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ABSTRACTS OF SPEAKERS

1. New roles of pattern recognition receptor-induced transcription factor signalling in monocyte responses

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Detection of microbial structures through pattern recognition receptors (PRRs) is crucial for proper function of innate immune cells, including monocytes. Efficient immune response and clearance of microbial pathogens requires strictly regulated cooperation of PRR-induced transcription factors (TFs). While a number of studies have demonstrated the contribution of nuclear factor of activated T-cells (NFAT) to myeloid cell responses, the exact role of NFAT in transcriptional program in activated monocytes is still unknown.

We have analyzed the transcripts regulated by NF-κB and NFAT in stimulated monocytes, enriched from the blood of healthy donors. Using commercial kits for flow cytometry, we optimized intracellular flow cytometry (IC-FC) protocol in order to show the translocation of NFAT in monocytes from cytoplasm to nuclei upon various stimuli. Based on the mRNA expression analysis in zymosan stimulated monocytes, we hypothesized that NFAT co-regulates some immune response genes including *CSF2, TNF* and *PTX3,* but not *IL6*. Using IC-FC method, we identified non-classical monocytes as the major NFAT2-expressing subset. Preliminary results obtained from TF activity assessment indicate that this high-throughput method could be a valuable tool for studying TF pathway activation in the cell subset of interest. Further studies will aim to identify NFAT2 regulated genes and interaction partners.

2. Cellular and molecular interaction of MAIT cells in mucosal tissues and their role in inflammatory bowel disease

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Inflammatory bowel disease (IBD) manifests as chronic inflammation and is characterized by a deregulated immune response. The identification of the cellular immune players involved represents a major approach unraveling the pathogenesis of IBD and may suggest new therapeutic strategies. Recently, MAIT cells have been identified as a possible key player in IBD. Activated MAIT cells produce cytokines including IL-26, a newly discovered cytokine involved in the pathology of IBD. IL-26 is not expressed in mice and therefore its effect on the course of inflammation has not been yet sufficiently investigated.

Our laboratory described a model of intestinal inflammation based on human iPSCs-derived gut organoids. I would like to follow up on this research and my main goals are: 1) to create a model of IBD using organoids 2) to optimize methods of organoid dissociation and to use it to describe cellular subpopulations 3) to use the model to explain the effect of IL-26 on the course of inflammation 4) isolate human MAIT cells 5) by in vitro activation of MAIT cells to describe specific molecular pathways that may be involved in the pathology of IBD 6) co-cultivation of organoids with MAIT cells and description of their interaction.

3. The role of microRNA and protein-coding genes in the biology of neural stem cells

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Small non-coding RNAs (miRNAs) are powerful regulators of gene expression. In human embryonic stem cells (hESCs), they have been directly linked to the maintenance of self-renewal and differentiation [1]. However, the role of miRNA in these fundamental stem cell properties of neural stem cells (NSCs) and their interplay with transcription factors, has not yet been sufficiently described despite their importance in development and disease. Therefore, our study aims to characterize the role of miRNA and transcription factors in NSCs' specific phenotype.

To this date, we have performed miRNA and mRNA sequencing of hESCs, NSCs and differentiating neurons, and selected candidate miRNAs with presumably important role in the self-renewal of NSCs. We have subsequently generated lentiviral vectors to manipulate their expression. Experiments are ongoing to analyze their effect on the self-renewing and differentiation properties of NSCs. Preliminary results suggest that one of these candidate miRNA families, miR-17-92, is directly regulated by C-MYC in our non-tumorigenic NSCs. Additionally, our data also point to an interesting observation that only a small selection of specific seed sequences seem to regulate the stem-cell specific properties, irrespectively of stem cell identity.

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4. Physiological oxygen levels together with FGF2 direct human pluripotent stem cells energy metabolism towards epigenetic landscape maintenance and enhancement of pluripotency by downregulation of ROS

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To this day *in vitro* cultivation of human pluripotent stem cells (hPSCs) poses challenges as they manifest inter- and intra-line variability in their competence to differentiate to a specific cell type. Therefore, the ability to increase their differentiation potential is vital for *in vitro* studies and regenerative medicine. Some of the factors that contribute to hPSCs pluripotency are Fibroblast growth factor 2 (FGF2), physiological oxygen levels (5% O_2) and aerobic glycolysis.

In this study, we describe that FGF2 activates pyruvate dehydrogenase (PDH) at 5% O_2, stimulating the TCA cycle and respiration. Our data indicate that Acetyl-CoA produced by activated PDH serves as a substrate for histone acetylation and we show that this epigenetic modification results in increased expression and protein levels of pluripotency marker Nanog. We further demonstrate that increased levels of reactive oxygen species (ROS) inhibit pyruvate dehydrogenase phosphatase 1 (PDP1), leading to phosphorylation and deactivation of PDH, and that FGF2 keeps ROS downregulated at 5% O_2, allowing the activation of PDH and production of Acetyl-CoA for histone acetylation. Targeting these processes and modulating hPSCs epigenetics through energy metabolism during *in vitro* cultivation might significantly improve our current cultivation protocols and enhance utility of hPSCs for research and medicine.

