy impacts hepatocyte functions and liver regeneration have been a long-standing debate in the field.

In this project, we take advantage of primary human Hepatocyte organoids to investigate how ECM proteins and their downstream signaling pathways can affect the cell cycle of hepatocytes, modulate their ploidy level and affect their gene regulation and regenerative abilities. To do so, we grow Hepatocyte Organoids in hydrogels with defined composition and in the presence of various inhibitors of ECM-receptors and found that Collagen-sensing impacts the ploidy level of Hepatocytes. Our first results hint at a role of the FAK/p53/p21 axis in this process, leading to an increased nuclear ploidy. We then aim to take advantage of this model to understand how gene expression is regulated in both cellular and nuclear polyploid cells, by combining sequencing and high-resolution imaging approaches. Taken together, our results uncover how extra-cellular micro-environment can influence polyploidy and how such change can be linked to disease progression and fibrosis.

Abstract No. P147**Neural progenitors adjust their cell cycle speed and intercellular communication to ensure normal brain development***Beatriz Lopez-Amo Calvo, Lara Harringer, Joyce Greisch, Frank Edenhofer, and Christopher Esk**

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During human brain development, cellular lineages are constantly altered by genetic or environmental perturbations, yet overall brain development in terms of growth and organization is largely maintained. Studies in brain organoids indicate that human neural progenitor cells (NPCs) can adjust progeny output in response to genetic or chemical tissue growth perturbation. How human NPCs carry out this replenishment response remains poorly understood. Here, we use a human chimeric brain organoid model of controlled, targeted depletion to dissect the cellular and molecular basis of replenishment. By combining microscopy, flow cytometry, time-resolved transcriptomics and lineage-resolved scRNA-sequencing, we demonstrate that tissue-perturbed organoids preserve overall cell type composition while undergoing a rapid expansion of replenishing, fast-growing lineages within ten days. This response is driven by accelerated cell-cycle progression and extensive rewiring of cell-cell communication, including developmental and extracellular matrix ligand–receptor pathways. Our findings reveal a compensatory strategy in which neural progenitors restore tissue homeostasis not via bulk self-expansion, but by dynamically adjusting proliferative behaviour and signalling interactions while broadly preserving developmental organization. These insights provide a framework for understanding tissue-mediated growth control and how human neural tissue withstands and repairs structural disruption, with implications for developmental neurobiology and regenerative strategies.

Abstract No. P148**Uncovering new CSF-contacting neuron subtypes as candidate regulators of the spinal cord stem cell niche**

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In the adult spinal cord, the stem cell niche lies in the ependymal zone surrounding the central canal and consists of quiescent cells. Following injury, progenitor cells re-enter the cell cycle and contribute to scar-forming glia. However, the markers of cells involved in this response and the regulators governing niche activation remain poorly understood.

We focus on cerebrospinal fluid-contacting neurons (CSF-cNs), as a controversial and understudied player in this niche. Their cell bodies reside in the periependymal layer, while an apical process extends into the central canal. CSF-cNs are GABAergic, express variety of chemo- and mechanosensory receptors, retain immature features, and migrate during postnatal development. Although their genetic ablation causes only minor locomotor deficits at baseline, it impairs recovery after spinal cord injury. Together with evidence that CSF-cNs suppress ependymal proliferation through dynorphin-dependent signaling, this identifies them as candidate niche regulators. We therefore asked whether molecularly distinct CSF-cNs populations may perform roles in niche regulation.

We isolated nuclei from genetically labelled neonatal mouse CSF-cNs, performed single-nucleus RNA sequencing, and validated markers in vivo by RNAscope across developmental stages. We identified five transcriptionally distinct populations, including two previously unrecognized spatially restricted groups: a ventral *Dcn*⁺ cluster and a caudal *Ren1*⁺ cluster. Both identities were maintained throughout development. Notably, ventral CSF-cNs were also *Pdyn*⁺, identifying a previously unreported source of dynorphin in proliferation control. We also identified a distinct *Ddc*⁺ population, suggesting a link to monoaminergic regulation of glial activation and neuroinflammation. Several clusters shared overlapping transcriptional programs, indicating a maturation continuum rather than exclusively terminally differentiated subtypes.

Our study provides a molecular framework for CSF-cNs heterogeneity and identifies potential targets within the spinal cord stem cell niche. These findings establish a basis for testing how distinct CSF-cN states regulate ependymal quiescence, activation, and progenitor fate after injury.

Abstract No. P149**Apically accessible intestinal organoids composed of stroma and macrophages - A model to test ingested chemical substances**

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The development of intestinal organoid cultures emerged as a potential replacement for animal experiments. However, the current state of organoid cultures is characterized by limitations in their capacity to accurately replicate in vivo conditions, which are attributed to the inaccessibility of the apical surface and the absence of stromal and immune cells. To overcome these constraints, we have developed a novel self-maintaining intestinal apical-out organoid (AOO) system that can be enriched with bone-marrow-derived macrophages. AOOs consist of a stromal-cell core covered by an epithelial layer and possess a constantly apically accessible surface. At an early stage, the epithelium exhibits regenerative properties. While covering the core the epithelium matures and finally comprises proliferative precursors, stem cells, and terminally differentiated secretory and absorptive cells. The AOOs display cellular turnover and allow continuous culture for several weeks. Embedded macrophages form inter-epithelial dendrites to sample environmental particles, migrate, and accumulate beneath the epithelial sheet in response to epithelial damage. This model underscores the utility of AOOs in analyzing tissue- and immune-cell responses to ingested and apically acting biological or chemical agents.

