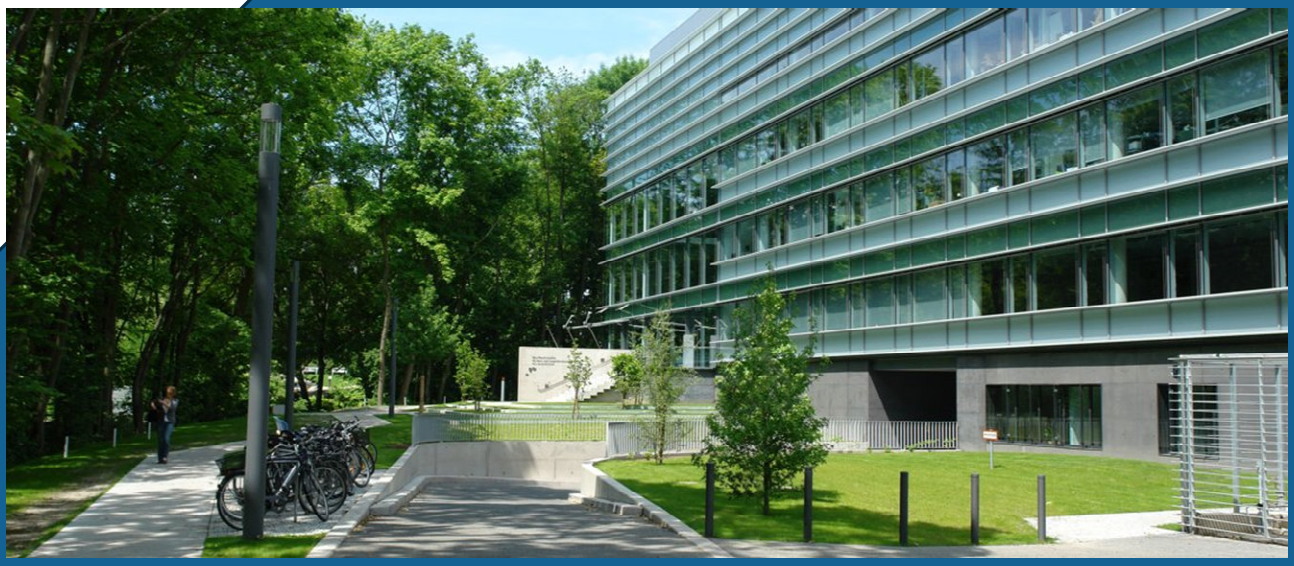
MAX PLANCK INSTITUTE
FOR HEART AND LUNG RESEARCH
W. G. KERCKHOFF INSTITUTE

SCIENTIFIC REPORT

2023



SCIENTIFIC REPORT

2023

IMPRINT

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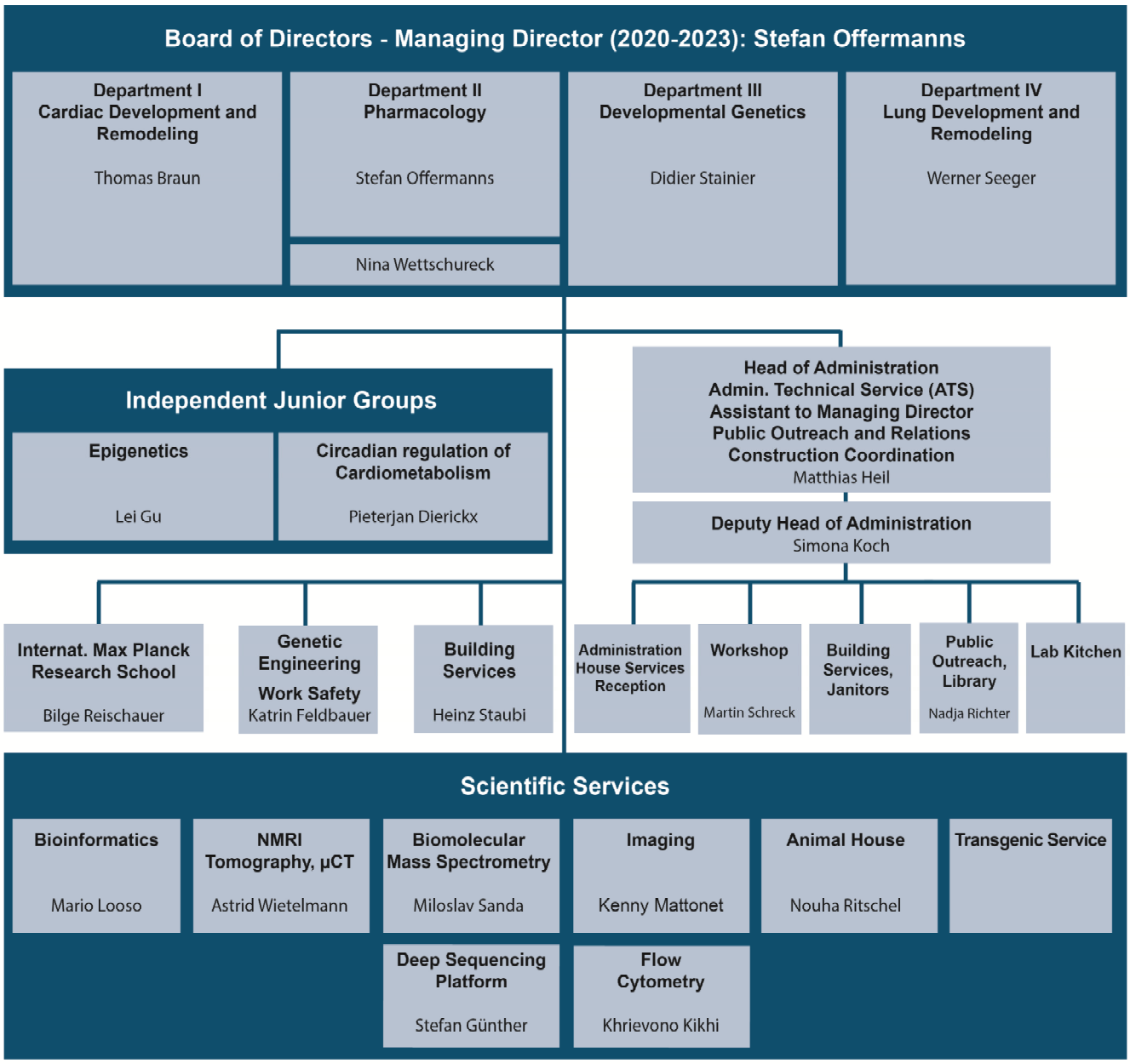

Version: December 2022

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Introduction

Founded in 1929 as an institute devoted to cardiovascular research by the William G. Kerckhoff foundation, the institute became a member of the Max Planck Society in 1951. In 2004 the former Max Planck Institute for Physiological and Clinical Research was renamed Max Planck Institute for Heart and Lung Research (MPI-HLR) and has currently four departments:

Department I: Cardiac Development and Remodelling (Thomas Braun)

Department II: Pharmacology (Stefan Offermanns)

Department III: Developmental Genetics (Didier Stainier)

Department IV: Lung Development and Remodelling (Werner Seeger)

The MPI-HLR currently hosts two independent junior research groups funded by the Excellence Cluster “Cardiopulmonary Institute”, which are headed by Lei Gu (Epigenetics) and Pieterjan Dierickx (Circadian regulation of Cardiometabolism), and the group of Nina Wettschureck (Department II / G-protein-mediated signaling).

Department IV, the junior groups and the administration are located in buildings at Parkstr. (built 1930/1969 and in part renovated 2013-2018) whereas the departments I-III reside in a separate building at Ludwigstr. which was opened in 2010.

Research at the institute is supported by several technical and scientific facilities: Bioinformatics (Mario Looso), biomolecular mass spectrometry (Miloslav Sanda), deep sequencing (Stefan Günther), flow cytometry (Kikhi Khrievono), microscopy (Kenny Mattonet), small animal imaging (Astrid Wietelmann), the animal facility (Nouha Ritschel) and a transgenic service group.

Since 2008, the MPI-HLR, together with the universities of Giessen and Frankfurt, runs an International Max Planck Research School (IMPRS), which was recently renamed “IMPRS for Molecular Organ Biology”.

The institute has strong ties to the universities of Frankfurt and Giessen. All scientific groups of the Institute are involved in the Cluster of Excellence “Cardiopulmonary Institute” (CPI) together with the universities of Frankfurt and Giessen. This large interdisciplinary network is the successor of an excellence cluster on the same subject which was funded from 2006 to 2018. Scientists of the MPI-HLR are also closely collaborating with colleagues in Frankfurt and Giessen in 8 DFG funded Collaborative Research Centers and Graduate Schools as well as in the German Centers for Cardiovascular Research (DZHK) and for Lung Research (DZL) which are funded by the Federal Ministry of Education and Research.

Over the last decade, the MPI-HLR has established itself as a leading research institution in cardiovascular and lung biology. Current research at the MPI-HLR explores the development, function and diseases of organ systems. While there is a clear focus on the cardiovascular system and the lung, skeletal muscle, metabolic organs and tumor biology are also covered by different groups.

3. Scientific Departments

I. Cardiac Development and Remodelling

Thomas Braun (director)

Thomas Braun, MD, Ph.D. | Professor of Medicine, Justus Liebig University of Gießen; Director, Dept. Cardiac Development and Remodelling, Max Planck Institute for Heart and Lung Research.

University Education

1993	German “Habilitation” and Ph.D. in Cellular Biochemistry
1983 – 1987	School of Philosophy, School of Medicine, University of Hamburg, Germany;
1980 – 1983	School of Philosophy, School of Medicine, University of Goettingen, Germany.

Scientific Career

since 2004	Director of the Max-Planck-Institute for Heart and Lung Research, Bad Nauheim; Full Professor, Univ. of Gießen, Medical Faculty, Dept. of Internal Medicine
2000 – 2004	Elected Dean for Research, Medical Faculty, Univ. of Halle-Wittenberg
1998 – 2000	Full Professor, Univ. of Halle-Wittenberg, Medical Faculty, Director of the Institute of Physiological Chemistry
1997 – 1998	Associate Professor, Univ. of Würzburg, Medical Faculty, Inst. of Medical Radiology and Cell Research
1993 – 1997	Group Leader, Dept. of Cellular and Molecular Biology, Univ. of Braunschweig;
1991 – 1992	Visiting Scientist, Whitehead Institute for Biomedical Research, Cambridge, USA;
1987 – 1992	Postdoctoral Associate, Dept. of Toxicology, Univ. of Hamburg;
1989	EMBO short term fellowship, Visiting Scientist at the Medical Research Council Cambridge, England
1989	Visiting Scientist at the Institute of Virology, Oxford, UK.; Elected member of the Saint Cross College Oxford.

Research activities/ qualifications/ Selected Awards & Honors

1989	EMBO Fellowship
since 2002	Elected Member of the German Academy of Natural Scientists, Leopoldina
since 2003	Member of the scientific advisory council of the Interdisciplinary Center for Clinical Research Cologne (CMMC)
since 2004	Scientific Member of the Max Planck Institute for Heart and Lung Research, Bad Nauheim
2004-2012	Member of the evaluation panel of the DFG
since 2012	Trustee Boehringer Ingelheim Fonds for Biomedical Research
since 2013	Member of the scientific advisory council of the International Clinical Research Centre, St. Anne’s University (ICRC) Brno, Czech Republic
since 2013	Elected Member Academia Europea
since 2016	Vice chair - CRC 1213, Pulmonary Hypertension and *Cor Pulmonale*
since 2019	Co Director CPI (Excellence Cluster, Cardio Pulmonary Institute)

Lab members of Department I: Cardiac Development and Remodelling

Lab Members – since 2019
(current / past)

Group leaders (in bold) and PostDocs

Prof. Dr. Thomas Böttger
Dr. Ewelina Betleja
Prof. Dr. Eva Bober
Dr. Laia Canes Esteve
Dr. Dong Ding
Dr. Jingjing Du
Dr. Claudia Garcia Gonzalez
Dr. Angelina Georgieva (maternity leave)
Dr. Alessandro Ianni
Dr. Pumaree Kanrai
Dr. Johnny Kim
Dr. Poonam Kumari
Dr. Xiang Li
Dr. Holger Lörchner
Dr. Giovanni Maroli
Dr. Hui Qi
Dr. Isabelle Salwig
Dr. Andre Schneider
Dr. Christian Schutt
Dr. Krishnamoorthy Sreenivasan
Dr. Shuichi Watanabe
Dr. Lei Wang
Dr. Maria Weiss
Dr. Jing-Jie Weng
Dr. Fan Wu
Dr. Xuejun Yuan
Dr. Ting Zhang
Dr. Jiasheng Zhong

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Farshid Amiri
Hanine Ahmad
Andreea Bostean
Natalie Brachmann
Lucia Camacho Pulido
Yanpu Chen
Julia Detzer
Yvonne Eibach
Lucy Fleming
Marie Elisa Almeida Goes
Sarina Geissler
Theresa Gerhardt
Xinyue Guo
Lu Han
Selina Hache (maternity leave)
Salma Hachim
Sara Hettrich
Ina Klockner*
Alix Kleine-Birkenheuer
Silke Kreher
Keynoosh Khalooghi
Justin Law
Shan Lin
Hang Liu
Julia Detzer
Chiara Mura
Yundong Peng
Mareike Pötsch
Johanna Schubert
Juan Segarra
Maximilian Staps
Shahriar Tarighi
Melissa Valussi
Stanislaus Wüst
Tonghui Xu
Michail Yekelchyk
Qing Yin

Visiting/Guest Scientists

Prof. Dr. Hossein Baharvand
Prof. Dr Ursula Just
Prof. Dr. Jochen Pöling
Dr. Christian Wächter

Technical Assistants

Monika Euler
Katja Kolditz
Susanne Kreutzer
Sonja Krüger
Kerstin Richter
Birgit Spitznagel
Sylvia Thomas
Marion Wiesnet
Roxanne Wagner
Barbara Zimmermann

Administrative Assistant

Susanne Martin

Introduction

Research in the department mainly focuses on tissue regeneration and underlying cellular processes such as differentiation/dedifferentiation/redifferentiation, stem cell quiescence/activation, and inflammatory responses. We target contractile tissues (cardiovascular system and skeletal muscle) and the lung, striving to unravel new mechanistic principles specifically in the field of epigenetics, metabolism, non-coding RNAs, cellular surveillance, and cellular signaling (Fig. 1).

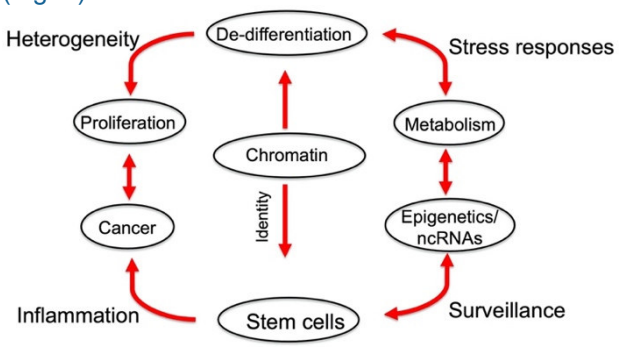

Fig. 1. *An integrated approach to study basic mechanisms required for organ development, remodeling, and regeneration.*

The ultimate goal is to connect and integrate different regulatory levels and prove physiological relevance in vivo, enabling precise control of disease-relevant processes. Understanding the temporal and spatial regulation of signaling events during development, homeostasis and disease is a major aim. Our research mainly concentrates on the identification of stem/progenitor cell populations and their niches and on genetic/ epigenetic and metabolic regulatory processes, required for regeneration. Repair and regeneration are intertwined with innate immune processes and vessel formation. Therefore, we are particularly interested in the characterization of innate immune cells and their regulation during repair and regeneration. Eventually, we will identify new approaches to improve heart, skeletal muscle and lung regeneration.

Heterogeneity within cell populations is important for development, tissue homeostasis and disease. However, the plasticity and the impact of individual cells, or clusters of cells, is far from clear. We have made progress to explore the graded responsiveness of cell populations to stress signals and the differential contribution of individual stem/progenitor cells, as well as parenchymal and non-parenchymal cells to control repair/regeneration versus organ malfunction and fibrosis. Novel genetic cell tracing techniques and fate mapping together with single cell sequencing, in situ sequencing, and bioinformatic analyses are employed to assess the relative contribution of distinct subsets of cells to tissue maintenance and repair under different conditions. Our multi-modal approach has already provided several novel insights into the pathophysiological processes mediating cardiovascular, skeletal muscle and lung diseases and will unravel new epigenetic and non-epigenetic pathways that determine cellular phenotype and fate.