5. Histone variant macroH2A1.1 enhances DNA repair and reprogramming efficiency of human iPSCs

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Genome integrity maintenance is essential for the generation of induced pluripotent stem cells (iPSC). In this respect, increasing DNA damage repair (DDR) could improve the efficiency of iPSC reprogramming. However, how DDR is orchestrated during somatic cell reprogramming has not been fully understood. We evaluated the splicing isoforms of histone variant macroH2A1, macroH2A1.1 and macroH2A1.2, as potential regulators of DDR. MacroH2A1 splicing isoforms were introduced in HepG2 cells as mtagGFP tagged proteins and isolated using GFP-trap, to pin down putative binding partners. Using liquid chromatography tandem mass spectrometry, we identified Poly-ADP Ribose Polymerase 1 (PARP1) and X-ray cross-complementing protein 1 (XRCC1) as macroH2A1.1 exclusive interacting partners. MacroH2A1.1 overexpression as mCerulean3 tagged protein in U2OS-GFP reporter cells enhanced prominently Non-Homologous End-joining (NHEJ) DNA repair pathway. MacroH2A1.1 Knock-out in mouse embryonic fibroblasts (MEFs) increased their basal DNA damage levels. We confirmed the exclusive interaction of macroH2A1.1, but not macroH2A1.2, with PARP1/XRCC1 in Human Umbilical Vein Endothelial Cells (HUVEC) undergoing cell reprogramming towards iPSCs. MacroH2A1.1 overexpression as 6His-tag protein enhanced HUVEC DDR, thus improving iPSCs reprogramming. Overall, our results highlight the role of macroH2A1.1 as an epigenetic target to improve iPSC genome stability and therapeutic potential.

6. Characterization of pathogenic *Escherichia coli* isolates and their possible treatment by bacteriocinogenic probiotics

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Pathogenic *E. coli* causes infections ranging from traveler´s diarrhea to life threatening extraintestinal infections. Intestinal colibacterioses spread mainly in children from countries with low hygienic standards. Additionally, spreading of pathogenic *E. coli* in livestock breeding presents worldwide problem, which significantly affects the economy of food producers. Pathogenic bacteria evolved various abilities to colonize diverse environment in the host with success, e.g. presence of virulence factors and also bacteriocinogeny.

The aim of this study was to characterize pathogenic *E. coli* from human and livestock. To determine what characteristics are important in pathogenicity, the set was compared with characteristics of commensal *E. coli* of the same origin. Presence of virulence factors differed among *E. coli* isolated from various origins and pathologies as well as their bacteriocinogeny, suggesting a possible role of certain bacteriocins in pathogenesis. Moreover, part of the pathogenic strains was tested for their susceptibility to different types of bacteriocins in order to find most promising combination active against certain pathotype. Finally, three commensal *E. coli* strains with specific bacteriocin production were selected based on previous results. Their activity towards pathogenic *E. coli* causing diarrhea was successfully proven in piglet model.

7. Morphological and immunohistochemical study of HNSCC focused on perineural invasion - a single institutional study with five year follow up

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Head and neck carcinoma (HNSCC) is a carcinoma with squamous differentiation arising from mucosal epithelium. It affects oral cavity, mobile and fixed tongue and oropharynx. It represents the 6th most common cancer in the world.

We retrospectively analyzed 487 patients with HNSCC who underwent curative surgery with bilateral cervical block dissection in period 2006-2016. We focused on evaluation of stage, nodal status, PNI, BVI and LVI. Moreover, we added new parameters such as the worst pattern of invasion [1], tumor budding and lymphocyte infiltration.

Most of our cases exhibited 4th degree of WPOI (212 cases). PNI was present with an increasing frequency of classification WPOI (3:12,9%, 4:26,9% 5:55,6%). Tumor budding correlated with incidence of PNI, where 85% of HNSCC with PNI developed HG budding. Brisk (49,5%) and non-brisk (42,9%) immune response manifested by TIL correlated with these morphological signs.

Moreover, we analyzed expression of BerEP4, SOX2, Oct3/4, BDNF, TrkB, Laminin and CD44 in samples with perineural invasion. Loss of laminin expression was observed in all affected nerves of our samples. In several cases, nuclear positivity of SOX2 was detected. Next, we plan to focus on the association of these factors expression pattern to the stage and morphology of PNI.

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8. Increasing complexity of lung organoids as tissue models

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Organoid culture allows *in vitro* recapitulation of developmental processes of various types of tissue, including lung tissue. The majority of current protocols however omits the vasculature, despite its importance for the tissue from both functional and developmental perspective.

Our goal is to create more complex model of lung tissue, where lung epithelium could develop along with the vasculature. We perform *in vitro* vascularization of various hydrogels in 3D, with the ability to control the parameters of created endothelial network. We culture organoids consisting of lung progenitor cells, in which we specifically focus on branching, as a dominant morphological change relevant for lungs. We are able to print complex 2D or 3D structures with cell-laden hydrogels to control spatial organization of cells in our co-culture models.

Here we present the current state of our aim – to combine these three approaches into one tissue model. Such model would be especially useful for studying the interaction between the lung epithelium and endothelial cells, and their involvement in morphogenetic and developmental process of human lungs.

9. Utilization of microfludic chip for formation of uniform cerebral organoids from pluripotent stem cells

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Organoids dramatically increased changed the field for creating models with 3D cellular organization allowing recapitulating biological properties of human tissues or organs. Weak methodological point is initial formation of cell spheroids which suffer from labor-intensive manipulation, continuity of medium exchange, as well as non-uniform size of spheroids and final divergence of organoids. Microfluidics is very promising platform to overcome these disadvantages. Due to automation, microfluidic systems are capable of continual medium flow in spatial and temporal domains, which allows to create better controlled microenvironments as well as apply lower amount of medium,

In this work, we present microfluidic chip for cultivation of biological structures. On the basis of a concave microwell-based PDMS multilayer chip, it enables a parallel perfusion culture of large amount of cell aggregates. Upon in silico simulations, we optimized chip designs and characterized several specific conditions in a microwells including sufficient nutrient exchange in all microwells. We characterized cell behaviour of pluripotent stem cells such as proliferation rates, morphology of spheroids and differentiation during long-term cultivation in these microfluidic devices. All these information we use for creating of microfludic system with automatic perfusion to form and cultivativate cerebral organoids.