Abstract No. P150**Optimization of pluripotent stem cell spheroid growth in liter scale bioreactors via continuous medium perfusion**

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The development of pluripotent stem cell (PSC) culture protocols in multi-liter bioreactors is important for efficiently scaling PSC yields to manufacturing levels. We have previously demonstrated that PSCs can be expanded as spheroids in 3 L stirred-tank bioreactors (SBRs). These systems use impellers that continuously mix the culture medium but also generate shear stress, which may negatively affect PSC growth. Additionally, protocols that rely on gravity sedimentation of PSC spheroids and manual aspiration of spent medium become impractical beyond ~3 L culture scale. SBRs can be combined with perfusion systems to continuously supply fresh medium and eliminate the need for manual medium exchange. Here, we describe our ongoing efforts to optimize PSC spheroid growth in perfusion-fed, multi-liter bioreactor cultures by balancing stir speed, shear stress, and medium exchange rates. PSCs were seeded into dual pitch blade horizontal impeller-based SBRs at low stir speeds (i.e., low RPM) to promote spheroid formation. The RPM was then gradually increased to prevent spheroid aggregation and maintain culture homogeneity. Unsurprisingly, shear stress was detrimental to spheroid growth at higher speeds; however, this effect was mitigated by the addition of Pluronic. Specifically, 0.1–0.2% Pluronic enabled the formation of larger, more uniform spheroids, resulting in increased cell yields. We further evaluated spheroid growth using tangential flow depth filtration (TFDF) and alternating tangential flow (ATF) perfusion systems for medium exchange. Both systems supported spheroid expansion with similar efficiencies. Under optimized conditions, we observed yields of up to 25 billion PSCs in 2 L cultures ($\sim 12 \times 10^6$ IIs/mL; ~ 80 -fold expansion) after 10 days, while maintaining high levels of pluripotency marker expression (>95% OCT4⁺/NANOG⁺ cells). Overall, these results demonstrate that PSC culture in perfused SBRs is a promising approach for meeting manufacturing-scale demands.

Abstract No. P151**Collagen-based biomaterials in organoid technology for regenerative medicine: Current advances and future perspectives***Hamideh Karimi^{1, 2,*}*¹ Ankara University, Ankara, Turkey² Graduate School of Health Sciences, Interdisciplinary Stem Cell and Regenerative Medicine Program, Ankara, Turkey

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Organoid technology, as an advanced 3D laboratory model, has become a revolutionary advance in research and a valuable tool for disease modeling, drug screening, and regenerative medicine, given the limitations of laboratory resources and ethical considerations. However, the reliance on Matrigel, given its tumor-derived origin and biosafety issues, still poses limitations to the development of therapeutic applications of organoids. In contrast, collagen-based biomaterials have been proposed as promising alternatives for organoid culture due to their biocompatibility, biodegradability, cell-remodeling ability, and structural similarity to natural extracellular matrix. This study was a narrative review of collagen-based biomaterials commonly used for organoid culture, including Matrigel and decellularized extracellular matrix. Collagen hydrogels, collagen-based composite scaffolds, gelatin methacrylate, recombinant collagen, and decellularized extracellular matrix in organoid culture were reviewed. Reports focusing on regenerative medicine applications, including liver, intestinal, lung, cardiac, neural, and musculoskeletal organoids, were analyzed. Particular attention was paid to extracellular matrix–cell interactions, mechanobiology, scaffold properties, and their potential for transfer to clinical tissue regeneration. Collagen-based biomaterials were shown to effectively promote organoid development, organization, stem cell retention, and tissue-specific differentiation. In addition to physiologically relevant chemical and mechanical cues, the use of collagen hydrogels and decellularized extracellular matrix–derived matrices promoted more advanced stages of organoid differentiation than did standard culture media. Composite collagen matrices enabled better tuning of stiffness, degradation, and activity, whereas bioengineered collagen derivatives enabled integration with bioprinting and organ-on-a-chip platforms. Importantly, across several tissue types, including liver, intestine, lung, heart, and neural tissue, the use of collagen matrices increased cell function and facilitated regeneration. Collagen-based biomaterials can be considered promising substitutes for extracellular matrices in future organoid-based regenerative medicine applications.

Farrell, L. et al. (2025). *Organoids with a type 1 collagen scaffold to model bacterial cancer therapy*. *Cells*, 14, 524. <https://doi.org/10.3390/cells14070524>

Feng, B. et al. (2023). *Collagen-based biomaterials in organoid technology for reproductive medicine: composition, characteristics, and applications*. *Collagen and Leather*, 5, 35. <https://doi.org/10.1186/s42825-023-00142-6>

Keywords: Collagen-based biomaterials, Organoids, Biomaterials, Regenerative medicine, tissue regeneration

Abstract No. P152**Human iPSC-derived cerebral organoid model for investigating cellular senescence in APOE-associated Alzheimer's disease**

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Aim: Cellular senescence is a hallmark of aging that contributes to Alzheimer's disease (AD) by promoting chronic neuroinflammation through the senescence-associated secretory phenotype (SASP). However, human models integrating patient-specific genetic risk with age-related cellular phenotypes remain limited. We aimed to establish a human iPSC-derived cerebral organoid model to investigate cellular senescence in APOE-associated AD and facilitate the evaluation of senescence-targeted therapies.

Method: Cerebral organoids were generated from patient-derived APOE4/4 late-onset AD iPSC lines and genetically matched APOE3/3 control iPSC lines using a robust differentiation protocol. Organoid development was assessed by immunofluorescence analysis of neural progenitor and cortical markers to confirm reproducible neural differentiation and cortical identity. Organoids were maintained under long-term culture conditions, and cellular senescence was induced using DNA damage paradigms.

Result: Robust and reproducible cerebral organoids were successfully established from both APOE4/4 and APOE3/3 iPSC lines. Organoids exhibited consistent cortical organization and reproducible expression of neural progenitor and cortical markers across independent differentiations. This platform enables comprehensive assessment of cellular senescence using complementary readouts, including senescence-associated β -galactosidase activity, expression of canonical senescence and DNA damage markers, and quantitative profiling of SASP-associated inflammatory mediators. Furthermore, the model provides a framework for evaluating senescence-targeted interventions, including senolytic and senomorphic compounds.

Conclusion: We established a patient-derived human cerebral organoid platform that integrates APOE-associated genetic risk with a disease-relevant three-dimensional brain model to investigate cellular senescence in Alzheimer's disease. This physiologically relevant platform enables mechanistic studies of genotype-dependent brain aging and supports the preclinical evaluation of senescence-targeted therapies.