Healthy and fully functional organs critically dependent on cellular surveillance mechanisms. The molecular nature of these mechanisms is complex, and stress responses as well as imbalanced control mechanisms contribute to tissue damage. During disease development and aging, quality control processes might become distorted, thereby amplifying tissue injury and accelerating pathogenesis. Surveillance mechanisms and stress responses act at different levels (transcription, translation, degradation, etc.), which are tightly interconnected. Quality control mechanisms either facilitate repair processes or trigger degradation of defective cellular components, thereby maintaining the stability of genetic information (e.g. by DNA repair), ensuring the quality of mRNAs and non-coding RNAs (ncRNAs), and eliminating dysfunctional proteins or organelles (e.g. via proteasomal degradation or autophagy). These processes are particularly important in the cellular adaptation to stressors. At the same time, surveillance and maintenance of cellular homeostasis are intimately connected with cellular metabolism, which generates metabolites that play important roles as cofactors for controlling epigenetic processes, signaling pathways, and cellular quality control. Maladaptive regulation of surveillance pathways, actively contribute to chronic disease states. We are deciphering adaptive versus maladaptive responses of surveillance pathways in cardiovascular and pulmonary diseases. Specifically, we focus on (i) mechanisms of RNA surveillance by RNA modifications and exploring the control and functional consequences of alterations in pre-mRNA splicing and RNA stability in stress responses and in a disease context; (ii) the role of metabolism and available intercellular nutrients in guiding cellular quality control and epigenetic processes driving regeneration. The following pages provide some examples of completed and ongoing projects, conducted in the past three years

Epigenetic regulation in development and disease

Development and regeneration of muscle tissues involves a series of orchestrated molecular and cellular events which are tightly regulated by epigenetic mechanisms. Epigenetic modifiers that govern distinct transcriptional programs directing cell/tissue functions play important roles in different pathogenic processes, including skeletal muscle disorders and cardiac or pulmonary diseases.

The smooth muscle cell (SMC) is highly plastic cell type that undergoes multiple phenotypic transitions characterized by the expression of distinct markers, defining contractile and synthetic states. Changes in DNA methylation contribute to profound and reversible phenotype changes of SMCs in response to external cues. We discovered that TET3, which successively oxidizes 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and other derivatives, is indispensable for faithful transcriptional initiation at canonical start sites within highly expressed cell identity genes to maintain SMC contractility. Loss of TET3-dependent 5hmC production in SMCs resulted in accumulation of spurious transcripts, which stimulated the endosomal nucleic acid-sensing TLR7/8 signaling pathway and caused phenotypic changes from the contractile to the synthetic state, thereby provoking massive inflammation and airway remodeling resembling human bronchial asthma (Fig. 2). Furthermore, we found that 5hmC levels are substantially lower in human asthma airways compared to control samples (Fig. 2). Our results indicates that suppression of spurious transcription is crucial to prevent chronic inflammation leading to asthma (Fig. 3; Wu et al., Nature Genetics, 2023).

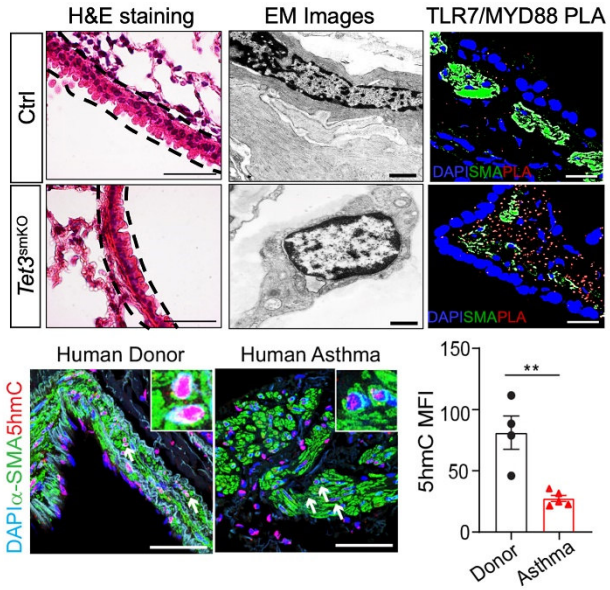

Fig. 2. SMC-specific Tet3 inactivation causes an asthma-like condition. *Upper panel: SMC-specific inactivation of Tet3 leads to an asthma-like condition including airway remodeling, shift of SMCs from the contractile to the synthetic state, and activation of TLR7 signaling. Lower panel: 5hmC level is markedly reduced in bronchial smooth muscle cells of human asthma patients.*

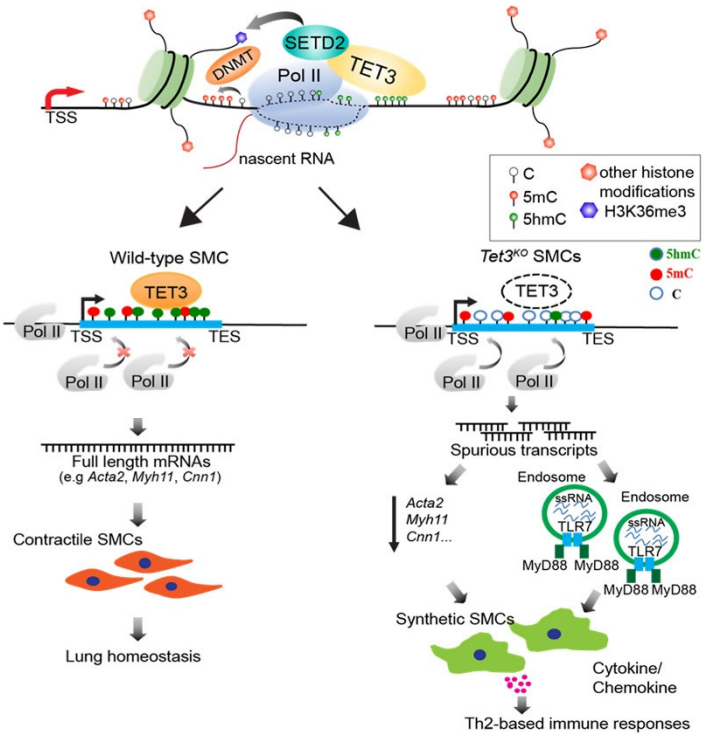

Fig. 3. Spurious transcription, causing innate immune responses, is prevented by 5-hydroxymethylcytosine. *TET3-mediated formation of 5hmC stabilizes recruitment of SETD2 to the RNA Pol II-containing elongation machinery, promoting H3K36me3 formation, which prevents ectopic entry of RNA Pol II. Intragenic entry of RNA Pol II initiates formation of spurious transcripts, which activate endosomal TLR7 signaling and innate immune responses. Resulting T helper type 2 (Th2) cell-based immune responses account for the massive inflammation and pathology of Tet3-deficient airways (Wu et al. Nat. Genet. 2023).*

Disruption of epigenetic processes contributes to life-threatening cardiopulmonary diseases such as Pulmonary Hypertension (PH) and Chronic Obstructive Pulmonary Disease (COPD). By screening a cohort of human PH and COPD patients for changes of histone modifications, we discovered a strong reduction of the histone modifications H4K20me2/3 in human COPD but not in PH patients, which depends on the activity of the H4K20 di-methyltransferase SUV4-20H1 (Fig. 4). Loss of *Suv4-20h1* in ISL1+ cardiopulmonary progenitor cells (CPPs) causes a COPD-like/PH phenotype in mice, including formation of perivascular tertiary lymphoid tissue and goblet cell hyperplasia, hyper-proliferation of smooth muscle cells/myofibroblasts, impaired alveolarization and maturation defects of the microvasculature due to disruption of the SOD3-dependent redox balance. These alterations lead to pulmonary hypertension, massive right ventricular dilatation and premature death (Qi et al., Circulation, 2021).

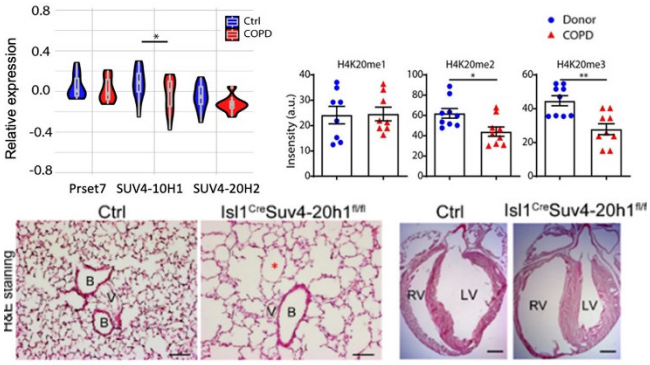

Fig. 4. H4K20me2 deposition in cardiopulmonary progenitor cells prevents pulmonary hypertension and COPD. *Upper panel: Reduction of H4K20me2 and SUV4-20H1 in lungs of COPD patients. Lower panel: Inactivation Suv4-20h1 in Isl1+ cardiopulmonary progenitor cells causes lung emphysema and right heart dilation in mice (Qi et al, Circulation, 2021).*

The NAD⁺-dependent SIRT1-7 family of protein deacetylases plays a vital role in various molecular pathways related to stress response, DNA repair, aging and metabolism. Increased activity of individual sirtuins often exerts beneficial effects in pathophysiological conditions whereas reduced activity is usually associated with disease conditions. We demonstrated that SIRT6 deacetylates H3K56ac in myofibers to suppress expression of utrophin, a dystrophin-related protein stabilizing the sarcolemma in absence of dystrophin. Inactivation of *Sirt6* in dystrophin-deficient *mdx* mice reduces damage of myofibers, ameliorates dystrophic muscle pathology, and improves muscle function, leading to attenuated activation of muscle stem cells (MuSCs). ChIP-seq and locus-specific recruitment of SIRT6 using a CRISPR-dCas9/gRNA approach revealed that SIRT6 is critical for removal of H3K56ac at the downstream utrophin Enhancer (DUE), which is indispensable for utrophin expression (Fig. 5). Our study indicates that epigenetic manipulation of utrophin expression is a promising approach for the treatment of Duchenne Muscular Dystrophy (DMD) (Georgieva et al., Nat. Comm., 2022).

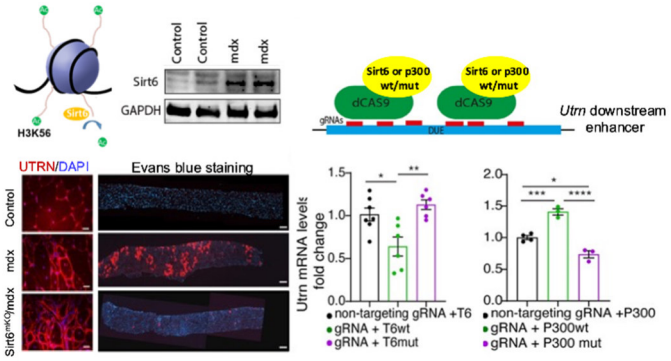

Fig. 5. Inactivation of Sirt6 ameliorates muscular dystrophy in mdx mice by releasing suppression of utrophin expression. *Inactivation of Sirt6, which is upregulated in mdx muscles, leads to H3K56 hyperacetylation. Sirt6 inactivation in mdx muscles activates Utrn expression and improves membrane integrity. CRISPR-dependent recruitment of SIRT6 to the Utrn downsteam enhancer (DUE) abolishes but p300 recruitment stimulates Utrn expression in muscle cells (Georgieva et al. Nat. Comm., 2022).*

Epigenetic mechanisms ensure the fitness of stem cell expansion during regeneration.

Tissue regeneration is based on proper and efficient amplification of adult stem cells, requiring timely and error-free DNA replication. Recent findings suggest that untimely transcription is potentially hazardous, hindring replication fork progression and generating replicative stress, which eventually causes genome instability. However, it remains mainly *unknown whether and how chromatin-dependent processes are involved in the coordination between DNA replication and RNA transcription and* whether transcription-replication collisions (TRCs) play a causative role in tumor initiation. We discovered that lack of the H4K20-dimethyltransferase KMT5B in muscle stem cells (MuSC) de-represses S-phase transcription by increasing H4K20me1 levels, which induces TRCs and aberrant R-loops in oncogenic genes (Fig. 6). The resulting replication stress and aberrant mitosis activate P53 signaling, promoting cellular senescence, which turns into rapid rhabdomyosarcoma formation when p53 is absent (Fig. 6). Inhibition of S-phase transcription ameliorates TRCs and formation

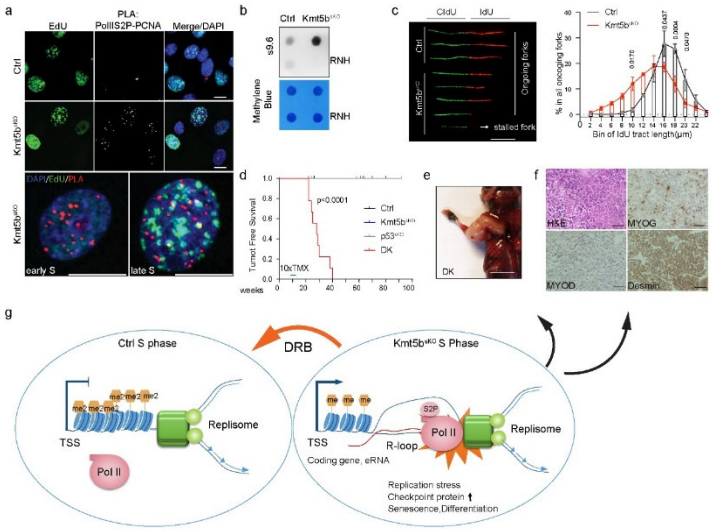

Fig. 6. KMT5B prevents genome instability in MuSC leading to rhabdomyosarcoma formation. *(a) Proximity ligation assay (PLA), visualizing collision of RNA Pol II and*

DNA Pol. (b) Quantification of R-loops in Ctrl and Kmt5b^{sKO} MuSCs (c) Stallment of replication forms in Kmt5b^{sKO} MuSCs using DNA fiber assays. (d) Rhabdomyosarcoma formation in Ctrl, Kmt5b^{sKO}, p53^{sKO}, and DK. (e) Image of a DK mouse with a rhabdomyosarcoma. (f) H&E and immunostaining for MYOD, MYOG, and desmin of a rhabdomyosarcoma. (g) Simplified model of the role of KMT5B in coordinating transcription and replication in MuSC (Zhang et al., Nat. Comm., 2022).

of R-loops in *Kmt5*-deficient MuSCs, validating the crucial role of H4K20me1-dependent, tightly controlled S-phase transcription for preventing collision errors. The study uncovers decisive functions of KMT5B for maintaining genome stability by repressing S-phase transcription and provides the first evidence to connect TRCs with tumorigenesis in vivo (Zhang et al. Nat. Comm, 2022).

Cell fitness during massive expansion of stem cells can also be maintained by eliminating non-competitive, potentially harmful cells. We found that a substantial fraction of MuSCs undergoes necroptosis during chronic skeletal muscle regeneration, which is required for efficient regeneration of dystrophic muscles. Inhibition of necroptosis strongly suppresses MuSC expansion in a non-cell-autonomous manner by interactions between fit and unfit MuSCs. Prevention of necroptosis in MuSCs of healthy muscles is mediated by the chromatin remodeler CHD4, which is attenuated in dystrophic muscles. CHD4 directly represses the necroptotic effector *Ripk3*. Loss of *Ripk3* repression by inactivation of Chd4 causes massive necroptosis of MuSCs, abolishing regeneration. This study demonstrates how programmed cell death in MuSCs is tightly controlled by epigenetic mechanisms to achieve optimal tissue regeneration (Sreenivasan et al. Cell Reports 2020).

Reversible dedifferentiation of adult cardiomyocyte promotes heart regeneration.

Cardiomyocytes are critically for the function of the heart. In adult mice and humans, the rate of cardiomyocyte division is extremely low, preventing heart regeneration. Induction of cardiomyocyte dedifferentiation to restablish an embryonic or fetal state may enable cardiomyocyte proliferation and/or improve survival under hypoxic conditions. We found that miR-1/133a suppresses FGFR1 and OSMR to inhibit cardiomyocyte dedifferentiation and cell cycle entry in adult hearts (Fig. 7). Inhibition of miR-1/133a in cardiomyocytes enhance tolerance to hypoxia and decreases scar size after infarction (Valussi et al, Sci. Adv. 2021).