10. Acceptor splice sites recognition through SRSF1 binding at the intron-exon border

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Acceptor splice sites (3'ss) are usually recognized through U2AF heterodimer. Its smaller subunit (U2AF35) interacts with sequence at the intron-exon border of the majority of exons and mediates contact with the splicing enhancers bound by SR proteins during the spliceosome assembly [1, 2]. In addition, previous studies demonstrated that not all 3'ss are dependent on U2AF binding [3]. Therefore, additional mechanisms of the 3'ss recognition have been proposed. In this study, we analysed hypothesis that some 3'ss may be recognized directly by splicing regulator SRSF1 bound to splicing enhancers at the intron-exon border.

Minigene assay of our model exons and their variants, *BRCA2* exon 12 and *VARS2* exon 17, indicated that the exon recognition better correlated with the predicted SRSF1 binding affinity than to the 3'ss quality assessed as predicted 3'ss strength or U2AF35 consensus. The knockdown experiments showed that both SRSF1 and U2AF35 were involved in the 3'ss recognition of model exons and, additionally, the RNA affinity purification confirmed the SRSF1 binding to our model 3'ss. These results suggested that SRSF1 bound to model 3'ss while U2AF35 may or may not bind to the 3'ss sequence. Overall understanding of the 3'ss recognition may contribute to determine the detailed mechanism of the splicing regulation.

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11. Differentiation of pluripotent stem cells using odontoblasts-specific transcription factors

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Teeth are made of three types of tissue: dental pulp, enamel and dentin. Tooth's morphology, function and mechanical properties are determined by dentin, which is the most abundant hard dental tissue. While being indispensable, dentin has only a very limited reparative regenerative capacity. Currently, there are no widely used dental treatments utilizing native biological mechanisms for facilitating tissue regeneration or reparation. Additionally, attempts to obtain odontoblasts – the dentin producing and repairing cells in vitro remain generally unsuccessful, likely due to their highly specialized, postmitotic phenotype. We designed a novel approach of direct differentiation of pluripotent stem cells into odontoblasts by harnessing developmental trajectories constructed using single-cell RNA-seq data from mouse continuously growing incisor. Here we show the changes in the expression patterns of mouse pluripotent stem in response to overexpression of several, recently described, odontoblast-specific transcription factors after both short- and long-term exposure using qPCR and immunohistochemistry. We anticipate our results to highlight the role of the overexpressed transcription factors in odontoblast development (differentiation) and introduce their possible utilization in regenerative dentistry.

12. CAR T-cells for neuroblastoma treatment

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CAR T-cells (Chimeric Antigen Receptors T-cells) are genetically modified T-cells able to target virtually any existing protein structure located at any cell within organism. This ability can be utilized in targeting the tumor-specific antigens of the cancer cells and in elimination of these cells. Majority of existing research focus on the blood cell malignancies (lymphomas, multiple myelomas, etc.), therefore application of this technology on the solid tumors is yet to be expanded into clinical practice. Disialoganglioside GD2 is an antigen expressed on several types of tumors (neuroblastoma, melanoma) with only minor expression in the healthy tissues. These properties and number of potential patients makes the GD2 a promising target for the CAR T-cell therapy. Main goal of this research is to establish and optimize the production and quality control testing of GD2 specific CAR T-cells under current Good Manufacturing Practice (cGMP) rules in clinically clean environment at Cell and Tissue Engineering Facility.

13. Temporal changes in the genetic diversity of treponemal strains in Papua New Guinea

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Yaws is one of the diseases responsible for afflicting children of Papua New Guinea (PNG) with exudative skin ulcers. The WHO-initiated yaws eradication program aims to achieve success by 2030 [1]. This disease impedes normal child growth and development. The etiological agent of yaws is *Treponema pallidum* subsp. *pertenue* (TPE). An ulcer with similar clinical features is caused by *Haemophilus ducreyi* (HD). Both pathogens are responsible for most skin ulcers in Papua New Guinea. Mass drug administration (MDA) with azithromycin is used to treat both yaws and HD ulcers [2].

Patient samples from Namatanai, PNG were tested for TPE DNA. Additionally, multi-locus sequence typing of TP0548, TP0488, and TP0858 genes were used for allelic profiling. Identification of mutations associated with macrolide resistance and detection of HD DNA were performed [1].

Our results categorized Namatanai samples as allelic profiles J11, S22, and T13 showing a decreased molecular diversity of TPE strains over one and a half years after MDA. J11 emerged as the most predominant strain over S22 and T13[1]. Single-locus sequence typing of HD was conducted on TPE negative samples. While the TPE strains showed reduced genetic diversity, the strain diversity of HD in Namatanai after MDA has remained nearly the same. Besides, four known and three novel HD genotypes were also detected.

[1] Lucy N. John, Camila G. Beiras, Wendy Houinei, Monica Medappa, Maria Sabok, Reman Kolmau, Eunice Jonathan, Edward Maika, James K. Wangi, Petra Pospíšilová, David Šmajs, Dan Ouchi, Iván Galván-Femenía, Mathew A Beale, Lorenzo Giacani, Bonaventura Clotet, Eric Q. Mooring, Michael Marks, Marti Vall-Mayans and Oriol Mitjà (2021, September 6th). A Trial of Three Rounds of Mass Drug Administration with Azithromycin for Yaws. New England Journal of Medicine (in press)

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14. Exploring ERK3/MAPK6 in breast cancer progression

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One of the mitogen activated protein kinases (MAPK) genes that is highly expressed in breast cancer is MAPK6(ERK3) [1]. Interesting research is emerging about ERK3 potential in cancer, particularly, in breast and lung cancer in which ERK3 shows a pro-invasive role [2,3,4,5,6].