Abstract No. P153**Spatiotemporal lineage progression of pancreatic cells during mouse development revealed by spatial transcriptomics***Yung Hae Kim*, HongKee Moon, and Anne Grapin-Botton*

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The pancreas is an organ with dual functions: the exocrine pancreas produces and transports digestive enzymes to the small intestine, whereas the endocrine pancreas produces and secretes hormones to regulate blood glucose. Both exocrine and endocrine cells arise from pancreatic progenitor cells at distinct stages during development. The morphogenetic organization of a branched ductal network with terminal acini and nearby islets of Langerhans coincides with cell differentiation in the embryonic pancreas. Snapshots of emerging cell types have been captured by a variety of techniques, including single-cell RNA sequencing, in situ hybridization, and immunofluorescence, but each approach has limitations. Cell dissociation methods sacrifice spatial information and can lose fragile cell populations, while imaging based methods are restricted to a limited set of pre-selected genes or proteins.

Here, we apply high-resolution spatial transcriptomics (Visium HD) to map morphogenesis and differentiation of mouse embryonic pancreas during development (E10.5, E11.5, E12.5, E14.5, E16.5, E18.5). We optimized a protocol that preserves RNA quality in the later stages of pancreas (E16.5 and E18.5), overcoming the challenges posed by the high RNase activity in acinar cells and the slow penetration of fixative. Using cell segmentation on haematoxylin and eosin stained images, our preliminary data confirm lineage progression of pancreatic progenitors, early acinar progenitors, early and late islets, acinar cells, as well as surrounding mesenchyme. Ongoing integration of data across developmental stages and experiments will generate a comprehensive, spatially resolved atlas of pancreatic lineage progression. This atlas will serve as an unbiased benchmark for spatiotemporal studies of pancreatic development and may reveal previously unrecognised sub clusters within cell type-specific populations.

Abstract No. P154**Matrix stiffness as a modulator of cellular behavior: mechanical regulation in 2D and 3D systems***Maibritt Kretschmer^{1,*}, Sven Hendrix¹, and Stefan Zahler²*¹ Medical School Hamburg² Ludwig-Maximilians-Universität Munich

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The extracellular matrix (ECM) is a central regulator of cellular behavior, influencing processes such as adhesion, migration, differentiation, and tissue organization through biochemical and mechanical cues. Among its many properties, matrix stiffness has emerged as a key regulator of mechanotransduction pathways such as Notch, which governs vascular morphogenesis while also playing fundamental roles in stem cell maintenance, lineage specification, and organoid development. Although the influence of ECM stiffness has been extensively investigated in 2D culture, its contribution to 3D microenvironments relevant to organoid systems remains less defined due to the complexity of matrix design and limited tunability. This study investigates how matrix stiffness modulates endothelial behavior in different culture systems and highlights the importance of considering mechanical properties when establishing physiologically relevant organoid and in vitro models.

Using synthetic PDMS-based substrates in 2D and natural PhotoCol hydrogels in 3D, matrix stiffness was systematically modified and mechanically characterized. Cellular morphology, cohesion, sprouting, and mechanosensitive signaling, including Notch pathway activation, were assessed. In 2D, substrate stiffness influenced endothelial adhesion, morphology, and signaling. In 3D, spheroid architecture and sprouting behavior were strongly stiffness-dependent, with matrix mechanics either promoting coordinated outgrowth and migration or preventing sprouting and compromising structural integrity, accompanied by corresponding changes in Notch activity.

These findings demonstrate that endothelial self-organization is tightly regulated by the mechanical microenvironment and support a role for mechanosensitive pathways such as Notch in mediating these responses. Given the central role of Notch signaling in stem cell maintenance and differentiation, these results emphasize the importance of considering mechanical cues when developing physiologically relevant stem cell-derived organoids and advanced in vitro tissue models.

Abstract No. P155**From regenerative niches to organ reconstruction: a multi-scale framework for human liver regeneration**

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The liver is unique among adult organs in its capacity to restore mass, architecture, and function following injury. Despite decades of research, the mechanisms coordinating regeneration across biological scales remain incompletely understood, especially in humans. A major challenge is to integrate the molecular, cellular, and spatial processes that determine regenerative success or failure.

To address this challenge, we are establishing a multi-scale framework for human liver regeneration that combines longitudinal human samples, patient-derived models, and experimental systems, enabling mechanistic interrogation of tissue repair. Our objective is to identify the cellular states, microenvironmental signals, and tissue-level interactions that drive regeneration and coordinate organ rebuilding.

Three-dimensional tissue reconstruction using light-sheet microscopy with a cell-type-resolved proteomic panel has enabled detailed characterisation of healthy and diseased human liver architecture, revealing the spatial organisation of the liver lobule, including the biliary network, vascular structures, and parenchymal compartment.

To investigate its singular organisation at molecular resolution, we developed a liver-specific spatial transcriptomic framework based on MERFISH. Using a custom-designed 1,000-gene panel, we resolved lobular architecture, metabolic zonation, and cellular identities in healthy livers. The sensitivity and specificity of this approach enable the reconstruction of inter- and intracellular communication networks and provide a foundation for characterising regenerative mechanisms at subcellular resolution.

To experimentally investigate the earliest events of liver repair, we established a tractable system of primary human hepatocyte-derived organoids from healthy adult and neonatal liver samples. Our model maintains the hepatocytes in a quiescent functional state while preserving their capacity to respond to regenerative stimuli and re-enter the cell cycle.

Together, these complementary approaches enable the characterisation of regenerative niches across scales, from molecular signalling and cellular interactions to tissue remodelling and organ reconstruction. Ultimately, this work seeks to define the principles governing human liver regeneration and provide a foundation for future regenerative medicine strategies.