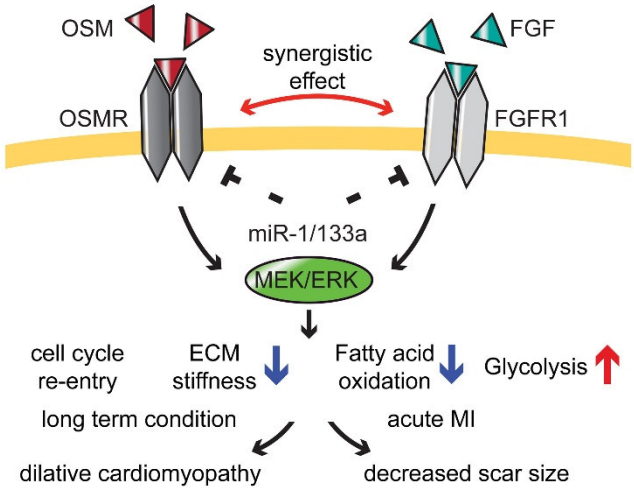

Fig. 7. miR-1/133a prevent dedifferentiation of cardiomyocytes. *Model of the putative regulatory mechanisms of cardiomyocyte dedifferentiation controlled by OSMR, FGFR1, and miR-1/133a. miR-1/133a suppresses expression of FGFR1 and OSMR receptors and thereby locks cardiomyocytes in a differentiated state. Inactivation of Osmr or Fgfr1 normalizes gene expression and prevents cardiomyocyte dedifferentiation in miR-1/133a dKO hearts (Valussi et al., Sci. Adv., 2021).*

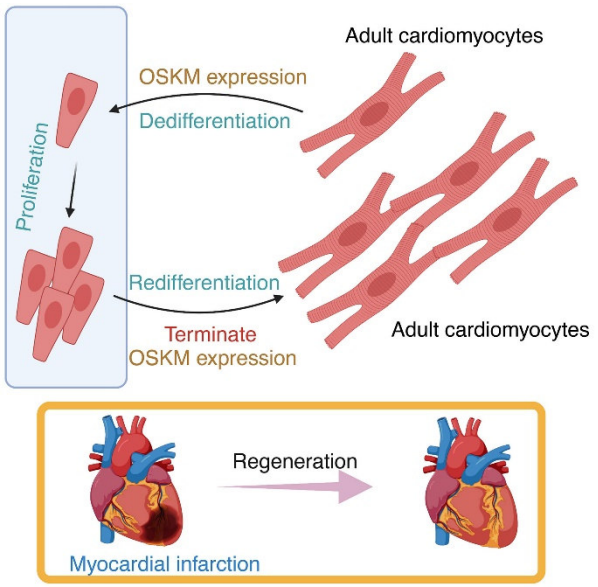

Fig. 8. Temporially-restricted, cardiac-specific expression of OSKM enables heart regeneration in mice. *OSKM overexpression converts adult cardiomyocytes into a fetal-like state, allowing proliferation of cardiomyocytes, which partially repair damage after caused by myocardial infarction (Chen et al., Science, 2021).*

Enhanced dedifferentiation by heart-specific expression of Oct4, Sox2, Klf4, and c-Myc (OSKM) induces proliferation of adult cardiomyocytes, conferring regenerative capacity to adult hearts. Transient, CM-specific expression of OSKM extends the regenerative window for postnatal mouse hearts and induces

a gene expression program in adult CMs that resembles that of fetal CMs. Extended expression of OSKM in CMs leads to cellular reprogramming and heart tumor formation. Short-term OSKM expression before and during myocardial infarction ameliorates myocardial damage and improves cardiac function, demonstrating that temporally controlled dedifferentiation and reprogramming enable cell cycle reentry of mammalian CMs and facilitate heart regeneration (Fig. 8, Chen et al, Science, 2021).

Molecular and physiological functions of noncoding RNA.

Non-coding RNAs are a large group of regulatory molecules with diverse molecular mechanisms and multiple physiological functions. We studied the role of microRNAs (miRNAs) and of long non-coding RNAs (lncRNAs) in contractile tissues, which led to exciting new insights in the mechanisms and functions of ncRNAs in skeletal muscle and the heart. While lncRNAs affect biological functions via diverse molecular mechanisms, miRNAs in principle function by a common molecular mechanism, i.e. destabilisation and repression of translation of mRNAs.

In skeletal muscle, miR-1/133a are strongly expressed together with the related miR-206/133b miRNA suggesting redundant functions of these miRNAs. We found that *miR-1/206/133* are crucial regulators of a signaling cascade comprising DOK7-CRK-RAC1, which is critical for stabilization and anchoring of postsynaptic AChRs during NMJ development and maintenance. We discovered that posttranscriptional repression of CRK by *miR-1/206/133* is essential for balanced activation of RAC1. Failure to adjust RAC1 activity severely compromises NMJ function, causing respiratory failure in neonates and neuromuscular disorders in adult mice. We conclude that *miR-1/206/133* serve a specific function for NMJs but are dispensable for skeletal muscle development (Klockner et al., Nat. Comm. 2022).

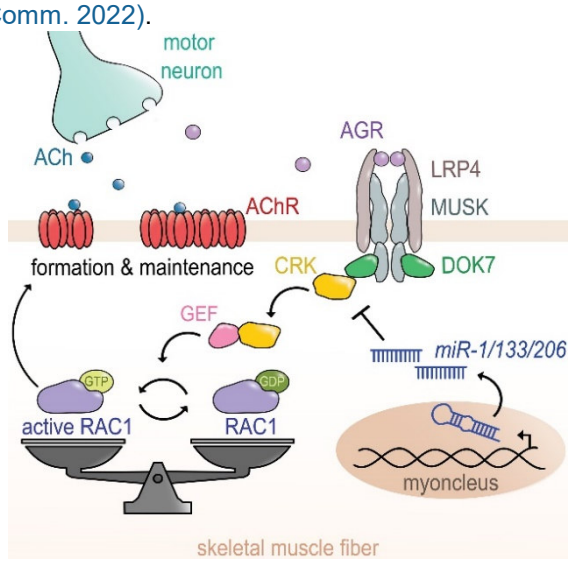

Fig. 9. Regulation of CRK-RAC1 activity by the miR-1/206/133 miRNA family is essential for neuromuscular junction function. *The muscle-specific miRNAs of the miR-1/206/133 family are crucial regulators of a postsynaptic signaling cascade comprising DOK7-CRK-RAC1, critical for stabilization and anchoring of AChRs during NMJ development and maintenance (Klockner et al., Nat. Comm., 2022).*

Our work on the mechanisms and functions of lncRNAs uncovered a role of a skeletal muscle-specific lncRNA in controlling the abundance of muscle stem cells, essential for muscle eutrophy and regeneration, by regulating activity of the chromatin remodeller INO80 (Schutt et al., EMBO J.). We disclosed a molecular mechanism, in which the lncRNA linc-MYH directs configuration of the chromatin remodeller complex.

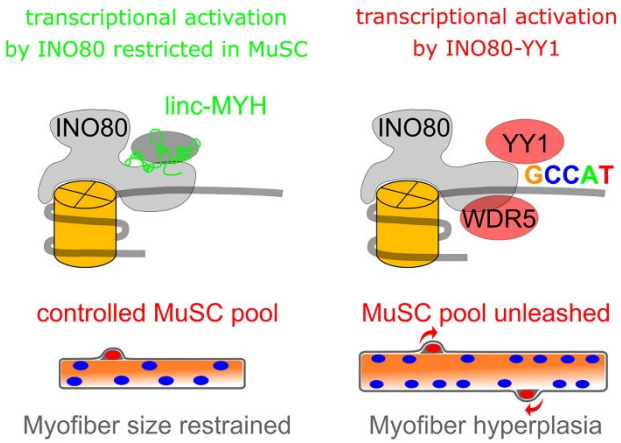

Fig. 10. *The lncRNA linc-MYH controls transcriptional activity of the chromatin remodeller INO80 in trans by preventing association of INO80 with the transcription factor YY1. Loss of linc-MYH promotes INO80-YY1 transcriptional activity extending myoblasts proliferation and increasing the MuSC pool (Schutt et al., EMBO. J., 2020).*

Safeguarding mechanisms in vascular smooth muscle cells and cardiomyocytes

BMP9 and BMP10 are released into the blood stream from the liver and the right atrium of the heart, respectively, suggesting a role in the function of vessels. We found that inactivation of BMP9/10 dramatically diminish the SMC layer in virtually all arteries and decrease blood pressure due to loss of contractile vascular SMCs (VSMCs). Increased expression of BMP10 induces an ALK1-dependent phenotypic switch of VSMCs from the synthetic to the contractile state. Deletion of ALK1 in SMCs recapitulated the phenotype of BMP9/10 mutants in pulmonary arteries but not in aortic or coronary arteries. RNA-seq and single molecule RNA-FISH uncovered heterogeneous expression of BMP type one receptors in distinct vessels, indicating that BMP9/10 signaling is modulated by heterogeneous expression of BMP type one receptors in a vessel bed specific manner (Fig. 11; Wang et al., Circulation, 2021).

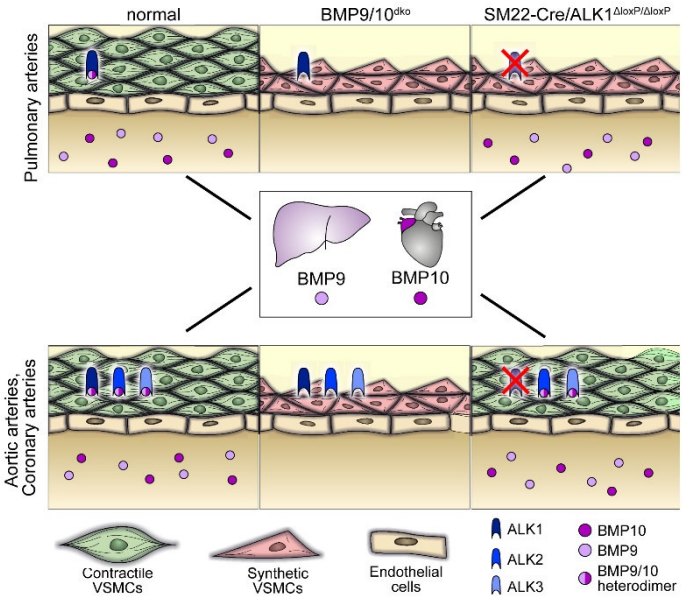

Fig. 11. Model of the role of BMP9 and BMP10 for inducing a contractile state in VSMCs in an ALK recetor dependent manner *(Wang et al., Circulation, 2021).*

In the lung, the presence of bronchial SMCs prevents selective manipulation of VSMCs. To circumvent this problem, we have successfully generated novel binary recombinase systems to specifically label and manipulate VSMCs by using a combination of the Cre/LoxP (ACTA2-Rox-Stop-Rox-CreERT) and Dre/Rox (NG2-Dre) systems (Fig. 12).

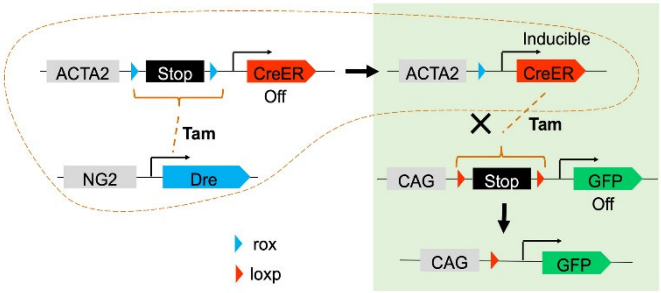

Fig. 12. A binary recombinase system based on knock-ins of Cre and Dre recombinases into the Acta2 and Ng2 genes to specifically manipulate genes in VSCMS *(unpublished).*

Transcriptional profiling of fluorescence-labelled VSMCs revealed a significant heterogeneity in different vessel beds. We also interrogated the role of the splicing factors RBPMS1 and RBPMS2 in SMCs. Concomittant deletion of RBPMS1 and RBPMS2 in all SMCs results in a dramatic reduction of contractile intestinal SMCs, preventing analysis of RBPMS1/2 functions in VSMCs. However, use of the newly developed binary recombinase system uncovered that the loss of RBPMS1/2 in VSMCs strongly enhanced formation of contractile VSMCs in pulmonary arteries, which points to opposing roles of RBPMS1/2 in VSMCs versus non-vascular SMCs. Further analysis indicates that RBPMS1/2 promotes alternative splicing events in the heart, which are essential for heart development and cardiomyocyte identity. RBPMS1/2 deficient cardiomyocytes show multipolar spindle defects and chromosomal mis-segregation, causing nuclear abnormalities and arrest of cardiomyocyte proliferation.

SIRT7-dependent deacetylation of NPM promotes p53 stabilization following UV-induced genotoxic stress

The tumor suppressor p53 maintains genomic integrity in response to genotoxic stress, by promoting cell-cycle arrest, facilitatating DNA repair, or triggering apoptosis. P53 prevents expansion of cells that have accumulated potential pro-tumorigenic mutations. Inhibition of p53 upon exposure to a variety of DNA-damaging agents, such as ultraviolet (UV) irradiation, accelerates tumorigenesis. p53 is maintained at low levels under basal conditions but cellular stressors lead to its stabilization, allowing p53 to elicit antitumor functions. The main regulator of p53 levels is the E3 ubiquitin ligase MDM2. MDM2 binds to p53 and promotes ubiquitination and subsequent proteasomal-dependent degradation of p53. Disruption of MDM2-p53 binding following stress is a key

mechanism to rapidly increase p53 levels and is induced by different signaling pathways. One main regulator of the MDM2-p53 axis is the abundant nucleolar protein nucleophosmin (NPM). Under normal conditions, NPM is mainly sequestered in the nucleolus, but it is rapidly excluded in response to stressors. Release of NPM from the nucleolus enables NPM to associate to MDM2, thus disrupting MDM2-p53 binding and promoting p53 stabilization. We discovered that deacetylation of NPM by the nucleolar NAD+-dependent histone/protein deacetylase sirtuin 7 (SIRT7) plays a decisive role in activating the NPM-MDM2-p53 pathway in response to UV irradiation (Fig. 13; Ianni et al., PNAS, 2021).

Under physiological conditions, SIRT7 and NPM form a molecular complex in the nucleolus. Exposure to UV-irradiation dramatically increases the catalytic activity of SIRT7 due to phosphorylation by the ATR kinase, a major activator of the DNA damage response. The enhanced enzymatic activity enables SIRT7 to deacetylate NPM at two distinct residues: lysine 27 and lysine 54. Deacetylated NPM leaves the nucleoli, strongly binds to MDM2, thus promoting p53 accumulation both in vitro and in vivo in the skin. Essentially, SIRT7 exerts its tumor suppressive functions in the skin exposed to UV irradiation through stabilization of p53.

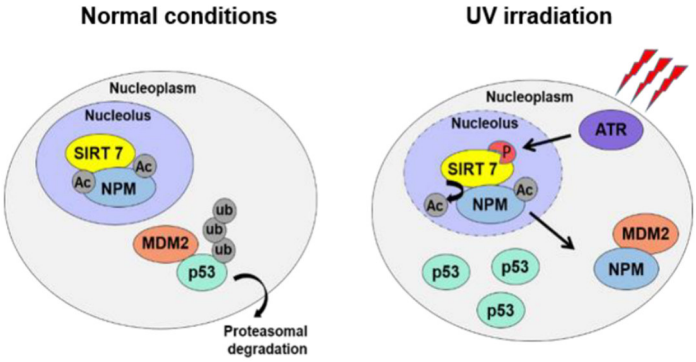

Fig. 13. Model of the putative mechanism employed by SIRT7 to promote p53 stabilization following UV-induced genotoxic stress *(Ianni et al, PNAS, 2021).*

Reg3β-mediated clearance of cardiac neutrophil granulocytes after myocardial infarction

Activation of the innate immune system is instrumental for cardiac healing after myocardial infarction (MI). The phagocytotic removal of necrotic tissue and release of numerous cytokines paves the way for formation of granulation tissue and successful scar formation. However, excessive and prolonged immune response are detrimental, favoring progression to heart failure. The identity of factors orchestrating local resolution of reactive innate leukocyte subpopulations is mostly unknown. We previously identified a novel cytokine, Reg3β, which is rapidly secreted by stressed cardiomyocytes within the ischemic heart. Loss of Reg3β provokes delayed recruitment of macrophages but also prolonged persistence of neutrophils after MI, leading to cardiac rupture. We discovered a novel mechanism by which Reg3β removes cardiac neutrophils from infarcted hearts. Reg3β binds directly to a subpopulation of actived neutrophils and kills them. We found that Reg3β is internalized and transported via the endolysosomal pathway to primary LAMP1⁺ lysosomes. Engulfment of Reg3β by lysosomes increases intracellular ROS levels in a NOX2-dependent manner and destabilize lysosomes, resulting in cathepsin release and ROS-dependent lysosomal cell death. The results demonstrate that Reg3β serves a dual function after MI: (i) local clearance of neutrophils and (ii) attraction of macrophages, which finally eliminate dying neutrophils via efferocytosis and thereby contribute to the resolution of cardiac inflammation (Fig. 14).