This role of ERK3 in cancer progression needs to be further studied, specially its signaling cascade, which is poorly understood [7]. The questions we hope to answer are: How is ERK3 involved in cancer progression? What is its signaling pathway? Is it a good candidate as therapeutic target?

We started by determining certain growth factors that can phosphorylate ERK3 and examined its endogenous expression as well as its half-life across different breast cancer cell lines. Then we determined how ERK3 might affect the classical signaling cascade of epidermal growth factor (EGF)/ receptor tyrosine kinase/MAPK or non-classical signaling cascades, such as transforming growth factor (TGFβ). We further studied ERK3 by using functional assays to determine its role in proliferation and migration. Next, we will further explore its signaling cascade by use of immunoprecipitation and mass spectrometry. Also to explore other possible phenotype changes, in actin polymerization and focal adhesions, as well as using spheroids to study invasion capabilities in 3D models.

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15. Vascularization of the wrist and dorsal capsulotomy Pronator quadratus muscle free flap – vascular pedicles of the radial artery

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The radial-based dorsal capsulotomy is commonly used surgical approach to the radiocarpal joint when the incision is made along the radiocarpal (RCL) and the intercarpal ligaments (ICL). The radial forearm flap is used for the reconstruction of the wide variety soft-tissue surgical defects and can include pronator quadratus muscle (PQM) flap and its vascular pedicles of the radial artery. The aim of the study was to provide detail description of arteries supplying dorsal portion of the radiocarpal joint capsule in relation to incisions and anatomical location of arterial branches of the radial artery perfusing PQM.

The anatomical study was based upon analysis of fresh cadaver upper extremities. The radial, anterior interosseous and ulnar arteries were cannulated and injected by colored silicon rubber followed by macroscopical and microscopical dissection.

The dorsal portion of the wrist joint capsule is vascularized from the radial dorsal carpal branch, supplying branches run upwards and across the ICL. Based on these results we suggest the capsulotomy with incisions respecting the position of the radial branches crossing the ICL. The most constant and widest radial vascular branches of the PQM are mostly localized in distal radial quarter of the muscle. The PQM flap should be harvested and raised from the distal radial pedicels.

16. Signaling of receptor tyrosine kinases involves primary cilia

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The primary cilium plays key regulatory functions in many cellular processes, either by spatial compartmentalization of the signaling molecules, or precise fine-tuning of their action. This is highlighted by the growing list of pathologies that are now being classified under the umbrella term of ciliopathies. Ciliopathies are often systemic disorders, and commonly present with symptoms that include developmental disorders of the skeleton, congenital heart defects, renal malformations, cognitive disorders and obesity. Several membrane receptors and their downstream effectors have been shown to reside in the primary cilium. In our study, we focused on 38 members of the receptor tyrosine kinase (RTK) family, and 24 of their pathology-associated variants, and assessed their interaction with primary cilia. We found that a considerable number of RTKs localize to the ciliary compartment, while noting differences between wild-type and disease-associated variants. Our data show importance of the ciliary RTK localization for a precise regulation of its signaling. These findings expand our understanding of the RTK signaling and the primary cilium compartment in development and disease.

17. Regulation of primary cilia by Ciliogenesis associated kinase 1 (CILK1)

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Primary cilium is a microtubule-based organelle protruding outside of the cell, transducing extracellular cues and regulate cell signaling. Proper function of cilia is important in development as well as homeostasis of the tissues. Ciliogenesis and maintenance of the cilia is a dynamic and complex processes, and loss-of-function of the critical regulators produces signaling defects that manifest in a pleotropic group of disorders collectively termed ciliopathies[1].

CILK1 (Ciliogeneis associated kinase 1), an RCK (ros cross-hybridizing kinases) family member, is an evolutionarily conserved and ubiquitously expressed serine/threonine kinase that functions in development of multiple tissues. CILK1 has been shown to regulate cilia formation, architecture and signaling. Loss of CILK1 activity leads to ciliopathies such as short rib-polydactyly syndrome and endocrine-cerebro-osteodysplasia due to deregulation of ciliary structure and signaling[2-4].

In this study, we used microinjection of small amounts of active CILK1 to challenge primary cilia at the physiological level of CILK1 expression. We also implemented a conditional knockdown system to study the effects of CILK1 downregulation, and the molecular mechanism of CILK1 action. Understanding these mechanisms would allow for better therapeutic options for the ciliopathy patients.

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18. Combination of biomolecule nanopositioning and superresolution microscopy for studying of cellular behavior

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The cellular behavior is controlled by cell interactions with surrounding microenvironment, which is composed from interactions with neighboring cells, extracellular matrix, and soluble factors. Several artificial culture systems were developed to understand those interactions. Generally, at macroscale, culture surface is based on random biomolecules distribution or at microscale, adhesive proteins are distributed to micro sized spots. However, cell interactions naturally occur within very small, nanoscale distances, which needs to be addressed. Recently several technologies allowing precise nanopatterning have been proposed.

We optimized electron beam lithography, that allowed us to control nanodistribution of biomolecules. We successfully achieved three different densities of lines within hexagonal nanopatterns, i.e., 1000, 500 and 250 nm. Recombinant tyrosine kinase receptor EphA2 was tagged with HaloTag molecule and specifically immobilized towards those nanopatterns. Our results showed for the first time the interactions of human pluripotent stem cells with nanodistributed EphA2. Further, we proved that the cells are sensitive to nanodistribution, while they express variability in formation of ephrinA1 ligand cluster. Finally, we demonstrate, that such a type of biomaterial may help to understand cellular signaling mechanism at nanosclae level.