Abstract No. P156**Transcription factor-guided co-differentiation of human iPSCs generates isogenic multilineage cardiac microtissues with a distinct endothelial identity**

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Human vascularized cardiac tissues for disease modeling and drug screening typically require separately differentiating and mixing multiple cell types, often from different iPSC lines or donors, limiting isogenic applications and precluding developmental co-emergence between lineages. Self-organizing cardiac organoid approaches, conversely, offer limited control over cellular composition, and endothelial fractions remain below physiological levels. Here, we developed a lineage-biased co-differentiation protocol combining wildtype human iPSCs with doxycycline-inducible ETV2 and NKX3.1 lines at defined ratios, jointly differentiated toward cardiomyocytes with staged growth factor supplementation. Single-line differentiation confirmed that ETV2 or NKX3.1 overexpression redirects cell fate toward endothelial cells or mesenchymal progenitors, respectively, within the cardiomyocyte differentiation milieu. At day 14, co-differentiation produced multilineage cultures comprising approximately 50% cardiomyocytes, 20% endothelial cells, and 20% mesenchymal progenitor populations. Single-cell RNA sequencing revealed that endothelial cells emerging alongside cardiomyocytes exhibit a transcriptional identity distinct from endothelial cells generated by single-differentiation: canonical arterial markers (DLL4, EFNB2, GJA4, CXCR4) were downregulated, while ENG, MMP2, SPARC, VIM, NR2F1, NR2F2, APLN, and ESM1 were upregulated, a profile suggestive of cardiomyocyte-instructed adaptation toward a capillary-like or remodeling-competent phenotype. These cells were then harvested for cardiac organoid generation. After two weeks of cardiac organoid culture, co-differentiation-derived endothelial cells showed improved network integration, mural coverage by PDGFR β + cells, and cardiomyocyte-vessel proximity compared to organoids assembled from separately differentiated cell mixtures. GFP-labeling confirmed the ETV2 line as a substantial source of the endothelial population in the organoids. The resulting vascular density scaled tunably with ETV2-iPSC input ratio, demonstrating protocol controllability. This platform enables generation of isogenic multilineage cardiac tissues from a single differentiation, positioned for patient-specific vascularized disease modeling. Ongoing work integrates these cells into perfusable cardiac-on-chip platform to assess long-term vascular and contractile function against sequentially differentiated cell mixtures.

Abstract No. P157**Characterization of early human cortical neurogenesis in MCT8-deficient cerebral organoids**

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Allan-Herndon-Dudley syndrome (AHDS) is a **rare X-linked disease** associated with severe cognitive and motoric developmental delay. It is caused by mutations in the *SLC16A2* gene, encoding for the thyroid hormone (TH) transporter MCT8. TH is critical for human brain development but, to date, the precise developmental effects of MCT8-deficiency on **human fetal neurogenesis** remain elusive. Against this background, we engineered **different MCT8-deficient hiPSC lines** and generated **cerebral organoids (hCOs)** to characterize cell type-specific effects of MCT8-deficiency on human cortex development. Using highly multiplexed **single cell RNA-seq**, we profiled the transcriptome of three MCT8-deficient hCO models *versus* isogenic controls under different TH culture conditions and at different time points. Quality of hCO cultures was stringently controlled by IF-based assessment of cytoarchitectural features at DIV 21, 46, 58 and 70. MCT8-deficient hCOs showed no differences in cortical architecture on DIV 46 yet a delay of TH-dependent upper layer neuron differentiation at DIV 58. When using scRNAseq to assess effects of MCT8 deficiency on cell type-specific gene expression at DIV 70, we observed both TH-dependent and TH-independent response patterns in neuronal progenitor cells and deep layer neuron subtypes. Interestingly, instead of an absent TH response, due to dysfunctional TH transport, so far defined as key driver of the pathology of AHDS in the CNS, we detected an enhanced TH response pattern caused by MCT8 deficiency. Current therapeutic strategies focus on compounds circumventing the transport defect and mimicking TH. Our experimental strategy (isogenic pairs, triplicate analyses) allowed new insights into the role of MCT8 deficiency in the neurodevelopmental pathogenesis of AHDS and strongly imply that new therapy approaches need to be developed that take our findings into account.

Abstract No. P158**Choroid plexus organoids as a model system for evaluating blood-cerebrospinal fluid barrier properties in healthy patient-derived induced pluripotent stem cells**

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In diseases of the central nervous system, the cerebrospinal fluid (CSF) is a key target for studies on disease onset and progression, as well as treatment efficacy. One of the primary routes of access to the CSF is by crossing of the blood-CSF-barrier (B-CSF-B) of the choroid plexus (ChP). The ChP epithelium tightly regulates the barrier permeability, CSF secretion, and brain homeostasis. Emerging studies using organoids manage to reproduce the human B-CSF-B more accurately than humanized mouse models. In our study, we characterized patient-specific ChP organoid models as a mean evaluate B-CSF-B properties.

Therefore, embryoid bodies of healthy induced-pluripotent stem cell (iPSC) lines of different ages and genders were generated and differentiated into a 3D neuroepithelial tissue of ChP identity, which developed fluid-filled compartments. The integrity of the B-CSF-B was evaluated using immunofluorescence staining of epithelial markers that are essential for barrier function and CSF secretion, as well as permeability assays.

The ChP organoids exhibited morphologically consistent expansion of neuroepithelium from culture day 10 onwards, with observable development of fluid-filled compartments on day 25-30 across different cell lines and culturing methods. Immunostaining evaluated the presence of key markers of the ChP epithelium, such as Transthyretin (TTR) and tight junction proteins. The analysis further investigated proliferation and apoptosis within the organoids. Molecules with known barrier permeability properties showed the degree of barrier integrity and function within the organoids.

This investigation demonstrates the applicability of ChP organoids to assess the properties of the B-CSF-B within healthy patient-derived iPSC lines.

Abstract No. P159**Modeling an immune-competent 3D liver portal niche to study T cell-mediated alloimmune injury and tolerance induction**

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Liver transplantation is the only curative treatment for end-stage liver disease, but prevention and treatment of allograft rejection, including acute T cell-mediated rejection (TCMR), still depend on systemic immunosuppression with long-term side effects. Acute TCMR occurs primarily in the portal region, where biliary epithelium, vasculature, and mesenchyme are densely organized, and is driven by alloreactive T cells that particularly target biliary epithelial cells (BECs). Regulatory T cells (Tregs) can promote immune tolerance, and increased Treg numbers correlate with graft acceptance, but their therapeutic potential and most relevant mechanisms remain unclear. Preclinical testing of Treg-based therapies relies largely on animal models, which have ethical and translational limitations, while current in vitro systems are mostly two-dimensional not capturing immune-tissue interactions.