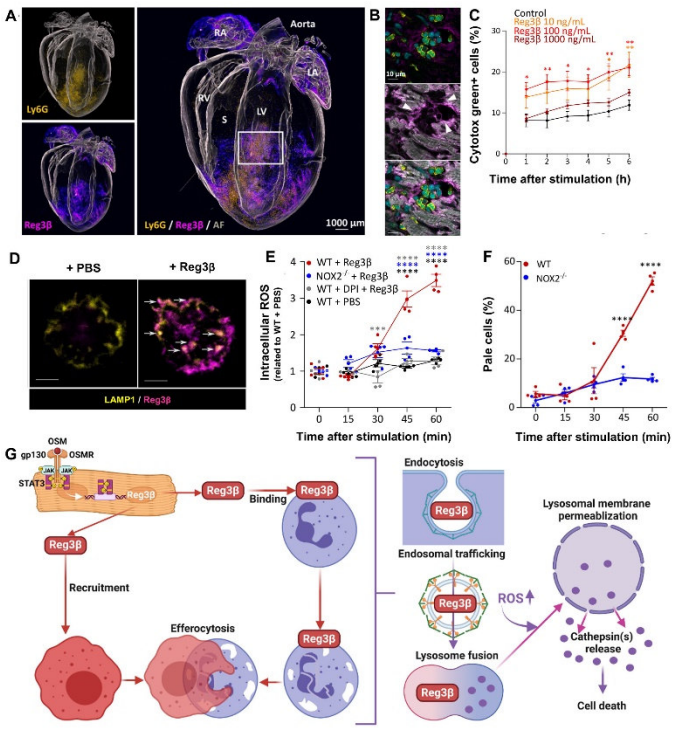

Fig. 14. Reg3β removes neutrophils from the ischemic myocardium. *(A, B) Visualization of Reg3β-binding to neutrophils. (C) Induction of neutrophil cell death by Reg3β. (D) Localization of Reg3β in lysosomes. (E, F) Reg3β increases ROS and kills neutorphils in a NOX2-dependent manner. (G) Model of Reg3β-dependent removal of neutrophils after myocardial infarction (unpublished).*

Cellular constituents of lung regeneration and tumorigenesis

Due to constant exposure to potentially harmful airborne pollutants and pathogens, lungs require a high regenerative capacity to rapidly replace lost or damaged cells. Efficient epithelial regeneration relies on the cooperation of multiple – mostly regional – stem/progenitor cell populations. To analyze stem cell niches in a spatial context, we combined advanced genetic mouse models with optical clearing and 3D-imaging of entire lungs to explore organ-wide regenerative processes. During bronchiolar repair, we identified airway bifurcations and bronchioalveolar duct junctions (BADJs) as starting points for epithelial recovery. BADJs are known to harbor rare bronchioalveolar stem cells (BASCs), which lack a singular genetic marker but co-express bronchiolar and alveolar genes. To trace BASCs and resolve their contribution during epithelial regeneration, we developed "split-effectors" enabling the selective manipulation of dual-marker expressing cell types *in vivo* (Salwig et al., EMBO J., 2019). Our study defined BASCs as crucial components of bronchioalveolar repair, highlighting their bi-potent differentiation potential. Currently, we investigate which key signaling pathways control cell-fate decisions, directing BASC into either bronchiolar or alveolar directions, depending on local needs. Since neoplastic lesions frequently arise in the vicinity of BADJs, we explore the tumor-initiating and -propagating potential of BASCs in Kras-induced lung adenocarcinoma (Fig. 15).

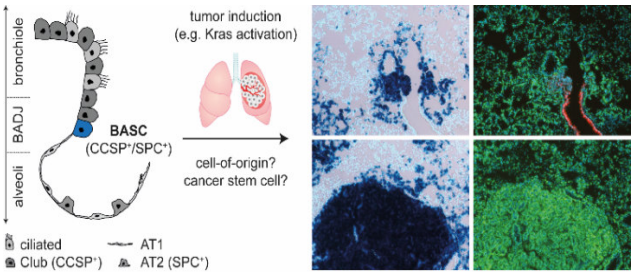

Fig. 15. Bronchioalveolar stem cells are amenable to cancerous transformation and give rise to lung adenocarcinoma *(unpublished).*

Our observation that distinct epithelial cells function as anatomically-segregated antigen presenting cells (Shenoy et al., Nat. Comm., 2021) may point towards altered immunosurveillance depending on the cell origin of tumors. While BASCs are the main cellular source for distal airway regeneration, our data indicate the existence of alternative stem/progenitor cells located at airway branch points. By single cell and *in situ* sequencing, we explored secretory cell heterogeneity and identified a subset of Club cells, co-expressing genes characteristic for ciliated cells. Split-effector based *in vivo* targeting revealed that such branch-point double-positive cells (BDPCs) are instrumental for the generation of multiciliated cells during postnatal lung development. Surprisingly, genetic ablation of BDPCs not only severely impairs formation of ciliated cells but also exhausts secretory Club cells. The exhaustion of Clun cells persists until adulthood and pre-disposes for inefficient regeneration following Influenza A virus (IAV) infection. BASCs/BDPCs appear to receive specific emergency calls either directly from damaged epithelial cells or *via* mesenchymal cells (MSCs). To study cellular crosstalk, we performed single cell profiling of healthy and diseased lungs and discovered that MSCs serve as an important signaling hub during the course of IAV infection. Interestingly, *in vitro* bronchioalveolar lung organoid cultures (Vazquez-Armendariz et al., EMBO J, 2020) suggest that MSCs isolated from IAV infected lungs lose their supportive function in promoting growth, differentiation and maintenance of epithelial progenitor cells. We therefore interrogate the beneficial effects of exogenously applied MSCs/MSC-derived factors after IAV infection of mice to develop novel therapeutic interventions.

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External Funding (2019-2022)

2010-2022: DFG/SFB TRR 81 (T. Braun/X. Yuan) Delineating the interplay of epigenetic modifiers and transcription factors in the control of muscle stem cell homeostasis and high order chromatin organization.

2016-2024: DFG/SFB 1213 – A02 (T. Braun/X. Yuan) Deciphering the role of BMP9-BMP10 and YAP-TEAD signaling in vascular smooth muscle heterogeneity and pulmonary hypertension.

2016-2024: DFG/SFB 1213 – B02 (T. Braun) Epigenetic control of pulmonary vessels and the right heart in development and disease: the role of Suv4-20h1.

2019-2023: DFG/SFB TRR 267 (T. Braun/T. Böttger) ncRNAs controlling chromatin and remodeller complexes in the cardiovascular system.

2016-2024: DFG/SFB 1531 (T. Braun/J. Pöling) Control of transport and clearance of cells of the innate immune system by REG proteins in the diseased heart.

2018-2022: DFG KFO309 (T. Braun) Novel Strategies for Therapeutic Programming and Delivery Of Mesenchymal Stem Cells To Improve Outcome Of Influenza Virus-Induced Lung Injury.

2021-2024: LOEWE, iCANx. (T. Braun) Lung (Disease) Crosstalk: Tumor and Organ Microenvironment.

2018-2022: DFG-GRK 2355 (T. Böttger/A. Schneider) Regulatory networks in the mRNA life cycle: from coding to noncoding RNA.

2016-2025: DZHK Deutsches Zentrum für Herz- und Kreislaufforschung. (T. Braun) Partner Site Project-Characterization of cellular heterogeneity and immune cell clearance in the cardiovascular system by mass spectrometry- and NGS-based biomolecular analysis.

II. Pharmacology

Stefan Offermanns (director)

Stefan Offermanns, Prof. Dr. med. | Director, Dept. of Pharmacology, Max Planck Institute for Heart and Lung Research / University Professor in Medicine, Goethe University Frankfurt

University Education, Scientific Career, Activities and Awards

1991	Final clinical examination
1990	M.D. degree
1984 – 1990	Studies in medicine at the Free University Berlin
1990 – 1993	Postdoc, Institute of Pharmacology, FU Berlin
1994 – 1997	Postdoc, Division of Biology, California Institute of Technology, Pasadena, USA
1997 – 2000	Group Leader (Heisenberg Program) at the Institute of Pharmacology, FU Berlin
2000 – 2009	Professor of Pharmacology and Director of the Institute of Pharmacology, University of Heidelberg
since 2008	Director and Scientific Member Max Planck Institute of Heart and Lung Research and Professor at the Goethe University Frankfurt
1998	Scientific Award for Basic Medical Science (SmithKline Beecham Foundation)
1999	Rudolf-Buchheim Award (German Pharmacological Sciety)
2006	Member of the German Academy of Sciences Leopoldina
2008	Feldberg Prize (Feldberg Foundation)
since 2010	Committee for Collaborations Fraunhofer Society / Max Planck Society
since 2012	Scientific Committee, Einstein Foundation Berlin
since 2014	Board of Directors, Feldberg Foundation
since 2015	Chair, Stipend committee, Minerva Foundation (German-Israeli exchange)
since 2016	Managing Board, Georg-Speyer-Haus
since 2017	Steering Committee Cardiopulmonary Institute (CPI)
2018	Dr. h.c. Semmelweis University Budapest (Hungary)
since 2018	Managing Board Frankfurt Cancer Institute
since 2020	Chair, Scientific Advisory Board, FMP, Berlin

Nina Wettschureck (group leader)

Nina Wettschureck, Prof. Dr. med. | group leader, Dept. of Pharmacology, Max Planck Institute for Heart and Lung Research / University Professor in Medicine, Goethe University Frankfurt

University Studies

1990 – 1996	Studies of medicine at Goethe University Frankfurt / Main
1996	M.D. degree, Goethe University Frankfurt / Main

Scientific Career

1996 – 1998	Dept. of Internal Medicine V. University of Heidelberg
1998 – 2000	Postdoctoral Research Fellow, Institute of Pharmacology, FU Berlin
2000 – 2009	Postdoctoral Research Fellow, Institute of Pharmacology, University of Heidelberg
2007	"Habilitation" for Pharmacology and Toxicology, University of Heidelberg
since 2009	Group Leader at the Max Planck Institute for Heart and Lung Research
since 2012	Professor for Molecular Pharmacology at the Goethe University Frankfurt / Main

Lab members of Department II: Pharmacology

Lab Members – since 2019
(Current / Past)

PostDocs
Dr. Wessam Alnouri
Dr. Sebastian Barthel
Dr. Rémy Bonnavion
Dr. Jorge Carvalho
Dr. Haaglim Cho
Dr. Elena Dyukova
Dr. Malar Gurusamy
Dr. Wie Huang
Dr. András Iring
Dr. Young-June Jin
Dr. Paula-Sofia Yunes Leites
Dr. Rui Li
Dr. Guozheng Liang
Dr. Haruya Kawase
Dr. Anna Monori-Kiss
Dr. Akiko Nakayama
Dr. Sophie Ramas
Dr. Shulan Pi
Dr. Sabrina Sapski
Dr. Sarah Tonack
Dr. Lei Wang
Dr. Hongjiao Xu
Dr. Rui Xu

PhD Students
Hanna Baltrukevich
Isabell Brandenburger
Xinyi Chen
Yannick Jäger
Sayali Joseph
Swarnali Kundu
Sabrina Kurz
Alan LeMercier
Jenoghyeon Kwon
Chien-Cheng Lai
Shangmin Liu
Kenneth A. Roquid
Jingchen Shao
Niharika Shiva
Hilal Taskiran
Adriana Vucetic
Tianpeng Wang
Shenglan Zeng

Staff Scientist
Dr. Boris Strilic

Science / Lab Coordinator
Dr. Nadine Rink
Dr. Constanze Vitzthum

Technical Support
Oxana Bechtgoldt
Veronika Handzik
Kathrin Heil
Ulrike Krüger
Dagmar Magalei
Claudia Ullmann
Martina Winkels

Administrative Assistant
Svea Hümmer

Visiting/Guest Scientists
Shuang Cao
Dr. Xiaofei Lyu
Yue Shi
Dr. Kerstin Troidl
Prof. Dr. ShengPeng Wang
Prof. Dr. Thomas Worzfeld

Introduction – Organization and Scientific Concept

The Department of Pharmacology has two research groups headed by Stefan Offermanns and Nina Wettschureck. The department pursues both basic science projects and projects with the aim to solve medical problems. Basic science projects focus on the analysis of the molecular mechanisms of particular cellular signaling pathways and on the understanding of fundamental physiological processes in the vascular and metabolic system of the mammalian organism. More medically oriented scientific projects deal with the mechanisms of pathophysiological processes and drug actions, in particular in the cardiovascular and metabolic system as well as in cancer with the option to carry the projects to a translational level.

Research during recent years in the department can be divided into the following research areas:

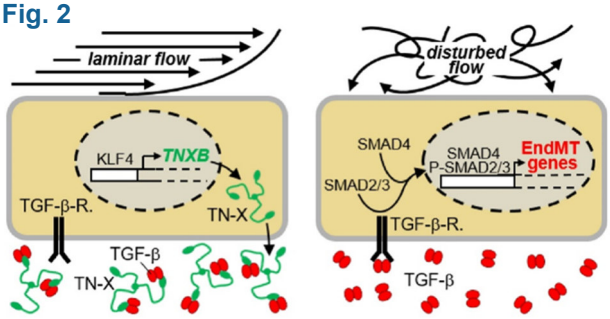
OFFERMANNS GROUP

1. Cardiovascular Signalling

Various projects in this research area have focused on **mechanosensing and mechanotransduction in endothelial cells**. Fluid shear stress exerted by the flowing blood is crucial for the development of blood vessels but is also one of the major regulators of vascular tone by inducing nitric oxide (NO) formation in endothelial cells, which relaxes vascular smooth muscle cells. Blood flow is also a key factor in the development of atherosclerosis, which mainly occurs in regions of arteries exposed to disturbances in blood flow. Understanding how laminar and disturbed flow promote atheroprotective and atherogenic signaling, respectively, may provide new insights into the pathophysiology of cardiovascular diseases such as arterial hypertension and atherosclerosis. In the past, we have characterized a central signaling pathway that links endothelial sensing of fluid sear stress exerted by laminar blood flow to the regulation of NO-dependent vasodilatation. This pathway involves the mechanosensitive cation channel Piezo1, the endothelial release of ATP and adrenomedullin which activate the G_q/G_{11}-coupled purinergic $P2Y_2$ receptor and the G_s-coupled adrenomedullin receptor, respectively (Wang et al., 2016; Iring et al., 2019) (Fig. 1). Interestingly, disturbed flow activates the same initial mechanosensing and mechanosignaling processes, but the two G-protein-mediated signaling pathways do not synergize but rather have opposite effects. While disturbed flow induced activation of $P2Y_2$ and G_q/G_{11} results in integrin-dependent activation of NF-κB (Albarran-Juarez et al., 2018), the activation of the adrenomedullin receptor and G_s-mediated cAMP formation has an anti-inflammatory effect by inhibiting NF-κB activation (Nakayama et al., 2020) (Fig. 1).

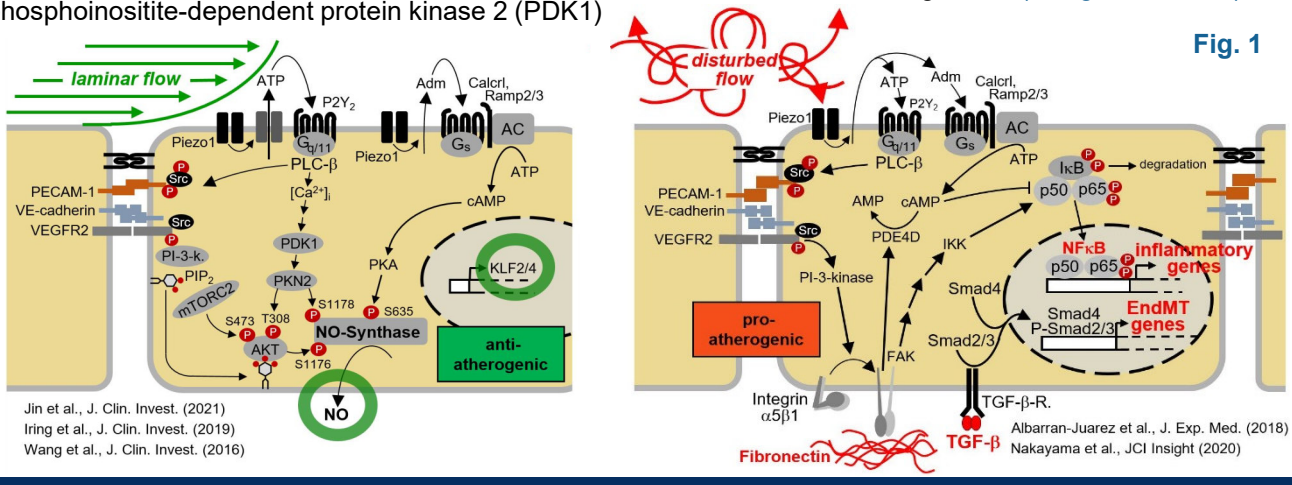
More recently, we identified protein kinase 2 (PKN2) as a central mediator of laminar flow-induced activation of endothelial NO synthase (eNOS) (Jin et al., 2021) (Fig. 1). PKN2 is activated through Piezo1 and G_q/G_{11}-mediated signaling as well as Ca^{2+} and phosphoinositite-dependent protein kinase 2 (PDK1) and directly phosphorylates human eNOS at the newly identified site serine 1179. PKN2 also leads to eNOS phosphorylation at serine 1177, which involves phosphorylation of AKT synergistically with mTORC2-mediated AKT phosphorylation (Fig. 1). These data could be verified using mice with endothelium-specific deficiency of PKN2 (Jin et al., 2021).