19. Role of RAD51 in metabolism of G4 structures

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G-quadruplexes (G4Q) are one of alternative DNA secondary structures which can be formed in human cells. Although high-throughput sequencing identified more than 700,000 potential G4Q forming sequences, biological functions of these structures are still not uncovered [1]. They are generally believed to be involved in regulation of replication, transcription and telomere maintenance. Once formed quadruplex is extremely stable, thus can block replication fork progression [2]. Not only quadruplexes but also other endo- as well as exogenous factors may obstruct replication, thus cells developed several mechanisms to deal with it. RAD51 protein is well described as a key protein of homologous recombination used to repair DNA double-strand breaks, however, it has recently been shown to participate in protection and even restart of stalled replication fork, a process implicated in genome instability associated various diseases [3]. This work identified RAD51 interaction with G4 and is focusing on characterization of the biological role of this interaction.

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20. Xeno- and feeder-free derivation of two sibling lines of human embryonic stem cells

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Human embryonic stem cells (hESCs) represent an unlimited source of cells for the differentiation into the derivates that are suitable for a variety of biomedical applications (e.g. cell therapies, disease modeling, and drug testing). Nevertheless, allogeneic background (e.g. mouse embryonic fibroblasts) and undefined culture conditions (e.g. fetal bovine serum) are limitations of current derivation protocols of hESCs. Therefore, here we present the derivation of the two sex-discordant sibling hESC lines, MUNIe008-A and MUNIe009-A, using the mechanical biopsy of vitrified-thawed embryos (surplus for fertility treatment) under xeno- and feeder-free conditions. The presented approach is applicable for the derivation of high-quality clinical-grade hESC lines for a variety of clinical applications.

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21. eIF4F inhibition induces LKB1-independent AMPK activation in *BRAF*^{V600E}-mutant melanoma cells

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The eIF4F eukaryotic translation initiation complex has been reported as the nexus of melanoma resistance to drugs targeting BRAF and MEK kinases. Furthermore, simultaneous inhibition of BRAF and eIF4F synergized in killing cancer cells [1].

Our research focusing on the crosstalk between the AMPK and ERK pathways showed that AMPK activity modulates ERK signaling in *BRAF*^{V600E}- and *NRAS*-mutant melanoma cells [2]. We also observed that eIF4F inhibition induces AMPK activation in melanoma cells. Therefore, we decided to characterize the link between AMPK, ERK, and eIF4F signaling by performing proteomic analysis of changes in *BRAF*^{V600E}- and *NRAS*-mutant melanoma cells in response to MEK and eIF4F inhibition. Interestingly, among targets downregulated after eIF4F inhibition, we found known AMPK regulators: MO25, an essential part of the main AMPK-activating complex (LKB1-STRAD-MO25), and PP2Aα, an AMPK-inhibiting phosphatase.

RNA interference-mediated knockdown of LKB1 revealed LKB1-independent AMPK activation in melanoma cells upon eIF4F inhibition. Moreover, we confirmed LKB1-independent activation of AMPK upon eIF4F inhibition also in LKB1-deficient *BRAF*^{V600E}-mutant melanoma cells. Furthermore, PP2Aα downregulation seems to play an essential role in AMPK activation after eIF4F inhibition, as RNAi-mediated knockdown of PP2Aα and a selective small-molecule PP2A inhibitor, okadaic acid, both potently increased AMPK activity in melanoma cells.

Recent studies reported the existence of negative feedback between ERK and LKB1 in *BRAF*^{V600E}-mutant melanoma cells. ERK and its downstream target RSK phosphorylate LKB1, compromising its ability to activate AMPK [3]. However, our findings demonstrate that eIF4F and PP2A can control AMPK activity in *BRAF*^{V600E}-mutant melanoma cells in an LKB1-independent manner.

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22. Whole-genome sequencing of Cuban bejel

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Bejel is a nonvenereal neglected disease caused by *Treponema pallidum* subsp. *endemicum*. This disease affects usually children between 2–15 years old. The primary chancre appears in oral area, often remaining undetected [1]. Until now, only two whole genomes sequences of this bacterium are available [2, 3]. Although it is mostly present in hot dry climate, several cases were found out of these areas, e.g., in Japan [4], France [5] or Cuba [6], in some cases due to disease import from endemic areas. The main aim of this work was to sequence and analyze four Cuban bejel samples, where there was no evidence of the import of the disease.

Genomes were obtained by pool segment genome sequencing and direct sequencing, resulting in broad genome coverage of 100%, 81.69%, 52.61% and 21.13%, respectively. Analysis of these samples revealed a non-clonal character of the genomes, with nucleotide variability ranging between 0.2-10.3 nucleotides substitutions per 100 kbp. Changes among genomes affected 27 genes. Analysis of finished genome also showed recombination events between *tprC* and *tprI*, in TP0488 as well as in the intergenic region between TP0127-129.

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23. NFAT signaling in human neutrophils

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Calcineurin (CN) - Nuclear factor of activated T-cells (NFAT) inhibitors are commonly used immunosuppressive drugs, but they frequently leave patients vulnerable to fungal infections. Pentraxin-3 (PTX-3), soluble pattern recognition receptor (PRR), recognizes and binds fungal conidia, which allows clearing the infection. We have previously shown that the expression of PTX-3 in mouse dendritic cells and neutrophils and in human monocytes is co-regulated by CN-NFAT[1–4]. The role of CN-NFAT pathway in human neutrophils remains elusive. The main aim of my PhD project is to dissect the activity of the NFAT signalling cascade in human neutrophil upon activation with different PRR ligands.

We have isolated human neutrophils and assessed their purity by flow cytometry as $CD66b^{+}CD16^{+}CD15^{+}$ cells. We have analysed mRNA expression of the main members of CN-NFAT pathway including Toll-like receptors, C-type lectin receptors, signal transductors, Calcineurin subunits and members of NFAT family. Moreover, we have evaluated effects of CN-NFAT inhibitors on neutrophil response induced by PRR ligands. Presented data show, that NFAT pathway is involved in response to PRRs by human neutrophils, and that CN-NFAT inhibitors impair PTX-3 production after neutrophil activation. Thus, we hypothesize, that the NFAT pathway is important in the regulation of neutrophils response to pathogen.