Here, we establish a human organoid model of liver TCMR focused on the portal area as the primary injury site. The model combines BECs, endothelial cells, fibroblasts, and immune cells. To overcome the limited self-organization of non-stem-cell-derived primary cells, we enable tissue assembly under bidirectional flow in a rotating bioreactor. Incorporation of pre-enriched alloantigen-specific allogeneic PBMCs induces immune-cell migration into organoids, as shown by liver-cell imaging, upregulation of T-cell activation markers (CD137, CD154), and production of effector cytokines (TNF α , IFN γ). Those responses were suppressed by tacrolimus, validating the model's therapeutic relevance. We will use this platform to compare clinical-grade Treg products, including CRISPR-Cas9-edited FKBP12KO Tregs developed at our institute, engineered to preserve Treg activity during tacrolimus exposure. Treg function will first be evaluated in standard suppression assays; using our platform, we will then increase the complexity of functional testing. Unbiased omics approaches will be applied to define mechanisms of action, including the relevant components of direct cell-cell interactions and soluble mediators, such as CTLA-4-mediated inhibition and IL-10/TGF- β secretion, which can be individually perturbed to verify their contribution to tolerance.

Zarrinrad G et al. Clinical development of tacrolimus-resistant regulatory T cells to enable simultaneous immunosuppression and immune regulation. Mol Ther Adv. 2026 Apr 9;34(2):201735. doi: 10.1016/j.omta.2026.201735. PMID: 42147444; PMCID: PMC13175770.

Abstract No. P160**Of mice and men: can cellular plasticity vary between species?**

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Recent findings in human suggest that transdifferentiation between hepatocytes and cholangiocytes is the primary regenerative process in chronically injured livers. Transdifferentiation is characterized by the direct conversion of one cell type into another. This form of cellular plasticity occurs in response to extracellular stimuli and does not involve an intermediate progenitor state. However, this process is limited to a small number of cells in patients and occurs only in end-stage disease. This contrasts sharply with observations in mouse models of liver injury which display large scale regenerative processes involving up to 40% of liver cells. This divergence in regenerative capacity suggests the existence of species-specific differences in cellular plasticity. To investigate this hypothesis, we derived intra-hepatic cholangiocyte organoids from both mouse and human livers, and characterized their plasticity by defining their ability to transdifferentiate into hepatocyte. Preliminary results suggest that mouse cholangiocytes display an increased capacity for transdifferentiation when compared to their human counterparts. We now aim to identify and validate the molecular mechanisms driving this divergence in cellular plasticity, with a focus on epigenetic regulation and gene regulatory networks. Finally, we will explore strategies to increase a plasticity-permissive state in human liver cells. This research could lead to new regenerative therapies in liver diseases, an area with a significant unmet medical need.

Abstract No. P161**Impact of epigenetic erosion on wild-type and P53-mutated cholangiocyte organoids**

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Metabolic Dysfunction–Associated Steatotic Liver Disease (MASLD) affects 1.3 billion people worldwide and this chronic disorder is a major contributing factor to liver cancer ¹. The Vallier laboratory has found that cholangiocytes and hepatocytes can transdifferentiate between each other during MASLD progression². This process is marked by the apparition of biphenotypic cells co-expressing cholangiocyte and hepatocyte markers. The apparition of these cells suggest an increase in cellular plasticity, which is likely induced by an epigenetic erosion driven by the diseased niche. On the other hand, bi-phenotypic cells are most abundant in end-stage disease, which carries a high cancer risk while they can also be found in both hepatocellular carcinoma and cholangiocarcinoma. Additionally, cancer-related genes such as *FOXO3* and *SOX4* are expressed in bi-phenotypic cells. Thus, we hypothesise that the epigenetic erosion associated with the emergence of bi-phenotypic cells could facilitate the progression toward tumorigenesis.

Here, we investigate this hypothesis by studying the impact of DNA methylation and Histone modification inhibition on the plasticity of wildtype and *P53*-mutated intrahepatic cholangiocyte organoids (ICOs) derived from primary tissues. For that we use small molecules and genetically engineered ICOs using RNP-based CRISPR. We then analysed the impact of these different conditions on cholangiocyte identity, proliferation and capacity to transdifferentiate in vitro.

Overall, this study will reveal the interplays between epigenetic regulations and somatic mutation in the development of cellular plasticity in pre-cancerous, late-stage MASLD. A deeper understanding of this process may ultimately contribute to improved strategies for the prevention and treatment of liver cancer associated with MASLD.

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Abstract No. P162**Molecular profiling of human intrahepatic cholangiocarcinoma across primary tissue and patient-derived tumoroids**

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Intrahepatic cholangiocarcinoma (iCCA) is a highly aggressive primary liver cancer with a poor prognosis and limited treatment options. The pronounced molecular heterogeneity of iCCA across genomic, transcriptomic, and epigenetic levels represents significant challenges for accurate diagnosis and for the development of targeted therapies.

To address this challenge, we established a deeply characterized patient-derived biobank comprising matched tumoral and non-tumoral tissues, along with corresponding organoid and tumoroid models, to better understand the mechanisms associated with iCCA. Using a multi-omics strategy that combines single-nuclei RNA sequencing, whole-genome sequencing, and DNA methylation profiling, we systematically characterize the molecular and cellular landscape of iCCA in-vivo. Comparing tumoral tissues with matching non-tumoral tissues and healthy cholangiocytes identifies novel disease-associated cell populations, cancer cell trajectories as well as genes and regulatory programs that underlie tumor heterogeneity and progression.

Preliminary transcriptomic analyses reveal a pronounced inter-patient heterogeneity, with tumoral cells displaying different transcriptional states across tissue samples. Furthermore, genomic analyses identified recurrent chromosomal alterations and frequently affected genomic regions across the patient cohort. In parallel, different media are being evaluated to purify the tumoral cell population in culture and optimize culture conditions. Preliminary data suggest that tumoroids can proliferate in the absence of selected factors, whereas the growth of matched non-tumoral organoids is restricted under these conditions. Ongoing comparative analyses of primary tissues and their derived in-vitro models will clarify the extent to which tumoroids recapitulate patient-specific tumor features.

Collectively, this study identifies key molecular signatures associated with iCCA, reveals how to improve the fidelity of tumoroid-based models and establishes a robust platform for biomarker discovery and future precision oncology approaches.

Abstract No. P163/T38**Lymphatic vasculature reprograms liver epithelium in assembloids and patients**

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Understanding how microenvironmental cues can hijack epithelial cell identity is central to regenerative medicine. Biliary atresia, an infant liver biliary epithelial disease, offers a compelling disease model in which niche signaling drives epithelial fate regression, a phenomenon with broad implications for organoid-based disease modeling and stem cell biology.