Fig. 2



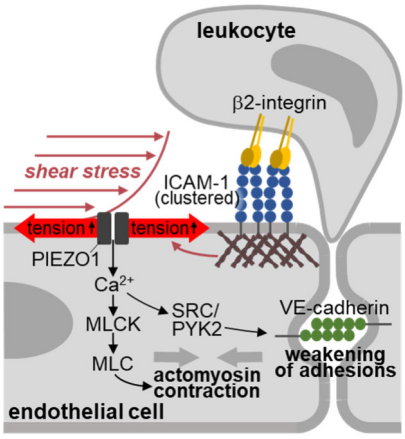
In a screen for laminar flow-induced endothelial gene expression, we have identified the extracellular matrix protein tenascin-X (TN-X), which turned out to be specifically induced in mouse and human arteries in response to laminar flow in a Krüppel-like factor 4 (KLF4)-dependent manner (Liang et al., 2022). In mice with endothelium-specific loss of TN-X (EC-Tnxb-KO), endothelial TGF-β signalling, expression of endothelial-to-mesenchymal transition (EndMT) as well as inflammatory marker genes were increased. We also observed increased vascular remodeling and advanced atherosclerotic lesions in these mice. This phenotype could be normalized by treating EC-Tnxb-KO mice with an anti-TGF-β antibody or after additional endothelial loss of the TGF-β receptors 1 and 2. In *in vitro* studies, we found that TN-X, through its fibrinogen-like domain, directly interacts with TGF-β and thereby interferes with its binding to the TGF-β receptor (Fig. 2). With these data, we identified a novel mechanism which mediates flow-induced inhibition of EndMT, endothelial inflammation and atherogenesis (Liang et al., 2022).

Fig. 1



The **extravasation of leukocytes** is a critical step in inflammation which requires localized opening of the endothelial barrier (Wettschureck et al., 2019). It had been known for a while that fluid shear stress promotes leukocyte extravasation. In an siRNA-based screen for transmembrane proteins involved in leukocyte extravasation, we identified Piezo1 as a critical mediator (Wang et al., 2022). In subsequent *in vitro* and *in vivo* studies, we found that mechanical forces generated by leukocyte-induced clustering of ICAM-1 synergize with fluid shear stress exerted by the flowing blood to increase endothelial plasma membrane tension to activate Piezo1. The subsequent increase in $[Ca^{2+}]_i$ and activation of downstream signaling events including phosphorylation of the tyrosine kinases SRC and PYK2 as well as of myosin light chain results in the opening of the endothelial barrier (Fig. 3). These data show that leukocytes and the hemodynamic microenvironment synergize to mechanically activated endothelial Piezo1 and downstream signaling to initiate leukocyte diapedesis (Wang et al., 2022).

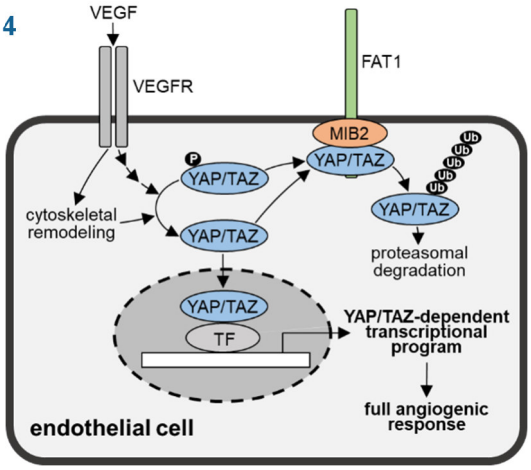
Fig. 3



Currently ongoing studies in the field of endothelial mechanosensing and mechanotransduction aim at the identification of the upstream regulation of laminar flow-induced KLF2 induction as well as at the elucitation of the role of protein kinase N1 in disturbed flow-induced induction of inflammatory genes.

Among the critical signaling pathways activated during **physiological and pathophysiological angiogenesis** is the YAP/TAZ signaling pathway. Whereas several mechanisms of endothelial YAP/TAZ activation have been described, it had remained largely unknown how YAP/TAZ activity is negatively regulated in endothelial cells. We have found that the protocadherin FAT1 acts as a critical upstream regulator of endothelial YAP/TAZ by limiting the activity of these transcriptional co-factors both during developmental and tumor angiogenesis by increasing the protein degradation of YAP/TAZ (Li et al., in revision). We have identified the E3 ubiquitin ligase Mind Bomb 2 (MIB2) as a novel FAT1 interacting protein which mediates FAT1-induced YAP/TAZ ubiquitination and degradation (Fig. 4). Loss of MIB2 expression in endothelial cells *in vitro* and *in vivo* recapitulates the effects of FAT1 depletion and causes decreased YAP/TAZ degradation, increased endothelial YAP/TAZ signaling and, ultimately, increased endothelial cell proliferation.

Fig. 4



Several current projects deal with the regulation of endothelial growth during **post-ischemic neovascularization**. Recent data have shown that ischemia-induced neovascularization after myocardial or hind limb infarction is due to the clonal expansion of pre-existing endothelial cells. Several clinical trials testing the therapeutic potential of known pro-angiogenic factors in ischemic diseases have not resulted in the expected beneficial effects, indicating that our understanding of the underlying mechanisms is still poor. In a currently ongoing project, we found the amyloid precursor protein (APP) and its close homologue APLP2 to be highly expressed in endothelial cells after myocardial infarction. In mice with endothelium-specific loss of APP and APLP2, ischemia-induced neovascularization after myocardial infarction was completely abrogated. This resulted in increased infarct size as well as in a strongly increased post-infarct lethality. Surviving animals had strongly reduced cardiac function compared to control mice. *In vitro* studies showed that APPsα, the product of non-amyloidogenic processing of APP, is able to induce endothelial cell proliferation and tube formation. A role of APPsα in postischemia neovascularization is also supported by our observation that the phenotype of APP/APLP2 endothelium-specific knock-outs is rescued in a knock-in mouse model expressing APPsα under the control of the *App* promoter. Current efforts focus on the mechanism by which APPsα promotes postischemic neovascularization and on preclinical tests to evaluate the therapeutic potential of systemically given APPsα to improve postischemia neovascularization after myocardial infarction.

Another project explores ways to improve postischemic neovascularization and is based on our discovery of the G-Protein-coupled receptor GPR182 as an atypical chemokine receptor for CXCL12 and

some other chemokines (see below) (Le Mercier et al., 2021). We found that tissue and plasma levels of CXCL12 are elevated in mice lacking GPR182 (see below). Since CXCL12 has been shown to promote postischemia neovascularization and collateral growth, we have tested the effect of a global and an endothelium-specific loss of GPR182 on the cardiac response to myocardial infarction. We found that loss of GPR182 resulted in increased CXCL12 levels in the myocardium, decreased infarct size as well as improved cardiac function after myocardial infarction. This makes GPR182 a promising target for the development of agents that improve postischemia neovascularization, and we have already secured funding to develop GPR182-blocking antibodies or nanobodies together with the Lead Discovery Center GmbH (Dortmund, Germany).

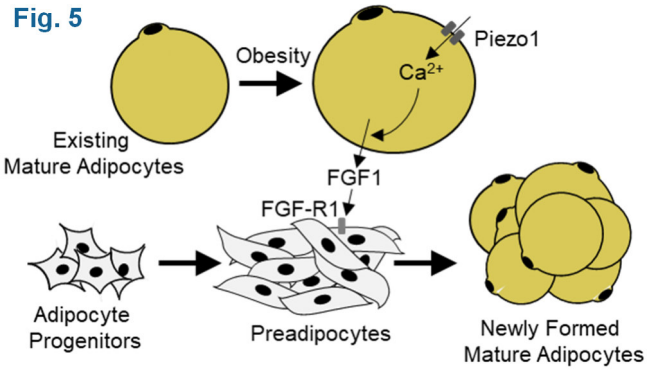
Other projects in the field of cardiovascular signaling have dealt with the mechanisms underlying the regulation of **vascular smooth muscle differentiation**. We found that YAP/TAZ are required to suppress osteogenic differentiation of vascular smooth muscle cells based on mice with induced smooth muscle-specific YAP and TAZ deficiency. In *in vitro* and *in vivo* studies we could identify disheveled 3 (DVL3) as a critical mediator of this effect. YAP/TAZ interact with DVL3 and thereby avoid nuclear translocation of DVL3, which upregulates osteogenesis-associated genes independent of canonical Wnt/β-catenin signaling (Wang et al., 2020b).

2. Metabolic signaling

The expansion of the **white adipose tissue** in obesity goes along with increased mechanical, metabolic and inflammatory stress. It has been poorly understood how adipocytes resist this stress. We found that in human and mouse adipocytes the transcriptional co-activators YAP/TAZ and YAP/TAZ target genes are activated during obesity by inflammatory mediators including TNF-α and IL-1β. In mice lacking YAP and TAZ in white adipocytes, feeding a high-fat diet results in severe lipodystrophy with adipocyte apoptosis. This is due to the upregulation of the pro-apoptotic factor BIM (Wang et al., 2020a), which normally is downregulated in adipocytes of obese mice and humans to protect against apoptosis. These data identify YAP/TAZ signaling as a major regulator of anti-apoptotic adipocyte regulation during obesity (Wang et al., 2020a).

In a parallel project, we were interested in understanding how white adipose tissue expansion in obesity results not only in adipocyte hypertrophy but also in the formation of new adipocytes in the process of obesogenic adipogenesis. We found that mature adipocytes express relatively high levels of the mechanosensitive cation ion channel Piezo1, which is activated when adipocytes increase in size during the development of obesity (Wang et al., 2020c). Mice lacking Piezo1 in mature adipocytes show defective differentiation of preadipocytes into mature adipocytes during obesity. This resulted in larger adipocytes, increased white adipose tissue inflammation and reduced insulin sensitivity. Under normal conditions opening of Piezo1 in mature adipocytes during obesity causes the release of the adipogenic fibroblast growth factor 1 (FGF1), which induces adipocyte precursor differentiation through activation of the FGF receptor 1 (Fig. 5). This provided evidence for an unexpected role of mechanosensing and signaling in adipocytes, which eventually controls adipogenesis during the development of obesity (Wang et al., 2020c).

Fig. 5



In the past, we have studied mechanisms of the auto- and paracrine regulation of **pancreatic β-cell function**, including the function of the β-cell-derived lipid mediator 20-HETE (Tunaru et al., 2018). A currently ongoing project deals with the role of the long-chain fatty acid receptor FFA4. Mice with global FFA4 deficiency have a reduced glucose tolerance due to impaired glucose-stimulated insulin secretion. In a series of experiments using conditional FFA4 knockout mice, we found that loss of FFA4 on pancreatic islet macrophages was responsible for this phenotype, which is accompanied by a reduced relative number of β-cells and an increase in the relative number of somatostatin-producing δ-cells. We are currently exploring how islet macrophages are involved in maintaining the normal distribution of endocrine cells in pancreatic islets.

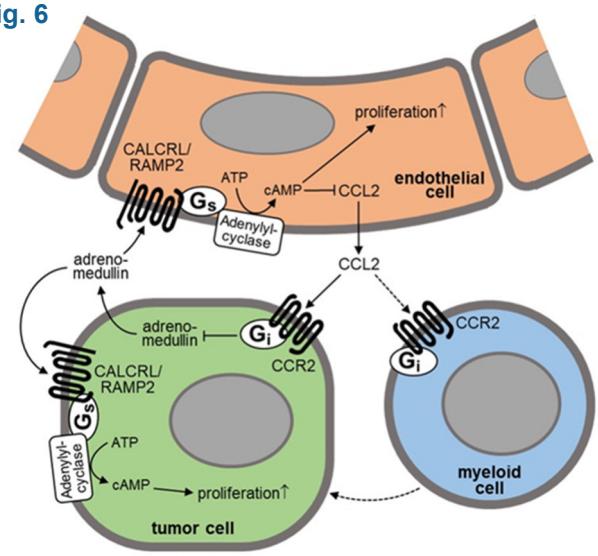
We have recently started to explore the **role of endothelial cells in the regulation of metabolic functions**. A major observation that initiated the studies was the finding that insulin signaling in endothelial cells is greatly increased in the absence of G_s-mediated signaling *in vitro* and *in vivo*. We found that under normal conditions, G_s-mediated increase in cAMP results via protein kinase A in decreased insulin-induced insulin receptor activation in endothelial

cells, reduced perfusion of metabolic organs and also decreased insulin receptor activation in metabolic target cells such as adipocytes or skeletal muscle cells. Endothelial G_s-mediated signaling is under the control of the G_s-coupled adrenomedullin receptor and, in obese type 2-diabetic mice, blockade of the adrenomedullin receptor increased insulin sensitivity and glucose tolerance. This suggests that increasing endothelial insulin signaling by inhibition of adrenomedullin formation or action could be a new therapeutic approach to treat insulin resistance in type 2 diabetes.

3. Tumor progression and metastasis

Within the **tumor microenvironment**, tumor cells and endothelial cells regulate each other in many ways. We have recently shown that tumor cell-derived adrenomedullin has a proangiogenic as well as a direct tumor-promoting effect and that endothelium-derived CC chemokine ligand 2 (CCL2) suppresses adrenomedullin-induced tumor cell proliferation (Nakayama et al., 2023). In an *in vitro* co-culture of tumor cells and endothelial cells, loss of the endothelial adrenomedullin receptor or G_s did not only reduce endothelial cell proliferation but also proliferation of tumor cells. By analyzing endothelial transcriptomic changes in response to adrenomedullin, we identified CCL2 as a critical angiocrine factor whose formation is inhibited by endothelial adrenomedullin signaling and which inhibits the formation of adrenomedullin by tumor cells through its receptor CCR2. These data identified CCL2 as an angiocrine factor, which controls adrenomedullin formation by tumor cells and which can decrease tumor growth (Nakayama et al., 2023) (Fig. 6).

Fig. 6



In the past, we have studied the interaction of tumor cells with endothelial cells in the context of extravasation at metastatic sites, and we have identified mechanisms mediating tumor cell extravasation during **metastasis formation** (Strilic and Offermanns, 2017; Strilic et al., 2016). In currently ongoing project, we study the survival of circulating tumor cells and have identified a transcription factor which is strongly upregulated in circulating tumor cells whereas it is downregulated after metastatic spreading. In another project, we are trying to identify local and systemic mechanisms which regulate tumor cell dormancy. To this end, we have performed siRNA-mediated screens of endothelial genes encoding transmembrane proteins or secreted proteins, and we identified several candidates, which are required for endothelial cell-induced tumor cell dormancy. In endothelium-specific knock-outs of some of these candidates, we found that tumor cell dormancy is also reduced under *in vivo* conditions, resulting in increased tumor cell metastasis. At the moment we are trying to understand the underlying mechanisms.

4. G-protein-coupled receptors (GPCRs)

The human genome encodes about 400 non-olfactory GPCRs. For about 150 GPCRs no ligand has been described so far, making them “orphan” GPCRs. A long-lasting effort of the department aims at the de-orphanization of these GPCRs. Most recently, we have found that GPR182, which is specifically expressed in lymphatic and microvascular endothelial cells (Le Mercier et al., 2021), binds CXCL10, CXCL12 and CXCL13 with nanomolar affinity. However, binding to GPR182 did not induce any downstream signaling. GPR182 shows a high constitutive activity to recruit β-arrestin, which results in a constant internalization of the receptor in the absence as well as in the presence of a receptor ligand (Le Mercier et al., 2021). Thus, GPR182 is an atypical chemokine receptor, which functions as a local regulator of chemokine levels by internalizing and thereby removing chemokines. In global and endothelium-specific GPR182-deficient mice, release of hematopoietic stem cells (HSCs) is increased, which suggests a role of GPR182 in establishing CXCL12 gradients in the bone marrow, required for regulating HSC homeostasis. Ongoing projects also study the role of GPR182 in postischemic neovascularization (see above).

Current efforts to identify new ligand receptor pairs are focused on various lipid receptors with an emphasis on specialized pro-resolving lipid mediators (SPMs), such as resolvins (Schebb et al., 2022).

Based on the observation that several of the proposed SPM receptors, including GPR18, GPR32 and FPR2, could not be confirmed, we screened our GPCR library and and found that PGE_2 receptor subtype EP4 is activated at relatively high concentrations by several resolvins and protectins in an orthosteric manner. In addition, these SPMs can modulate EP4 in an allosteric manner with much higher potency. inflammatory effects, we explored the potential role of EP4 in mediating anti-inflammatory and resolving effects of SPMs *in vitro* and *in vivo* and found that the effect of various SPMs are indeed mediated by EP4. We are currently exploring the precise mechanisms underlying the allosteric regulation of EP4 by SPMs as well as the *in vivo* relevance of this interaction.

5. Plexins

Work in this research area has focused on plexins of the B family. Based on previous studies, which showed that Plexin-B1 plays a role in osteoporosis as well as in multiple sclerosis, we have collaborated with LifeArc (London, UK) to develop a humanized antibody targeting Plexin-B1, which can be developed into a potential new treatment for osteoporosis and multiple sclerosis (Vogler et al., 2022).