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24. Fibroblasts are mechanical actuators in the process of mammary epithelial branching morphogenesis.

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Fibroblasts constitute the most prevalent cell type in the tumor mass of breast cancer, the most common life-threatening cancer disease among woman. Their contribution to the tumor growth, progression and metastasis is well documented. However, the distinct mechanisms how fibroblasts regulate normal development and homeostasis of the mammary gland are just being explored. Recent *in vitro* study from our laboratory revealed that mammary stromal fibroblasts induce mammary epithelial folding by exerting mechanical forces on the epithelium [unpublished]. To test if this mechanism acts also in living tissue, we are using genetically engineered mouse models for targeting fibroblasts *in vivo*. Here we show that fibroblast depletion and fibroblasts-specific loss of mechanical force-generating machinery lead to impaired phenotype of mammary gland. These results suggest that fibroblasts and their mechanical properties are essential for proper mammary epithelial branching morphogenesis.

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25. ERK signalling patterns in mammary gland morphogenesis and cancer

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Currently, breast cancer is the most common cancer in women and the second leading cause of cancer-associated death. Both development and cancerous transformation of mammary epithelium are governed by the same molecular interactions and signalling pathways. ERK signalling is well established as crucial for the development and homeostasis maintenance of various tissues, and its dysregulation has been linked to cancer progression. Besides oscillations in frequency and duration of ERK signalling at the single-cell, changes in these variables are also observable at the tissue level. Recent advances in bioimaging methods and the development of FRET-based biosensor mouse strains have enabled detailed spatiotemporal examination of protein dynamics in live cells. In this project, we utilise EKAREV-NLS biosensor mouse strain, developed for monitoring ERK activity, to connect characteristic activation patterns with distinct morphological and functional outcomes. To achieve this, we will perform confocal and light-sheet time-lapse microscopy of physiologically relevant 3D mammary epithelial organoids under different conditions. Further, selected time points will be analysed for relevant gene and protein expression patterns. Ultimately, our results will contribute to a better understanding of molecular mechanisms behind ERK-mediated changes in morphology and function of normal and cancerous mammary epithelium.

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26. Exploring the effects of photo-stimulation on the human retina using the retinal organoid model

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Retinal development is not only controlled by morphogens via cell to cell communication and by signalling pathways, but interestingly also by responses to light uniquely participating in shaping this tissue. However, the signalling pathways underlying these responses are largely unknown and our preliminary data has provided the evidence that light may influence differentiation programs. The aim of this project is to use human pluripotent stem cells-derived retinal organoids as a model to closely investigate photo-induced effect on retinal differentiation.

To address the general proposition of the project, we propose the following strategy: I) To photo-stimulate retinal organoids at different developmental stage with different wavelengths of light and assess the impact of this treatment on differentiation and functional outcomes. II) To assess the whole transcriptome and select candidate pathways and genes involved in photo-induced response. III) To perform functional studies on candidate components, to describe molecular mechanisms that underlie this process.

The proposed strategy combines the innovative approach of merging two different scientific fields: physics and stem cell biology, allowing to study photo-induced effects on retinal development from a uniquely different perspective.

27. Elucidation of genes able to upregulate CD20 using CRISPR/Cas9 screening

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CD20 molecule is used as the main target of monoclonal antibodies (mAbs) like rituximab (RTX) in the immunotherapy of B-cell malignancies. However, malignant B cells tend to downregulate CD20 on their surface, leading to mAbs resistance and therapy failure [1]. Unfortunately, mechanisms underlying this phenomenon remain largely unknown. We aim to identify genes that regulate surface expression of CD20 and thus can potentially enhance anti-CD20 mAbs efficiency.

We performed CRISPR/Cas9 screening on rituximab-resistant Ramos cells with downregulated CD20 on their surface. RTX-resistant cells were infected with a genome-wide CRISPR knockout library to obtain a population of cells with single-gene knockouts. After 3-week cultivation, the top 5% of cells that have mostly upregulated CD20 were sorted out. Using NGS and bioinformatical tool MAGeCK, we identified gRNAs that were significantly enriched in the sorted population. Several top hits were selected and isogenic knockout cell lines were prepared using new gRNA targeting each gene. The effect of genes depletion on CD20 expression was assessed by flow cytometry and qPCR.

Thorough validation and investigation of underlying molecular mechanisms will follow, as they are crucial to use these genes to modulate the expression of CD20 and thus potentially improve the efficacy of anti-CD20 mAbs.

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28. MinION as a tool for sequencing of variable genome sites of pathogenic treponemes

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Syphilis and yaws are diseases caused by two different subspecies of bacteria *Treponema pallidum.* Unlike the worldwide prevalent sexually transmitted syphilis, yaws is transmitted by skin to skin contact in humid tropical areas. *Treponema pallidum* was not successfully cultivated until 2018 [1]. Therefore, vast majority of information about these spirochetes comes mostly from genomic studies. Learning the differences in genetic information between these two subspecies of *T. pallidum* is crucial for understanding the differences in pathogenicity. These regions represent genes, that showed high sequence variability in other studies, paralogous genes and repetitive regions. This diversity could be the cause of different pathogenicity and could be involved in host immune system evasion [2, 3]. These regions are hard to sequence with traditional sequencing approaches (Sanger, Illumina) and are usually missing in draft genome studies.

In this pilot experiment, thirty-six different genes for three strains of *Treponema pallidum* (2 syphilis, 1 yaws), with known genome sequence, were sequenced using long-read sequencing technology (Oxford Nanopore, MinION). Different bioinformatic tools and approaches were tested to eliminate errors and produce consensus of specific sequences. Majority of errors were indels and over half of all errors were located in homopolymer regions.