Using a large, multi-center patient cohort (n=46), we integrated single-nuclei RNA sequencing (snRNA-seq), spatial transcriptomics (10X VisiumHD & Stereo-Seq), multiplexed immunofluorescence, and 3D light-sheet microscopy to resolve the hepatic lymphatic niche at single-cell and spatial resolution. To dissect niche-epithelial crosstalk, we employed human organoid and stem cell platforms: intrahepatic cholangiocyte organoids (ICOs), iPSC-derived hepatocytes (iHeps), and multicellular fetal liver-like organoids (FLOs), the latter recapitulating the cellular complexity of the developing liver.

Results:

snRNA-seq revealed profound transcriptional dysregulation of lymphatic endothelial cells (LECs) in patients with poor outcomes, corroborated spatially by LEC hyperplasia and 3D remodeling of the intrahepatic lymphatic network. Among upregulated LEC-derived mediators, the chemokine CCL21 emerged as a key niche signal enriched in patients requiring transplantation. To interrogate how this pathological secretome reshapes epithelial identity, we exposed FLOs to BA tissue-conditioned media, observing cholangiocyte dedifferentiation and delumenization into CK19⁺ ductular-reactive conglomerates — faithfully modeling the in vivo ductular-epithelial reaction in a dish. Targeted CCL21 treatment of ICOs and iHeps consistently upregulated SOX9 and LGR5, respectively, activating biliary progenitor and hepatic stemness programs - especially in patients with an inflammatory disease phenotype. Direct co-culture of patient-derived hepatic LECs with ICOs recapitulated this epithelial fate regression, establishing a lymphatic-to-epithelial axis of niche-driven reprogramming.

Conclusion:

We identify CCL21-high lymphatic endothelial cells that reprogram liver epithelium in immune-mediated liver epithelial disease toward a progenitor/reactive state, impairing epithelial function. Primary assembloid and iPSC-based fetal tissue-like organoids faithfully model this niche-epithelial crosstalk, offering tractable systems for dissecting fate decisions in the developing and diseased liver.

Abstract No. P164**Glucose uptake assay to study functional maturation of intestinal organoids**

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Glucose enters cells via two transporter families: passive GLUTs and active SGLTs. These days SGLT1 and SGLT2 are getting more in focus as therapeutic targets especially for diabetes. To test potential drugs, a reliable glucose uptake assay is needed. Traditionally, radioactive glucose analogues like ¹⁴C-AMG are used in glucose uptake assays but these have safety and cost drawbacks. The fluorescent probe 1-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-1-deoxy-d-glucose (1-NBDG), unlike 2-NBDG, is transported by both GLUTs and SGLTs and remains stable under alkaline conditions, enabling simpler, safer assays. Using human SGLT1- and SGLT2-expressing cell lines, researchers developed and validated a high-throughput screening method with 1-NBDG, showing results consistent with traditional radioactive assays.

In this project, self-synthesized 1-NBDG is used to set up a functional glucose uptake assay in human small intestinal organoids. The assay is validated using known SGLT1 inhibitors with either an image based or a spectrophotometric readout. When testing different differentiation conditions, a disconnection between the results gained during functional testing with the 1-NBDG assay and gene expression levels were discovered. This result highlights the need for a reliable functional glucose assay in addition to RT-qPCR for drug discovery and basic research.

Abstract No. P165**Modeling long-term host-microbe interactions with human intestinal organoid monolayers**

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Clinical studies have linked the presence of specific bacterial strains in the gut microbiome with beneficial outcomes, yet the mechanisms of action are often difficult to resolve. This is in part caused by the lack of human *in vitro* models that allow the mechanistic analysis of host-microbe-interaction, especially for a prolonged duration of time, such as weeks rather than hours. To investigate host-microbe-interaction *in vitro*, we use patient-derived intestinal organoids, which preserve gastrointestinal region specificity, epithelial cell heterogeneity, and innate immune features. By growing human intestinal organoids as monolayers and applying previously reported differentiation media, we generated an apically accessible intestinal epithelium composed of differentiated enterocytes and mucus-secretory cells. To enable long-term colonization, we focused on the probiotic bacterium *Limosilactobacillus reuteri* (*L. reuteri*). While contact with pathogenic bacteria, such as Enteropathogenic *Escherichia coli* (EPEC), leads to cell death within the first 3-6 hours, direct contact with *L. reuteri* induces only mild epithelial cell responses while maintaining barrier integrity. We observed that epithelial cell responses differ according to the enterocyte maturation state in the small intestine. This model will now be used to address the question, whether secreted mucus is necessary to physically separate the colonic epithelium from a lumen-like environment containing probiotics to support long-term co-culture. Our approach highlights adult stem cell-derived intestinal organoids as physiologically relevant models for dissecting strain-specific probiotic mechanisms across different regions of the host-microbe interface.

Abstract No. P166**CELLnROLL: a gentle, parallelized 96-well cell sorting platform for low-input organoid and co-culture applications***Sarah Scatigna*

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CELLnROLL is a novel cell sorting platform designed to address the limitations of conventional technologies when working with low-input and fragile samples. Standard cell sorting approaches often require large cell numbers, extensive preprocessing, specialized instrumentation, and frequently expose cells to significant shear stress during operation. These constraints restrict their use in emerging models such as tissue-derived organoids and co-culture systems, where sample availability is limited, cells are often fragile, and preservation of physiological states is critical for downstream applications. As a result, researchers are often forced to pool multiple samples to reach sufficient input, which dilutes the signal and obscures biological variability.

Here, we present CELLnROLL, a parallel cell sorting platform implemented in a standard 96-well plate format. The system distributes sorting reactions across individual wells, enabling simultaneous processing of multiple samples, targets, or conditions in parallel. This parallelized design allows scalable handling of low cell numbers while maintaining compatibility with standard laboratory workflows and infrastructure. The platform is microfluidics-free, avoids high-shear flow conditions, and completes sorting in as little as 10 minutes, helping preserve cells in their native physiological state.