Currently ongoing projects focus on the role of Plexin-B1 and Plexin-B2 in the regulation of pancreatic β-cells and in hepatic homeostasis. In mice with pancreatic β-cell-specific Plexin-B1/-B2 deficiency, we found increased glucose-stimulated insulin secretion in chow-fed mice but not in obese type 2-diabetic animals, suggesting that Plexin-B2/-B2 under normal conditions exert an inhibitory role on glucose-stimulated insulin secretion, which is inactive under type 2-diabetic conditions. Mice lacking Plexin-B1/-B2 in cholangiocytes show a strong increase in the number cholangiocytes, a condition called “ductular reaction”. This indicates that B family plexins play an important role in the regulation of cholangiocyte differentiation and homeostasis. We are in the process of identifying the underlying mechanisms.

WETTSCHURECK GROUP

Our research focusses on mechanisms regulating vascular function under basal conditions and after ischemic, inflammatory or mechanical damage. A central goal is to identify novel signaling pathways controlling vascular parameters such as smooth muscle cell (SMC) contractility, SMC differentiation, endothelial cell (EC) inflammation, or immune cell activation. This part of our work focuses on G protein-coupled receptors (GPCRs) without known ligand and function, so-called orphan GPCRs. Other aspects of our work deal with angiocrine factors upregulated during cardiac remodeling or the transcriptional and epigenetic regulation of the vascular GPCRome. In detail, we pursue the following projects:

1. Role of orphan GPCRs in SMC biology (J. Shao / T. Wang)

Single-cell expression analyses showed that the GPCR repertoire of vascular SMCs is highly plastic and undergoes characteristic changes during dedifferentiation (Kaur et al., 2017). We here focus on three receptors with yet unknown function in SMC biology, the orphan receptors GPRC5B, GPRC5C, and GPR153. We found that GPRC5B dimerizes with PGI_2 receptor IP, thereby modulating intracellular IP trafficking. In GPRC5B-deficient SMCs, membrane availability of IP is increased (Fig. 1A, B), resulting in increased IP-mediated relaxation in vessels from tamoxifen-inducible, SMC-specific *Gprc5b*-KO mice (iSM-*G5b*-KO) (Fig. 1C). In vivo, iSM-*G5b*-KOs are partially protected from angiotensin II (Ang II)-induced hypertension (Fig. 1D) and atherosclerosis development (Carvalho et al., 2020). Also the orphan receptors GPRC5C and GPR153 seem to regulate SMC contractility and differentiation *in vitro* and *in vivo*, and we are currently investigating the underlying molecular mechanisms.

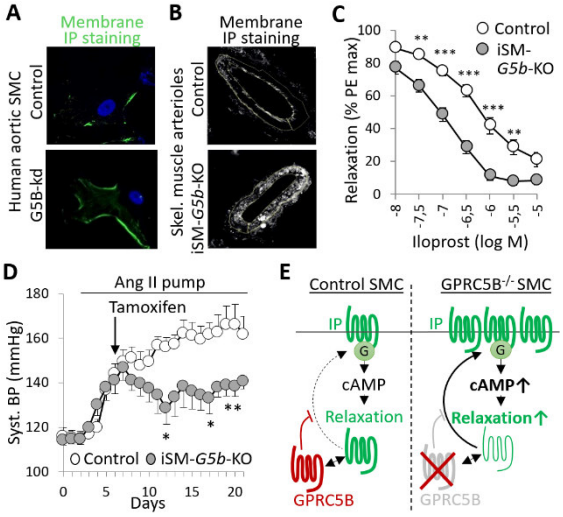


Fig. 1. ***A, B,*** *IP membrane staining in GPRC5B-deficient SMC.* ***C,*** *Vessel relaxation in response to PGI_2 analogue Iloprost.* ***D,*** *Blood pressure in Ang II-induced hypertension.* ***E,*** *Schematic overview of findings.*

2. Modulation of immune cell function by local (lipid) mediators (M. Gurusamy, J. Kwon)

We investigate in this project the role of orphan receptors P2Y10 and GPRC5B in the activation, differentiation, and migration of immune cells. P2Y10 is strongly upregulated in CD4 T cells during neuroinflammation, and we found that chemokine-induced migration and RhoA activation is reduced in P2Y10-deficient CD4 T cells (Fig. 2A, B). Mechanistically, we showed that putative P2Y10 ligands ATP and lysophosphatidyl serine (LysoPS) are released upon chemokine stimulation and facilitate RhoA activation and migration in a P2Y10-dependent manner. *In vivo*, loss of P2Y10-dependent facilitation of migration protects CD4-specific P2Y10-KOs in experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (Fig. 2C) (Gurusamy et al., 2021). The relevance of this positive feedback in other immune cell types and other inflammatory processes is currently under investigation. In a second project we are investigating the role of orphan receptor GPRC5B in myeloid cells, with a special focus on the question whether altered prostanoid signaling underlies increased phagocytosis and inflammatory activation in GPRC5B-deficient macrophages.

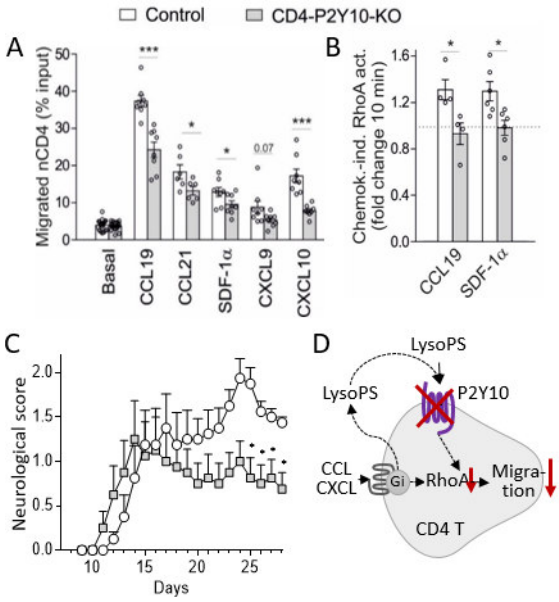


Fig. 2. ***A, B,*** *Chemokine-induced migration and RhoA activation in CD4 T cells harvested from control and CD4-P2Y10-KO mice.* ***C,*** *EAE development in control mice and CD4-P2Y10-KOs.* ***D,*** *Schematic summary of results.*

3. Function of orphan GPCRs with stress-induced expression in EC (J. Shao)

Inflammation and other pathological influences result in a characteristic change of the GPCR repertoire in vascular cell types (Kaur et al., 2017; Tischner et al., 2017). We here investigate selected orphan receptors that are upregulated in inflamed EC with a special focus on their role in vascular permeability control in the context of autoimmune or ischemic neuroinflammation.

4. Regulation of the vascular receptor repertoire under stress conditions (H. Taskiran)

The GPCR repertoire of individual cells is very plastic, but the mechanisms regulating the vascular GPCRome under physiological and pathological conditions are largely unclear. In this project, we investigate how the repertoire changes in different organ systems in response to ischemic, inflammatory or mechanical damage and how these changes are controlled. Using ATACseq and ChIP-seq, potential regulators will be identified and their functional relevance as well as pharmacological modulation will be investigated *in vitro* and *in vivo.*

5. Regulation of cardiac stress responses by EC (N. Shiva)

Single-cell expression analyses in murine models of cardiac damage showed that EC cells upregulate numerous secreted proteins with yet unknown function in cardiac hypertrophy and fibrosis. Among these putative angiocrine factors are a number of neuropeptides that have not yet been studied in the context of cardiovascular remodeling, and we are currently investigating their impact on fibroblasts, cardiomyocytes or immune cells *in vitro*. To adress the role of EC-derived neuropeptides in vivo, we have generated EC-specific knockout mice to study their relevance in damage-induced cardiac remodeling.

Key Publications (2016-2022)

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Reviews and Book Chapters

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Freissmuth M, Offermanns S, Böhm S. (2020) Pharmakologie & Toxikologie: Von den molekularen Grundlagen zur Pharmakotherapie (Springer-lehrbuch). Springer Medizin, Verlag Heidelberg. (3rd Edition)

External Funding (2019-2022)

2015–2021: NovoNordisk Foundation (Challenge Grant): “Novel Receptor Targets in the prevention and treatment of diabetes and obesity” (Schwartz / Offermanns / etc.)

2015–2020: LOEWE-Schwerpunkt „Translationale Medizin und Pharmakologie“ (LOEWE-TMP) –Teilprojekt: „Anti-Plexin-B1-Antikorper fur die Behandlung der Multiplen Skerlose“ (Offermanns/ Worzfeld)

2016–2020: SFB/CRC Transregio 128/2 “Multiple Sclerosis”: TP A3: “G-protein-coupled receptor signaling in immune cells and endothelial cells: implications for neuroinflammation” (Wettschureck / Waisman)

2018–2022: DFG SFB 834/3: Endothelial Signalling and Vascular Repair: TP A01: “Regulation of endothelial functions via G-protein-mediated signalling pathways” (Offermanns)

2018–2022: DFG SFB 834/3: Endothelial Signalling and Vascular Repair: TP A12: “G-Protein-coupled receptors in endothelial cells: from single-cell expression to new functions” (Wettschureck)

2019–2020: Deutsches Zentrum für Herz-Kreislauf-Forschung: DZHK – Shared Expertise “Microvascular shear stress-mediated NF-κB activation and its role in activity-induced hyperemia” (de Wit / Schwaninger / Wenzel / Offermanns)

2019–2022: Cardiopulmonary Institute (CPI): CPI-Flex Funds Project 3 – “mORF-encoded micropeptides in vascular physiology/pathophysiology” (Siragusa / Fleming /Offermanns)

2019–2022: Cardiopulmonary Institute (CPI): CPI-Flex Funds Project 5 – “Deciphering the ABC (airway brush cell) “(Kummer/Offermanns)

2020–2023: LOEWE-Verbundsprojekt „GPCR Ligands for Underexplores Epitopes“ (LOEWE-GLUE) – Teilprojekt B6: “*In vivo*-Funktionen und allosterische Regulation von FFA-Rezeptoren” (Offermanns)

2020–2023: LOEWE-Verbundsprojekt „GPCR Ligands for Underexplores Epitopes“ (LOEWE-GLUE) – Teilprojekt B7: “Analyse der biologischen Funktion und pharmakologischen Modulierbarkeit von Orphan-GPCRs" (Wettschureck)

2021–2024: DFG WE 2891/2-1: „Modulation der Neuroinflammatiion durch neue endotheliale GPCRs“ (Wettschureck)

2021–2025: DFG – SFB1039/3 „Lipid Signaling“: „Neue G-Protein-gekoppelte Rezeptoren fur kurzkettige Fettsauren und andere Lipide“ (A04) (Offermanns)

2021–2025: DFG – SFB1039/3 „Lipid Signaling“: „Neue G-Protein-gekoppelte Rezeptoren fur kurzkettige Fettsauren und andere Lipide“ (A04) (Wettschureck)

2021–2025: HMWK: „Aufbau eines Leistungszentrums für innovative Therapeutika (TheraNova) in Kooperation mit der Fraunhofer Gesellschaft – Verbundprojekt (Offermanns)

2022–2025: DFG SFB 1526 „Pathomechanismen Antikörpervermittelter Autoimmunerkrankungen (PANTAU): Erkenntnisse durch Pemphigoiderkrankungen“ – Teilprojekt B2: „G-Protein-gekoppelte Rezeptoren als Regulatoren der Granulozyten-Aktivität in Pemphigoid-Erkrankungen“ (Sadik / David / Wettschureck)

2022–2026: DFG SFB 1531 „Schadenskontrolle durch das Stroma-vaskuläre Kompartiment“ – Teilprojekt A06: „Schutz vor kardiovaskulären Schäden durch APP/APLP2“ (Offermanns)

2022–2026: DFG SFB 1531 „Schadenskontrolle durch das Stroma-vaskuläre Kompartiment“ – Teilprojekt A04: „Schadensbedingte Anpassung der Signalübertragung von G-Protein-gekoppelten Rezeptoren“ (Wettschureck)

2023–2024: CPI Translational Project “Therapeutic inhibition of GPR182 using nanobodies to improve recovery from myocardial infarction” (Remy Bonnavion)

Patents (2019-2022)

2022: Blockade von GPR182 zur Behandlung des akuten Myokardinfarktes: R. Bonnavion, H. Kawase, S. Offermanns, S. Ramas, MPG. (Patent filed under # 22 182 000.4)

2020: Dual Inhibition of Plexin-B1 and Plexin-B2: S. Offermanns, T. Worzfeld, MPG, Philipps Universität Marburg (WO2020165094A1)

III. Developmental Genetics

Didier Stainier (director)

Prof. Dr. Didier Stainier

University Education, Scientific Career and Awards

1981	I.B., UWC Atlantic College, Wales, UK
1982	Université de Liège, Belgium
1984	B.A. (Biology), Brandeis University, Waltham, USA
1990	Ph.D. (Biochemistry and Molecular Biology), Harvard University, Cambridge, USA
1990 - 1994	Postdoctoral Fellow, Massachusetts General Hospital, Harvard Medical School, Boston, USA
1995 - 2000	Assistant Professor
2000 - 2003	Associate Professor
2003 - 2013	Professor, Department of Biochemistry and Biophysics, University of California, San Francisco, USA
since 2012	Director, Department of Developmental Genetics, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany
since 2015	Professor, Department of Biological Sciences, Goethe University, Frankfurt
1982 - 1984	Wien International Scholar, Brandeis University
1983	Elihu A. Silver Prize for Undergraduate Research in Science, Brandeis University
1984	Dr. Joseph Garrison Parker Prize in Biology, Brandeis University
1984	*Phi Beta Kappa*, Brandeis University
1991 - 1994	Helen Hay Whitney Postdoctoral Fellow
1995 - 2000	Packard Foundation Fellowship in Science and Engineering
1996 - 1998	Basil O'Connor Scholarship, March of Dimes Foundation
2000 - 2003	Established Investigator Award, American Heart Association
2002	Mossman Award in Developmental Biology, American Association of Anatomists
2003	Outstanding Faculty Mentorship Award, UCSF
2003 - 2004	Annual Byers Award in Basic Science, UCSF
2003 - 2006	NIH DEV1 Study Section Chair
2008	Fellow American Association for the Advancement of Science
2008	J.W. Jenkinson Memorial Lectureship, Oxford University
2011	NIH Director's Wednesday Afternoon Lectureship, NIH
2013	Officier de l'ordre de Léopold de Belgique
2014	Ernst Caspari Lecture, University of Göttingen, Germany
2015	22nd Severo Ochoa Memorial Lecture, CBMSO, Madrid, Spain
2016	Member of Academia Europaea
2016	Member of EMBO (European Molecular Biology Organization)
2017	Inaugural recipient of the Christiane Nüsslein-Volhard Award, European Zebrafish Society
2019	President, International Zebrafish Society

III – Developmental Genetics

Lab Members – since 2019
(Current / Past / Running their own lab*)

PostDocs:

Dr. Mridula Balakrishnan
Dr. Arica Beisaw*
Dr. Maëlle Bellec
Dr. Anabela Bensimon Brito*
Dr. Samuel Capon
Dr. João Cardeira da Silva
Dr. Cansu Çirzi
Dr. Michelle Collins*
Dr. Christopher Dooley
Dr. Ryuichi Fukuda*
Dr. Sébastien Gauvrit
Dr. Felix Gunawan*
Dr. Hamzeh Haj Hammadeh
Dr. Zhen Jiang
Dr. Thomas Juan
Dr. Aosa Kamezaki
Dr. Hyun-Taek Kim*
Dr. Zacharias Kontarakis*
Dr. Rubén Marín Juez*
Dr. Tonatiuh Molina Villa
Dr. Paolo Panza
Dr. Rashmi Priya*
Dr. Sven Reischauer*
Dr. Pooja Sagvekar
Dr. Vahan Serobyan
Dr. Yuntao (Charlie) Song
Dr. Marco Tarasco
Dr. Guilherme Targino Valente
Dr. Jordan Welker
Dr. Chi-Chung Wu*
Dr. Lihan Xie

Students:

Marga Albu
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Overview

The Department of Developmental Genetics investigates questions related to organogenesis including cell differentiation, tissue morphogenesis, organ homeostasis and function, as well as organ regeneration. We study these questions in zebrafish as well as in mouse and are currently mostly focusing on the cardiovascular system, with some work in the lung. We utilize cell biological and genetic approaches to dissect cellular processes using high-resolution live imaging. One goal of our studies is to gain understanding of vertebrate organ development at the single-cell level, and beyond. In the past several years, we have also become interested in issues pertaining to transcriptional adaptation, a cellular response to mutant mRNA degradation that can in some cases contribute to genetic robustness, or in other cases make the mutant phenotype more severe.