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29. Exoribonuclease ISG20L2 in human disease and immunity

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The gene regulation is in eukaryotes regulated at several levels. One of the key mechanisms is mRNA stability. Human cells encode several types of exoribonucleases of which the XRN1 and exosome are the most prominent factors that degrade mRNAs. Whereas XRN1 is a 5' to 3' exoribonuclease acting as a monomer, the exosome is a multimeric protein complex with 3' to 5' exoribonucleolytic activity. The catalytic subunits are the DIS3 proteins DIS3, DIS3L and RRP6. However, mammalian cells encode other 3' to 5' exoribonucleases such as DIS3L2 or ERI1 that preferentially target oligouridylated aberrant RNAs. Defects in RNA degradation have been linked to several human diseases such as Perlman syndrome or neurodegenerative disorders, for example pontocerebellar hypoplasia.

In this project we focused on yet another 3' to 5' exoribonuclease called ISG20L2. ISG20L2 was shown to be involved in the maturation of 5,8 S rRNA [1]. We aim to investigate the detailed molecular mechanisms how 3' to 5' exoribonuclease ISG20L2 recognizes and process target RNAs. For this we assay its nucleolytic activity *in vitro* on different RNA substrates. These studies aim to explain how ISG20L2 participates in cellular RNA homeostasis and how defects in its function contribute to the disease development.

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30. Whole genome sequencing and analysis of *Treponema pallidum* subsp. *pertenue* of non-human primate origin

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Treponema pallidum subsp. *pertenue* (TPE) is the causative agent of yaws disease, endemic in tropical regions of Africa, Asia and Pacific. While this disease mainly affects human children, this bacterium also causes infection in wild non-human primate populations in Africa [1]. Previously, the only whole genome sequence coming from a non-human primate was strain Fribourg Blanc, isolated from a baboon. This strain was found to be highly similar to the human infecting strains [2]. Since then, one other strain of non-human origin has been sequenced (LMNP-1) [1]. To analyze the similarity between the strains of human and non-human primate origin, we determined whole genome sequences of eight different samples obtained from two different species (*Papio anubis* and *Chlorocebus pygerythrus*) from four different areas in Tanzania (Lake Manyara NP, Serengeti NP, Ruaha NP, Ngorongoro Conservation Area) [3]. These samples were selected from available set of samples based on genetic diversity. Non-human strains were compared to the strains of human origin and no consistent differences were found, suggesting that the non-human primate populations serve as a reservoir of TPE.

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31. Retinal organoids in microfluidic systems – formation and early-stage differentiation

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Organoids are self-organizing stem-cell derived 3D constructs that mimic in vivo structure and function. Since the development of the first retinal organoids roughly one decade ago, methods for retinal organoid cultivation have been modified to give rise to all major cell types of the neuroretina, including mature photoreceptors. However, several challenges remain, such as heterogeneity between organoids, poor photoreceptor maturation and the degradation of retinal ganglion cells in long time cultures. Microfluidic platforms have the potential to help overcome some of these by allowing for automation and precise control of the microenvironment. For this work we designed microfluidic device for the differentiation of human pluripotent stem cells towards the retinal organoids. We induced uniform organoids during 20 days within continuous medium perfusion and compared them with organoids grown in static conditions by analysis of morphology, proliferation and cell differentiation. Even though there are significant differences between these methodological approaches, the results are comparable.

32. A study of the effect of DAMPs on choroid plexus using an in-vitro model

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Following nerve injury, damage-associated molecular patterns (DAMPs) are released into the blood circulation. DAMPs might migrate through the blood-cerebrospinal fluid barrier (BCSF-B) and trigger toll-like receptors (TLRs) in the choroid plexus, which can potentially result in the immune reaction in the central nervous system. To study this mechanism an in-vitro model of choroid plexus (Z310 cells) was used and the proinflammatory reaction of choroidal epithelial cells in response to DAMPs was examined.

In-vitro experiments were provided on Z310 cells, which are primary choroidal epithelial cell culture established from rat choroid plexus tissue. Z310 cells were incubated with the pattern recognition receptors (PRRs) agonists, such as LPS (lipopolysaccharide), N-Formylated Peptide (fMLP), and CpG oligodeoxynucleotides (CPG ODN) for 24 hours. Cells were then used for immunocytochemistry and western blot analyses of expression of TLR4, TLR9, and cytokines IL-6, IL1-β, TNF-α.

Following fMLP agonist treatment we found upregulation of FPR2 expression in cytoplasm and of TNF-α on the cell membrane of Z310 cells. The expression of cytokines TNF-α was upregulated, following activation of PRRs via DAMPs. After tissue injury, DAMPs can migrate through BSCF-B and they have the ability to increase the pro-inflammatory profiling of the choroidal epithelial cells.

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33. Exosomal long non-coding RNAs as potential colorectal cancer biomarkers

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It is estimated that colorectal cancer (CRC) accounts for 8 % of all detected cancers. It is also the third most common cause of cancer-related deaths [1]. Despite the improved detection, a considerable number of the CRC patients suffer from poor prognosis [2]. Therefore, non-invasive, and clinically validated biomarkers that can detect cancer at an early stage are needed. This gap could be filled by long non-coding RNAs (lncRNAs) that are enveloped in exosomes circulating in blood.

Exosomes were isolated from human blood serum collected from CRC patients. Long ncRNAs were preamplified and then quantified by qRT-PCR.

We successfully detected cancer specific lncRNAs in exosomes that were isolated from CRC patient blood sera. ZFAS1, MALAT1, NEAT1 were detected both in exosomes and colonic tissue, HOTAIR and CRNDE-h were found only in the tissue.