CELLnROLL is particularly suited for applications involving limited sample input. The platform has demonstrated the ability to perform sorting with as few as 5,000 cells per well without impacting viability. In negative isolation of T cells from 10,000 PBMCs, CELLnROLL achieved a purity above 94%, while positive selection experiments reached near 100% purity, sufficient for sensitive downstream applications such as proteomics and sequencing.

By combining parallelization, low-input compatibility, and rapid, gentle processing in a scalable 96-well plate implementation, CELLnROLL provides a practical alternative to traditional cell sorting technologies. The platform is designed to enable future applications in organoid research, co-culture systems, immune profiling, and other settings where limited sample availability and the need for parallel processing are critical.

Abstract No. P167**Charité PRIMARY TISSUE PIPELINE: a central service platform to provide access to primary tissue for stem cell-based and biomedical research**

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Human model systems, such as organoids and engineered tissues, serve as proxies for specific aspects of organ function. Patient-derived tumor organoids can capture oncogenic signaling within a patient-specific background, which can reveal mechanisms of therapy resistance. Additionally, advances in omics-based transcriptome, proteome, and metabolome analysis technologies offer invaluable tools for uncovering disease mechanisms and optimizing therapies. When combined with clinical data, these methods can enhance the predictive value of preclinical research and expand the possibilities for personalized medicine, ultimately benefiting patients. A structured and comprehensive access to primary tissue is essential for these advancements.

As one of Europe's largest hospitals, Charité provides both patient care and performs extensive biomedical research. During surgeries and medical procedures, tissues and cells are excised and withdrawn from patients—though only partially utilized for diagnostics.

With the “Primary Tissue Pipeline”, we have established a central facility at the Charité – Universitätsmedizin Berlin that facilitates access to primary tissue for Charité researchers as well as external and industry partner. It serves as an interface between Charité as a hospital and the biomedical research community. The pipeline supports specific research projects by initiating and coordinating the sampling of human tissue. Specifically, it contacts relevant clinical departments, recruits patients and suitable biosamples, supports with application for ethical votes and builds up a project-specific logistic for sampling. The initiative serves as a central point of contact and takes on a pilot function in order to make the best possible use of the existing infrastructures at Charité.

This initiative enhances the stem cell based models and direct tissue cultures, promoting personalized medicine and regenerative medicine by bridging the gap between healthcare and research, while reducing reliance on animal models.

Abstract No. P168/T37**Characterization of naïve human neuroepithelial stem progenitor cells with unlimited self-renewal and broad neurogenic potential**

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Cell lines derived from early embryonic stages have significantly contributed to our understanding of mammalian development, yet they predominantly reflect late developmental stages, following neural tube closure. The current human neural stem/progenitor cell (NSPC) models, such as rosette-like or radial glial-like cells, either represent later neurodevelopmental phases or fail to sustain long-term clonal self-renewal. Here, we report the successful isolation and long-term expansion of a tissue-derived human NSPC population that transcriptomically mirrors the human neuroepithelium at five weeks of in vivo embryonic development. These embryonic neural stem/progenitor cells (eNSPCs) display a distinct naïve, non-polarized, pre-rosette phenotype, distinct from previously described rosette-like and radial glial-like NSPC models. Notably, eNSPCs exhibit virtually unlimited self-renewal capacity and retain multipotent differentiation potential into diverse neural lineages of both the central and peripheral nervous systems—including dopaminergic neurons, V2a progenitors and interneurons, and neural crest derivatives. Multimodal analyses on both RNA and protein level, including single-cell RNA sequencing, immunocytochemistry, flow cytometry and ATAC-sequencing, revealed that eNSPCs constitute a homogeneous population of multipotent stem cells, rather than a mixture of committed neural progenitors, with distinctive expression of early neurodevelopmental markers, lack of lineage commitment and broad differentiation potential. In comparison with other previously established NSPC models, eNSPCs exhibit a distinct transcriptomic profile that corresponds to earlier human developmental stages. Together, these findings establish eNSPCs as the earliest stabilized human NSPC model reported to date and highlight their value for studying early human neurodevelopment, lineage specification, disease modelling, and regenerative medicine.

Abstract No. P169/T39**Liver regeneration is programmed in the epigenetic landscape of cholangiocytes during fetal development**

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Liver regeneration during chronic disease involves transdifferentiation of intrahepatic cholangiocytes into hepatocytes. This process implies that cholangiocytes have a unique capacity to switch of cellular identity in extreme conditions. The molecular mechanisms which establish this cellular plasticity in the human liver remain unclear. To address this question, we first assessed the transdifferentiation capacity of adult intrahepatic and gallbladder cholangiocytes using organoids. Despite a shared transcriptome, gallbladder organoids failed to activate hepatocyte markers, indicating a limited regenerative capacity. In parallel, we profiled genome-wide histone modifications and DNA methylation in intrahepatic and gallbladder organoids and their foetal progenitors. Our integrated analysis revealed epigenetic features associated with a permissive state at hepatocyte marker loci in intrahepatic cholangiocytes. In particular, intrahepatic cholangiocyte organoids showed increased H3K27ac enrichment and decreased DNA methylation at hepatocyte genes compared with gallbladder organoids. These hepatocyte markers are activated upon transdifferentiation of intrahepatic cholangiocytes to hepatocytes, suggesting that both histone marks and DNA methylation establish a competence towards hepatocyte fate. Notably, this signature is already present in hepatoblasts, the foetal progenitors of both hepatocytes and cholangiocytes during liver organogenesis. In contrast, this signature is absent in foetal gallbladder cholangiocytes, indicating that adult liver regeneration is developmentally programmed. To confirm the functional importance of epigenetic programming, small molecule inhibitors of epigenetic modifiers were added in culture conditions inductive for transdifferentiation in vitro. Under these conditions, adult gallbladder organoids induced hepatocyte markers upon transdifferentiation, supporting the role of epigenetic mechanisms in cholangiocyte plasticity. Together, our findings show that cellular plasticity of intrahepatic cholangiocytes is epigenetically programmed during foetal development to enable regenerative processes in the adult liver.

Abstract No. P170**Modeling neurodevelopmental diseases in 3D dorsal forebrain organoids**

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Human cortical development relies on tightly coordinated regulatory programs controlling progenitor proliferation, neuronal differentiation, migration, and maturation. Disruption of these processes contributes to neurodevelopmental disorders (NDDs), including Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS) and Schaaf-Yang syndrome (SYS). BBSOAS is caused by haploinsufficiency of the transcription factor NR2F1, whereas SYS results from mutations in the imprinted gene MAGEL2, linked to regulation of post-transcriptional pathways.