History

My laboratory (initially at UCSF in the Department of Biochemistry and Biophysics and since 2012 at the Max Planck Institute for Heart and Lung Research) has been investigating organ development and function using both forward and reverse genetic approaches in zebrafish and more recently in mouse. These studies have focused on the cardiovascular system as well as on several endodermal organs including the pancreas, liver and gut, and more recently the mouse lung. A critical aspect of these studies involved the development and use of imaging techniques, including light sheet microscopy, in order to achieve single cell understanding of developmental processes, and thereby take full advantage of the attributes of the zebrafish system. More recently, through studies initially designed to investigate the phenotypic discrepancy between mutants and antisense treated zebrafish, we uncovered a phenomenon we termed transcriptional adaptation, which in some cases can lead to genetic compensation.

Structure of the report

1) Highlights of studies published since 2019
   a) Transcriptional adaptation
   b) Cardiac development and regeneration
   c) Vascular development
   d) Lung and pancreas development
2) Highlights of ongoing studies
   a) Transcriptional adaptation
   b) Cardiac development and regeneration
   c) Vascular development

1) Highlights of studies published since 2019

a) Transcriptional adaptation

Transcriptional adaptation is defined as the 'modulation of the transcriptome of a cell due to a mutation in a gene, independent of the mutation's effect on the encoded protein'.

During the course of our work designed to investigate the discrepancy between mutation-induced and morpholino-induced phenotypes, we studied the endothelial specific gene *egfl7*. We selected this gene in part because of the conflicting reports between the severe antisense-induced phenotypes (in zebrafish and *Xenopus* embryos as well as in human endothelial cells in culture) and the lack of phenotype in mouse and zebrafish mutants. In order to

understand how the antisense approach led to a much stronger vascular phenotype than that observed in the mutants, we carried out proteomic and transcriptomic profiling of informative samples. This analysis led to the identification of several genes (and proteins) that were upregulated in mutant but not antisense-treated embryos. These genes were shown to be able to compensate, at least partially, for the loss of *egfl7* function. Similar observations were made for the *vegfaa* gene; *vegfab* was upregulated in *vegfaa* mutants but not morphants (morpholino-injected embryos). These and other data were published in Rossi, Kontarakis et al. (*Nature*, 2015), and have attracted a lot of attention (1179 citations to date (Google Scholar)). Importantly, in these initial studies we also showed that the upregulation of *vegfab* in *vegfaa* mutants was not due to the loss of Vegfaa function.

These data led us to introduce the concept of transcriptional adaptation (TA) whereby mutations in a specific gene cause the transcriptional upregulation of other genes (which we term 'adapting genes'). Thus, in the context of this phenomenon, gene upregulation is not due to protein feedback loops. We covered this and related topics in a review entitled 'Genetic compensation: a phenomenon in search of mechanisms' (El-Brolosy and Stainier, *PLoS Genetics*, 2017), which has also attracted much attention (679 citations to date (Google Scholar)). As indicated in the title of this review, while genetic compensation has been observed very frequently, the underlying mechanisms are unclear. This is especially the case for TA, the phenomenon we discovered. Therefore, we went on to investigate potential underlying mechanisms, initially focusing on identifying the trigger for TA, and tested three possibilities: the mutant mRNA, the DNA lesion, and the lack of protein function. During this work, which was carried out using multiple models of TA in zebrafish embryos and mouse cells in culture, we observed a tight correlation between mutant mRNA degradation and TA, leading us to hypothesize that mutant mRNA degradation was the trigger for TA. To test this hypothesis, we generated RNA-less alleles and observed that these alleles do not exhibit TA. We also blocked mutant mRNA degradation, both genetically and pharmacologically, and under such conditions also observed a lack of TA. Additional data led to the model that sequence similarity with the mutant mRNA determines which genes get upregulated during TA (Fig. 1).

We anticipate that this phenomenon of upregulation of 'related' genes following mutant mRNA degradation is widespread and could account for several observations including haplosufficiency (whereby the wild-type allele would be upregulated following degradation of the mRNA encoded by the mutant allele), as well as haploinsufficiency (whereby a 'detrimental' gene would be upregulated). In terms of relevance to human diseases, there are numerous reports indicating that lesions leading to mutant mRNA decay cause less severe phenotypes than lesions not affecting mutant mRNA stability. These and other data and discussion points were published in El-Brolosy et al. (*Nature*, 2019; 645 citations to date (Google Scholar)).

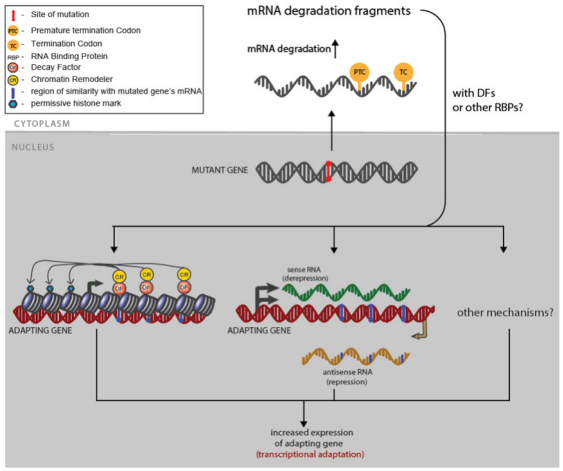

Fig. 1. *Current model of transcriptional adaptation (TA) to mutations. mRNA degradation fragments may act as intermediates to bring decay factors and chromatin remodellers to adapting gene loci, thereby triggering increased gene expression. Alternatively, mRNA degradation fragments may function by repressing antisense RNAs at the adapting gene loci, thereby allowing increased sense mRNA expression. Additional mechanisms are likely involved in TA, and possibly in a gene- and/or mutation-dependent manner.*

Since TA had only been investigated in vertebrates thus far, we wanted to determine whether this phenomenon was common across multicellular organisms. To this end, we turned to *Caenorhabditis elegans*, a very facile genetic system that also offers rapid knock down approaches. We initially focused on actin genes, some of which we had investigated in mouse cells as well (El-Brolosy et al., 2019). The *C. elegans* genome contains five actin genes and we examined several mutant alleles for four of them. We observed *act-3* upregulation in an *act-5* allele that exhibits mutant mRNA degradation and provided experimental evidence that this upregulation was due to TA. Notably, using an *act-3* reporter transgene, we observed in *act-5* mutants its ectopic expression in the intestine, a tissue that in wild-type worms expresses *act-5* but not *act-3*. This observation confirms our data in zebrafish and mouse cells that TA is a cell-autonomous phenomenon. In addition, through a targeted RNAi screen followed by genetic analysis, we found a role for small RNA biogenesis in this process (Serobyan et al., *eLife*, 2020).

In order to identify regulators of TA, we decided to carry out a forward genetic screen in *C. elegans* using a visual assay based on the intestinal expression of the *act-3* reporter in *act-5* mutants. However, in a pilot screen, we observed that 100% of the wild-type offspring from *act-5* heterozygous nematodes displayed intestinal expression of the *act-3* reporter. To further analyze the inheritance mode of this phenotype, we sequentially self-fertilized wild-type hermaphrodite offspring from *act-5* heterozygous nematodes and observed the intestinal expression of the *act-3* reporter up to six generations. These data indicated the intergenerational and transgenerational inheritance of TA, which we further confirmed in *C. elegans* and also observed in zebrafish. These, and other data, which reveal a means by which parental mutations can modulate the offspring's transcriptome, were recently published in Jiang, El-Brolosy, Serobyan et al. (*Science advances*, 2022).

b) Cardiac development and regeneration

Our work on the vertebrate heart aimed to 1) understand key aspects of cardiac development at single cell resolution, and 2) delve into relatively unexplored areas of cardiac regeneration.

- **Cardiac development**

Cardiac trabeculation is the process by which the heart increases its muscle mass before coronary vessels become functional; it involves the transformation of the cardiomyocyte monolayer to a multilayered structure. However, this formation of multiple layers is restricted in space as cardiomyocytes cannot be located more than 2-3 cell diameters away from the blood supply for oxygenation and nutrition. Thus, these trabeculae appear as finger-like projections in a 2-dimensional view, although they end up forming complex tubular structures when visualized in 3 dimensions (Fig. 2).

Cardiac trabeculation is a fascinating process at several levels: 1) it involves complex cell biological mechanisms whereby a subset of cardiomyocytes leaves the compact layer to form the trabecular layer; 2) it is subject to some interesting patterning within the heart as trabeculae form mainly in the ventricle but not in the atrium, and 3) it is regulated by genetic as well as 'epigenetic' factors, specifically flow/contractility. Indeed, in the absence of flow/contractility, trabeculation fails to occur, and in addition, trabeculae mainly form in the outer curvature of the ventricle but not in its inner curvature; interestingly, different types of flow are observed in these different parts of the heart, with laminar flow in the inner curvature and turbulent/perturbed flow in the outer curvature.

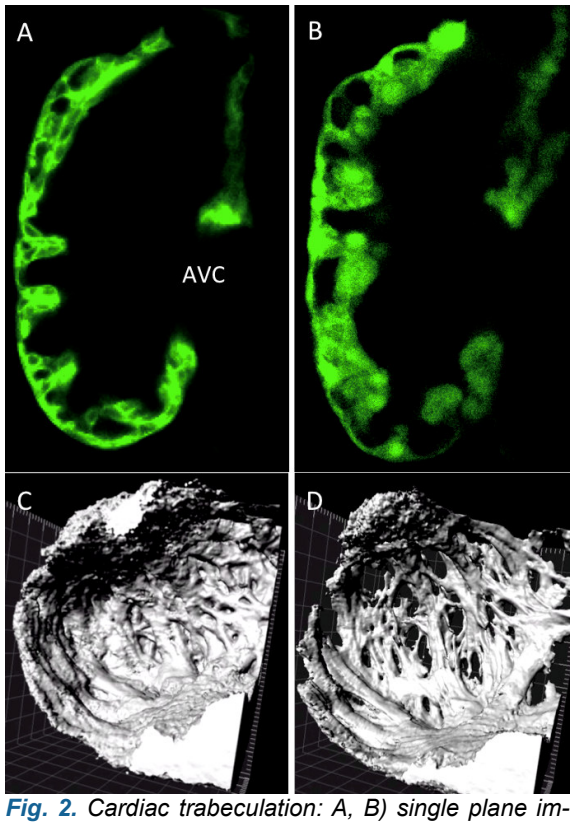

Fig. 2. *Cardiac trabeculation: A, B) single plane images of cardiac trabeculation in the ventricle of 5 days post-fertilization zebrafish larvae; AVC, atrioventricular canal. C, D) single frames from 4-D imaging of cardiac trabeculae as viewed from the cardiac lumen during ventricular contraction (C) and relaxation (D).*

Based on data obtained from proliferation, lineage tracing and transplantation studies, we proposed that cardiac trabeculation is initiated by directional cardiomyocyte migration rather than oriented cell division, and that Erbb2 cell-autonomously regulates this process (Liu et al., *Development*, 2010). In order to further understand cell biological mechanisms underlying the initial events of trabeculation, we developed methods to follow single cardiomyocytes in the contracting heart as they leave the compact layer and enter the trabecular layer. Focusing on the outer curvature of the ventricle, where trabeculation initiates, we observed that cardiomyocytes are surprisingly active as about half of them send out basal processes that extend on neighboring cardiomyocytes. As the basal processes become more extensive, the apical surface of these cardiomyocytes also progressively shrinks and ultimately the cells leave the compact layer and enter the trabecular layer.

Amongst the many questions that remain to be answered, we have been interested in the following aspects of trabeculation: 1) How are the cardiomyocytes 'selected' to become trabecular? 2) What signal(s) trigger the distinct behavior of the selected cardiomyocytes (extension of basal processes and shrinking of the apical membrane)? 3) What is the

role of the cardiac jelly (the extracellular matrix between the myocardial and endocardial layers) in this process? 4) How does blood flow and/or cardiomyocyte contractility affect this process? 5) Is the different level of mechanical stretching of myocardial/endocardial cells in the outer curvature vs the inner curvature involved in the differences in the level of trabeculation observed, or is it due to the different types of flow (turbulent vs laminar)? 6) What explains the lack of trabeculation in the atrial chamber?

We have been taking various approaches to address these questions including reverse genetics, the generation of transgenic lines to visualize specific intracellular structures in cardiomyocytes, and the optimization of imaging techniques.

For example, we recently addressed the question of how trabecular cardiomyocytes are selected. We tested the role of differential adhesion and differential contractility in this process and found that local tension heterogeneity drives organ-scale patterning and cell fate decisions during cardiac trabeculation in zebrafish. Proliferation-induced cellular crowding at the tissue scale triggers tension heterogeneity among cardiomyocytes of the compact layer and drives those with higher contractility to delaminate and seed the trabecular layer. Experimentally, increasing crowding within the compact layer cardiomyocytes augments delamination, whereas decreasing it abrogates delamination. Using genetic mosaics in trabeculation-deficient zebrafish models - that is, in the absence of critical upstream signals such as Nrg-Erbb2 or blood flow - we find that inducing actomyosin contractility rescues cardiomyocyte delamination and is sufficient to drive cardiomyocyte fate specification, as assessed by Notch reporter expression in compact layer cardiomyocytes. Furthermore, Notch signaling perturbs the actomyosin machinery in cardiomyocytes to restrict excessive delamination, thereby preserving the architecture of the myocardial wall. Thus, tissue-scale forces converge on local cellular mechanics to generate complex forms and modulate cell fate choices, and these multiscale regulatory interactions ensure robust self-organized organ patterning. These and other data were published by Priya et al. (*Nature*, 2020).

Cardiac valve formation has become an area of increased investigation in the lab because of the importance of this process as well as the clear advantages offered by the zebrafish model to study it. Valves, including those in the heart, prevent retrograde flow and the development of these complex structures is poorly understood, mostly because of the inability to watch them develop in real time. We are focusing most of these studies on the development of the atrioventricular (AV) valve and aim to investigate this process in real time and at single-cell resolution. Cardiac valves are formed by endocardial cells (EdCs) located at the AV canal, and we observed an initial event of collective migration of AV EdCs into the extracellular matrix (ECM), and their subsequent rearrangements to form the leaflets. We started by analyzing integrin-based focal adhesions (FAs), critical mediators of cell-ECM interactions, during valve morphogenesis. Using transgenes to block FA signaling specifically in AV EdCs as well as loss-of-function approaches, we found that FA signaling mediated by Integrin α5β1 and Talin1 promotes AV EdC migration and overall shaping of the valve leaflets. These and other data were published by Gunawan et al. (*J. Cell Biol.,* 2019). We have extended these studies by analyzing the formation of the valve interstitial cells (VICs), specialized ECM secreting cells that confer biomechanical strength to the cardiac valve, and especially the role of the transcription factor gene *nfatc1* in this process, and the resulting data were published by Gunawan et al. (*Circ. Res.*, 2020).

Additional cardiac development projects have investigated the role of several transcription factors including the epithelial-mesenchymal transition regulator Snail1 (Gentile et al., *eLife*, 2021) and the Paired-like homeodomain transcription factor Pitx2 (Collins et al., *PNAS*, 2019), the role of Crumbs2 in cardiomyocyte apicobasal polarity and adhesion (Jimenez-Amilburu and Stainier, *Development*, 2019), the role of mechanical forces in cardiomyocyte myofilament maturation (Fukuda et al., *Dev. Cell*, 2019), the role of metabolic modulation in cardiac wall morphogenesis (Fukuda et al., *eLife*, 2019), the role of Apelin signaling in the formation of endocardial protrusions during cardiac trabeculation (Qi et al., *eLife*, 2022), the role of the developing epicardium in cardiomyocyte growth (Boezio et al., *Dis. Model Mech.*, 2022), the role of endothelial TGF-beta signaling during outflow tract formation (Boezio et al., *eLife*, 2020), and the role of the ECM in mammalian cardiomyocyte cytokinesis (Wu et al., *Circ. Res.*, 2020).

- Cardiac regeneration

While adult mammalian hearts exhibit limited capacity to regenerate, the zebrafish heart is a very potent regenerator, and thus a very popular model to look at cellular and molecular mechanisms of regeneration (Fig. 3).

We are investigating several specific questions about the process of heart regeneration in zebrafish including revascularization after injury, the role of neutrophils and macrophages (and inflammation in general), the origin of the new cardiomyocytes and cardiomyocyte dedifferentiation, as well as the role of specific signaling pathways and transcriptional effectors.