We focused on the detection of cancer-specific lncRNAs in exosomes isolated from CRC patient serum samples and compared it to their expression in CRC tissue and adjacent normal mucosa. Some lncRNAs present in CRC tissue were also found in circulating exosomes. Our data show that exosomal lncRNAs can be detected even in low amount of patient samples and could potentially be used in the clinical application.

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34. Contribution of Fibronectin alternative splicing isoforms to myocardial fibrosis

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Cardiac fibrosis is a consequence of chronic insults to the myocardium, characterized by abnormal accumulation of extracellular matrix (ECM). Cardiac fibroblasts (cFbs) transdifferentiated into myofibroblasts are the main responsible of pathological ECM remodeling [1], which compromises heart function and leads to heart failure. The ECM protein Fibronectin (FN) is critical during the early stages of fibrosis, but the role of its splicing isoforms, expressed during wound healing, containing an extra-domain A (ED-A) and/or extra-domain B (ED-B), is not fully understood [2]. To investigate the splicing events in cardiac disease, we derived cFbs from induced pluripotent stem cells (iPSCs-cFbs) and optimized a TGFβ-induced model of cardiac fibrosis. TGFβ treatment increased the expression of FN containing extra domains, as detected by qPCR and immunostaining of decellularized matrices. Results were confirmed by comparing cFbs isolated from heart failure patients to healthy counterparts.

To better reproduce the cellular complexity and functionality of the human heart, we established a scaffold-free 3D *in vitro* culture system, which entails the co-culture of isogenic iPSCs-cardiomyocytes and cFbs.

3D organoids might be able to recapitulate the accumulation of fibrotic tissue occurring during heart disease and investigate the contribution of fibronectin isoforms to the establishment of the pathology.

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35. Novel Stem Cell Based Model For The Study Of Cardiac Arrhythmias

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Cardiac arrhythmias are cardiac rhythm abnormalities with incidence from 1,5% to 6,5% in adults and causing about 10% of sudden cardiac deaths [1]. Apart from arising from various conductive defects leading to various cardiomyopathies, arrhythmias can be also induced by drugs with arrhythmogenic effects such as caffeine and its derivatives used in clinical practice.

Beating embryoid bodies (EBs) were differentiated from human pluripotent stem cells (hIPSC) by previously established protocol [2]. Such EBs consist of various types of cardiomyocytes and other supporting cell types forming functional beating syncytium which enables formation of conductive communication between EBs in close proximity in vitro. Here we demonstrate that such novel system of two EBs seeded in close proximity monitored by live imaging in time by means of fluorescence of calcium indicator Fluo-8 AM can serve as novel stem cell based model to study arrhythmogenic effects of drugs such as caffeine.

In this work we therefore introduce a novel stem cell-based model allowing for monitoring a complex interaction of individual EBs in presence of arrhythmogenic drugs. When patient specific hIPSCs are used to generate the model the disease-associated arrhythmogenic effect can be studied and effective drugs can be screened for using such model.

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36. Generation of a patient-specific cardiac fibrosis model to analyze lncRNA contribution to heart disease

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Cardiovascular disease is the leading cause of death worldwide, being ischemic heart disease and endomyocardial fibrosis the primary reasons of end-stage heart failure (HF). Cardiac fibrosis is the pathological process mediated by cardiac fibroblasts (cFbs) [1] and determined by the maladaptive remodelling of the heart extracellular matrix (ECM). Although it underlies most of cardiac disfunctions, effective therapies for its inhibition or reversion are currently not available. Long non-coding RNAs (lncRNAs) are potent transcriptional regulators which might be involved in the cardiac fibrotic process and - therefore - have emerged as viable prognosis makers and therapeutical targets [2]. To investigate the role of lncRNAs during fibrotic progression, we differentiated induced pluripotent stem cells (hiPSCs) into cFbs (hiPSC-cFbs) and exposed them to a TGFb-induced fibrotic assay. Following the fibrotic trigger, a subset of fibrosis-related lncRNAs was found dysregulated. A similar trend was observed in primary cultures of cFbs derived from HF patients as compared to the same cells obtained from healthy donors. To better model cardiac fibrosis progression, iPS-cFbs were co-cultured with isogenic hiPSCs-derived cardiomyocytes (iPS-CMs) to generate patient-specific three-dimensional cardiac organoids. This model will be further exploited to test the role of individual lncRNAs by altering their expression through GapmeRs.

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37. The effect of Y1816C SORLA mutation on the development of Alzheimer's disease in stem cell-based models

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Alzheimer's disease (AD) is a neurodegenerative disorder affecting brain neurons and causing cognitive impairment. Alterations in the *SORL1* gene are associated with AD, as the compromised function of SORLA (protein coded by the *SORL1* gene) increases Amyloid-β levels [1,2]. However, it is currently unknown which *SORL1* variants are risk-increasing and which are benign.

We introduced the *SORL1* Y1816C knock-in (KI) mutation and two *SORL1* knock-out (KO) mutations into human induced pluripotent stem cells, which were differentiated into cortical neurons, neural rosettes, and 3D cerebral organoids. We then analyzed the effects of the mutations on pluripotency, neurodifferentiation, and AD-associated cell pathologies. Our results show that the *SORL1* Y1816C mutant behaves similarly to the *SORL1* KO. Both KI and KO mutants show enlarged endosomes, formation of cysts during early cerebral organoid neurodevelopment, and downregulation of NMDA receptor. We did not observe significant differences in Amyloid precursor protein (APP) levels in either mutant. In the near future, we plan to study changes in Amyloid-β secretion and electrophysiological changes of the NMDA receptor. Altogether, our data thus far describe for the first time the phenotype of *SORL1* Y1816C mutant and point to several interesting phenomena that might be leading to the development of AD.

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