We previously established CRISPR/Cas9-engineered hiPSC models to study both conditions in 2D hiPSC-derived cortical neurons by integrating multi-omics approaches. Using transcriptomic, epigenomic, chromatin accessibility, and chromatin conformation data, we characterized NR2F1-associated regulatory networks and inferred NR2F1 dosage-dependent gene regulatory networks, revealing stage-specific functions of NR2F1 in progenitor maintenance and neuronal lineage specification. MAGEL2-related defects were mapped by transcriptomics, proteomics, and ubiquitinomics, identifying imbalances in progenitor proliferation, neuronal maturation, migration, and synaptic development. Although these 2D models provide novel insights into the pathophysiology of these conditions, some of which may reflect broader pathogenic pathways shared with other NDDs, they do not capture key aspects of tissue architecture and cellular heterogeneity.

We are therefore currently characterizing 3D dorsal forebrain organoids (DFOs) derived from isogenic hiPSC lines modeling BBSOAS and SYS to study genotype-phenotype relationships in a context that more closely resembles the developing human cortex. We will apply single-cell multi-omics (combined transcriptomic and epigenomic profiling from the same cell) to resolve cell-type-specific regulatory states of NR2F1. As a next step, we plan to extend these analyses by studying MAGEL2, with the goal of incorporating emerging single-cell proteomics methods for both disorders to link regulatory states to protein-level changes across cell types.

Abstract No. P171**Hormone-responsive reproductive organoid interfaces for interpreting stem cell-derived EVs effects***Sevgi Unal Karakus^{1, 2}*¹Ankara University Stem Cell Institute, Turkey²Gazi University, Ankara, Turkey

Human reproductive success is governed by a temporally coordinated dialogue between a hormone-conditioned endometrial niche and an implantation-competent trophoblast lineage. Stem cell-derived extracellular vesicles (EVs) have increasingly been discussed as cell-free mediators of tissue repair, immunomodulation, angiogenesis, and endometrial regeneration. However, their biological effects are often evaluated outside spatially organized, hormone-responsive epithelial–stromal–vascular systems that more closely reflect the implantation microenvironment. This limitation restricts mechanistic interpretation, translational safety assessment, and the rational positioning of EV-based reproductive interventions.

In this review, hormone-responsive reproductive organoid and spheroid interface models are discussed as emerging platforms for interpreting the context-dependent actions of stem cell-derived EVs. Particular attention is given to estrogen- and progesterone-conditioned endometrial epithelial and stromal compartments, epithelial polarity, extracellular matrix remodeling, angiogenic support, inflammatory regulation, fibrosis-associated signaling, and trophoblast-facing epithelial competence. These model systems provide a biologically relevant framework for examining how EV cargo may influence endometrial receptivity, stromal remodeling, vascular support, and implantation-associated cellular communication.

Importantly, stem cell-derived EVs should not be regarded as uniformly regenerative biological agents. Vesicular signals capable of promoting repair, angiogenesis, and epithelial–stromal recovery may also modify stromal permissiveness, epithelial plasticity, or invasion-associated pathways under conditions of aging, chronic inflammation, genetic susceptibility, or tumor-adjacent remodeling. Therefore, reproductive organoid-based models may serve not only as platforms for regenerative screening but also as early safety filters for detecting undesired pro-remodeling or pro-invasive responses.

By integrating reproductive stem cell biology, EV-mediated communication, organoid engineering, and computational interpretation, hormone-responsive reproductive organoid interfaces may contribute to more precise, safer, and mechanistically grounded approaches for endometrial dysfunction, fertility preservation, and gynecologic disease prevention.

Keywords: Organoids; Stem cell-derived EVs; Endometrial receptivity; Reproductive niche; Hormone-responsive modeling

Abstract No. P172**Integrating computational and stem cell-derived modeling of metabolic liver disease identifies vitamin B2 networks as tractable targets**

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Human-induced pluripotent stem cell (hiPSC)-derived models are a widely used technology for drug testing; however, they often operate in isolation from computational drug predictions, limiting their full potential. Here, we integrate hiPSC-derived modeling into computational frameworks to improve disease network identification. We apply this strategy to metabolic dysfunction-associated steatotic liver disease (MASLD), identifying vitamin B2 networks as therapeutic targets. First, we developed MetaLiver, a digital systems biology platform harmonizing a MASLD-specific knowledge graph, bulk and single-cell transcriptomic patient data. MetaLiver positioned vitamin B2 networks as one critical target for hepatocyte dysfunction in MASLD. To iteratively refine and validate these predictions, we established and incorporated a hiPSC-derived MASLD escalation model into MetaLiver. The model induces metabolic crisis in hepatocyte-like cells (HLCs) by compounding free fatty acid excess, disease-aggravating extra-hepatic signaling molecules, and immune cell co-culture. Exposed HLCs mimicked metabolic injuries when benchmarked to patient transcriptomes, including mitochondrial dysregulation, reproduced across three hiPSC donors. Consistent with MetaLiver's prediction, metabolic crisis in HLCs disrupted vitamin B2 network gene expression, which was reversible by its effector metabolite flavin adenine dinucleotide (FAD). FAD supplementation rescued major MASLD hallmarks, e.g. steatosis, mitochondrial dysregulation, and pathologic transcriptional programs, including fibrogenic signaling. We validated the anti-steatotic and pro-mitochondrial effects in primary human hepatocytes. For translational exploration, FAD-affected network nodes identified the candidate compounds aspirin, norleucine, and 3P that recapitulated pro-mitochondrial effects. Ultimately, we identified vitamin B2 network hub genes, using Cox regression analysis on an independent patient cohort (n =460) for discriminating liver events. This 19-gene hub-signature outperformed the clinical fibrosis marker FIB-4, positioning these signature genes as critical bottlenecks. Our study demonstrates how integrating stem cell-derived models with computational drug prediction expedites therapeutic discovery and reveals previously unknown pathways. Importantly, MetaLiver enables integration of any iPSC-line transcriptomic data for donor-specific network and drug response predictions (digital twin).

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