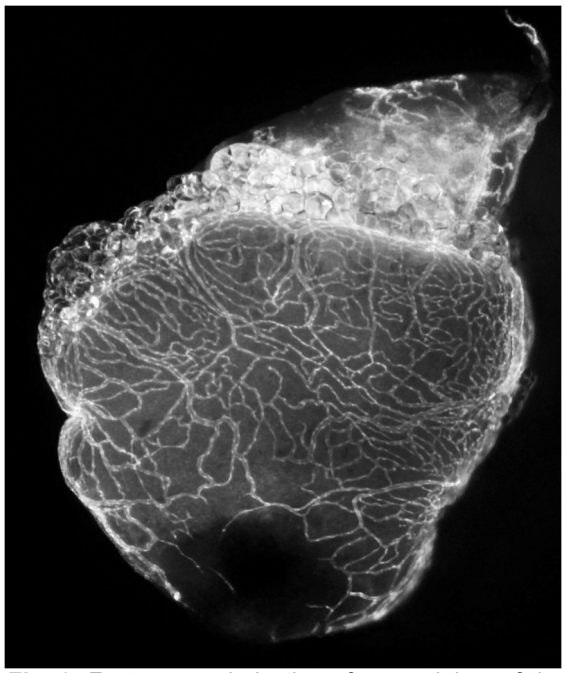

Fig. 3. *Fast revascularization after cryoinjury of the zebrafish heart. TgBAC(etv2:EGFP) ventricle at 18 hours post-cryoinjury. Coronary vessels have started sprouting into the injured area (darker region).*

For example, in order to identify pathways triggered specifically by injury, we compared the transcriptome of adult hearts after exercise and after injury. This and other analyses led us to a number of interesting genes/proteins including Interleukin-11. We generated gain- and loss-of-function lines for this ligand and its receptor and found that the Interleukin-11 signaling pathway promotes cellular reprogramming and limits fibrotic scarring during tissue regeneration. A manuscript reporting these and other data was published by Allanki et al. (*Science Advances*, 2021).

Despite the extensive work on zebrafish heart regeneration, we know little about how revascularization of the damaged area happens. We investigated this question using a number of transgenic lines and mutants and extended our initial report on the role of Vegfa signaling in this process (Marín-Juez et al., *PNAS*, 2016) by identifying additional regulators and underlying mechanisms (El-Sammak et al., *Circ. Res.* 2021). We further investigated coronary revascularization and found that it requires a coordinated multi-tissue response culminating with the formation of a complex vascular network available as a scaffold for cardiomyocyte repopulation. During a process we term “coronary-endocardial anchoring”, new coronaries respond by sprouting 1) superficially within the regenerating epicardium, and 2) intra-ventricularly towards the activated endocardium, in other words, new coronaries regenerate superficially, to engulf the injured area, as well as towards the cardiac lumen. By loss-and gain-of-function manipulations, we found that epicardial Cxcl12/Cxcr4 and endocardial Vegfa signaling regulate these two modes of coronary revascularization. We further found that coronaries form a vascular scaffold available for cardiomyocytes during regeneration as well as during development, and that perturbation of coronary revascularization affects cardiomyocyte replenishment. Our findings reveal how by orchestrating a multi-tissue response, the regenerative zebrafish heart grows a vascular scaffold that aids cardiomyocyte repopulation of the injured area. A manuscript reporting these and other data was published by Marín-Juez et al. (*Dev. Cell*, 2019).

Additional cardiac regeneration projects have investigated the role of glycolysis in cardiomyocyte proliferation (Fukuda et al., *EMBO Rep.*, 2020), the role of chromatin remodeling in promoting sarcomere disassembly and cardiomyocyte protrusion (Beisaw et al., *Circ. Res.*, 2020), as well as cardiac valve regeneration (Bensimon-Brito et al., *Dev. Cell,* 2020),

c) Vascular development

- Endothelial cell differentiation

In 1992, I identified a mutant that lacks most endothelial and most blood cells. I named this mutant *cloche* because of its bell-shaped heart (cloche means bell in French). This avascular and bloodless mutant has been used extensively as it provides a unique platform to investigate various developmental processes in the absence of a vascular system and/or blood cells. After isolating the *cloche/npas4l* gene in 2016 (Reischauer et al., *Nature*, 2016), we turned our attention to identifying its direct transcriptional targets (e.g., *etv2*, *tal1* and *lmo2*) (Marass et al., *Development,* 2019), and also asked the question of what happens to *npas4l*-expressing cells in the absence of *npas4l* function. Unexpectedly, we found that in *npas4l* mutants, *npas4l* reporter-expressing cells contribute to the pronephron tubules. Single-cell transcriptomics and live imaging of the early lateral plate mesoderm in wild-type embryos indeed reveals coexpression of endothelial and pronephron markers, a finding confirmed by creERT2-based lineage tracing. Increased contribution of *npas4l* reporter-expressing cells to pronephron tubules is also observed in *tal1* and *lmo2* mutants and is reversed in *npas4l* mutants injected with *tal1* mRNA. Together, these data reveal that Npas4l/Tal1/Lmo2 regulate the fate decision between the endothelial and pronephron lineages. These and other data were published by Mattonet et al. (*Science Advances*, 2022).

- Vascular development in mouse

Endothelial cells (ECs), which line blood and lymphatic vessels, are generally described to come from the lateral plate mesoderm despite experimental evidence for a broader source of origin, including the paraxial mesoderm (PXM). Current dogma suggests that following specification from mesoderm, local environmental cues establish the distinct molecular and functional characteristics of ECs in different vascular beds. We have generated experimental evidence to challenge this view, showing that lymphatic EC fate is imprinted during transition through the PXM lineage. We have shown that PXM-derived cells form the lymphatic endothelium of multiple organs and tissues, with a more restricted contribution to blood vessel endothelium. By deleting the lymphangiogenesis regulator gene *Prox1* specifically in PXM-derived cells, we have also shown that this lineage is indispensable for lymphatic vessel development. Collectively, our data, published by Stone et al. (*Dev. Cell*, 2019) establish lineage history as a critical determinant of EC specialization, a finding with broad implications for our understanding of vascular development and heterogeneity.

Additional vascular development projects have investigated the role of apelin signaling during angiogenesis (Helker et al., *eLife*, 2020).

d) Lung and pancreas development

Our work on lung development started with a forward genetic screen (in mouse) and we have been completing studies on some of the more interesting mutants that came out of the screen. For example, we have analyzed smooth muscle ECM and cytoskeletal organization during tracheal tube formation and specifically the role of the potassium channel KCNJ13 in these processes (Yin et al., *Eur. Respir. J.*, 2019), as well as the role of the WNT co-receptor RYK in goblet cell differentiation during lung development (Kim et al., *PNAS*, 2019) and repair (Kim et al., *PNAS*, 2022). We also started working with lung organoids, focusing on the differentiation of alveolar progenitors (Gkatzis, Panza et al., *eLife*, 2021).

Our work on pancreas development has focused on the role of the pancreatic vasculature in islet architecture and function (Mullapudi et al., *Development*, 2019), the role of the transcription factor Hhex in liver and pancreas development (Villasenor, Gauvrit et al., *Developmental Biology*, 2020), as well as the role of autonomic innervation on endocrine pancreas function (Yang et al., *eLife*, 2022).

2) Highlights of ongoing studies

a) Transcriptional adaptation

Current studies on transcriptional adaptation are focused on further investigating the underlying molecular mechanisms using multiple approaches in *C. elegans*, zebrafish, and mouse as well as in mammalian cell lines. The main questions we are trying to address include the identity of the adapting genes (i.e., how they are selected), and the mechanisms by which their expression becomes modulated. We have also recently started working in *Neurospora crassa*, another powerful genetic system, and are first testing whether transcriptional adaptation is present in that system.

b) Cardiac development and regeneration

Current studies of cardiac development are mostly focused on three processes: heart tube formation, cardiac trabeculation, and valve development, delving deeper into cellular and molecular mechanisms, and making full use of the live imaging allowed by the zebrafish system as well as the advances in genome engineering and transcriptomic analysis. We have also started investigating how the atrial wall develops, how the cardiac conduction system including the sinoatrial node are formed and how they function, and are also digging deeper into the role of the epicardium in cardiac development.

Current studies of cardiac regeneration are focused on several broad questions including coronary revascularization, the identification and functional analysis of endothelial-derived factors that modulate cardiomyocyte dedifferentiation and/or proliferation, the role of the innate and adaptive immune systems, as well as the role of cardiac innervation.

c) Vascular development

Current studies in vascular development are focused on the role of endothelial cells in alveolar development in the mouse lung; we are also investigating endothelial cell regeneration in zebrafish.

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External Funding (2019-2022)

International

2016 – 2020: Fondation Leducq Transatlantic Networks of Excellence, Grant No. 15 CVD 03. *Eliciting heart regeneration through cardiomyocyte division.* Joint application led by Ken Poss, Duke University, Durham, USA.

2016 – 2021: EU / ERC Advanced Grant, Grant No. 694455. *ZMOD: Blood Vessel Development and Homeostasis: Identification and Functional Analysis of Genetic Modifiers.*

2022 – 2026: EU ERC Advanced Grant, Grant No. 101021349. *TAaGC: Transcriptional Adaptation and Genetic Compensation.*

National

2012 – 2023: Deutsches Zentrum für Lungenforschung e.V. (DZL) / Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt

2018 – 2021: DFG / SFB 834/2 – A11 (C. Helker/D. Stainier). *Control of Vascular Guidance by Apelin Signalling.* Joint application led by Ingrid Fleming, Goethe University, Frankfurt.

2019 – 2022: DFG / SFB CRC 1366 – A4 (R. Marín Juez/D. Stainier). *Angiocrine Control of Heart Regeneration.* Joint application led by Hellmut Augustin, European Center for Angioscience (ECAS), Medical Faculty Mannheim, Heidelberg University and DKFZ, Heidelberg.

2020 – 2024: DFG / SFB CRC 1213 - B01 (S. Reischauer/D. Stainier). *Analysis of ventricular hypertrophy under physiological and pathological conditions in zebrafish.* Joint application led by Norbert Weissmann, Justus Liebig University, Giessen.

Dr. Mridula Balakrishnan (DZHK Start-up Grant)

Dr. Arica Beisaw (NIH Grant, CPI Flex Grant, DZHK Promotion of Women Grant, DZHK Shared Expertise Grant)

Dr. Maëlle Bellec (EMBO Fellowship)

Dr. Michelle Collins (DZHK Start-up Grant, CPI Start-up Grant)

Dr. João Cardeira da Silva (CPI Flex Grant)

Mohamed El-Brolosy (Boehringer Ingelheim Foundation Fellowship, Otto Bayer Fellowship, Birnstiel Award, Otto Hahn Medal, Peter Hans Hofschneider Prize)

Hadil El-Sammak (Boehringer Ingelheim Foundation Fellowship)

Dr. Ryuichi Fukuda (Daiichi-Sankyo Foundation Fellowship, Sumitomo Foundation Grant, DZHK Shared Expertise Grant)

Dr. Sébastien Gauvrit (Sturge-Weber Foundation: Lisa's Research Grant)

Dr. Felix Gunawan (EMBO and CIHR Fellowships)

Gabrielius Jakutis (Boehringer Ingelheim Foundation Fellowship)

Dr. Zhen Jiang (CPI Start-up Grant)

Dr. Thomas Juan (EMBO Fellowship, DZHK Start-up Grant)

Dr. Zacharias Kontarakis (EMBO Fellowship)

Dr. Rubén Marín Juez (DZHK Shared Expertise Grant)

Sapna Kumari Meena (DAAD Fellowship)

Dr. Paolo Panza (CPI Flex Grant)

Dr. Rashmi Priya (EMBO and Humboldt Fellowships, CPI Start-up Grant)

Dr. Chi-Chung Wu (Croucher Foundation Fellowship, CPI Flex Grant, CPI Start-up Grant, DZHK Start-up Grant)

IV. Lung Development and Remodelling

Werner Seeger (director)

Werner Seeger, Prof. Dr. med. | Director, Dept. of of Lung Development and Remodeling, Max Planck Institute for Heart and Lung Research / University Professor in Internal Medicine, Justus Liebig University Giessen (JLU)

University Education

1986	Habilitation Internal Medicine, JLU
1980	Doctorate Medicine, JLU
1972–1979	Studies of Medicine, Westfälische-Wilhelms University Münster and Justus Liebig University Giessen (JLU)

Scientific Career

Since 2020	Chair of the Institute for Lung Health, JLU/DZL, Giessen, Germany
Since 2019	President of the international “Pulmonary Vascular Institute” (PVRI)
Since 2016	External Scientific Member and Research Laboratory Leader at the Biomedicine Research Institute in Buenos Aires, Argentina (IBIOBA)
Since 2015	Member of the Forum for Health Research (Forum Gesundheitsforschung) of the German Ministry for Research and Education
Since 2011	Chair of the German Center for Lung Research (DZL)
Since 2010	Chair of the Universities of Giessen and Marburg Lung Center (UGMLC)
2007–2010	Chairman of the Medical Commission of the German Council of Science and Humanities
Since 2007	Director of the Department of Lung Development and Remodeling at the Max Planck Institute for Heart and Lung Research, Bad Nauheim
Since 2006	Medical Executive Director of the University Hospital Giessen and Marburg (UKGM)
2006–2013	Member of the Health Research Council (Gesundheitsforschungsrat)
2003–2009	Member, German Council of Science and Humanities (The Wissenschaftsrat)
Since 2006	Coordinator of the Excellence Cluster Cardio-Pulmonary System/Cardio-Pulmonary Institute (ECCPS/CPI)
2002–2009	Member, Senate Committee “Clinical Research”, German Research Foundation
Since 2000	Medical Executive Director of the Department of Internal Medicine, JLU
Since 1999	Chair, TransMIT GmbH, Medical Technology
1997–2009	Head, Collaborative Research Center SFB 547 “Cardiopulmonary Vascular System”
Since 1996	Professor of Internal Medicine, Section Head of Respiratory and Critical Care Medicine, JLU (professorship prolonged until 2024 by the University President)
1991–1996	Head, Clinical Research Group “Respiratory Failure”, JLU
1987–1991	Assistant Professor of Medicine, Department of Medicine, JLU
1982–1987	Internship and Residency, Department of Medicine, JLU
1979–1982	Postdoctoral Fellow, Department of Pathological Biochemistry, JLU

Lab members of Department IV: Lung Development and Remodelling

Lab Members – since 2019
(Current / Past / (guest))

Group leaders
Prof. Dr. Soni Pullamsetti (guest)
Prof. Dr. Rajkumar Savaj (guest)
Dr. Rory E. Morty

PostDocs:
Dr. Rajender Nandigama
Dr. Sascha Seidel
Dr. Sandra Medrano Garcia
Dr. Poonam Sarode
Dr. Kati Turkowski
Dr. Annika Karger
Dr. Prakash Chelladurai
Dr. Chanil Valasarajan
Dr. Anoop Cherian
Dr. Manju Nandigama
Dr. Giovanni Maroli
Dr. Argen Mamazhakypov
Dr. Pouya Sarvari

Doctoral Students:
Dr. med. Philipp Arndt
Joshua Ayoson
Dr. med. Michael Cekay
Öznur Cetin
Dr. vet med. Siavash Mansouri
Spyridoula Barmpoutsi
Joshua Olapoju
Selma Dizdarevic
Maria José Alonso
Jamal Nabhanizadeh
Edibe Avci
Golnaz Hesami
Sreenath Nayakanti
Zahraa Msheik
Samuel Olapoju
Leili Jafari
Nassima Mordjana
Fabian Tobor
David Brunn
Ylia Salazar
Xiang Zheng

Technical Assistants:
Jeanette Knepper
Yanina Knepper
Uta Eule
Jana Rostkovius
Daniel Grella
Laura Kahnke

Overview of Department IV

Respiratory diseases rank second only to cardiovascular diseases in terms of mortality, incidence, prevalence, and socio-economic burden. The ***Department for Lung Development and Remodelling (IV)*** addresses diseases that affect the lung parenchyma, with its vascular, interstitial, and alveolar compartments. Particular attention is paid to the development of new treatment concepts, thus shaping the *translational focus* of the Department. To this end, it is closely linked with the ***University of Giessen Lung Center* (UGLC).** All topics addressed within the Department represent research fields jointly pursued by the UGLC and Department IV. Department IV/UGLC holds a central position in the ***Excellence Cluster Cardio-Pulmonary Institute* (CPI)**, the ***German Center for Lung Research* (DZL)** with the University of Marburg being included in the local site (UGMLC) and in the newly founded **Institute for Lung Health (ILH)**. External laboratories established in the framework of international cooperations include the "High Altitude Research Guest Laboratory" at the University of Lhasa, Tibet, China; and the "Max Planck Heart and Lung Guest Laboratory" at the *Instituto de Investigación en Biomedicina de Buenos Aires (IBIOBA)*, in Buenos Aires, Argentina.

The competence of Department IV/UGLC in **lung-focused translational science** is further demonstrated by the fact that discoveries of this group have led to **worldwide clinical approval of novel therapies**, namely inhaled iloprost and inhaled treprostinil for pulmonary arterial hypertension (PAH), the phosphodiesterase inhibitors sildenafil and tadalafil for PAH, and the soluble guanylate cyclase stimulator riociguat for PAH and chronic thromboembolic pulmonary hypertension (CTEPH; first drug in this field). Further investigator-initiated phase I/II/III trials under the responsibility of Department IV/UGLC scientists, addressing reverse-remodeling and/or regenerative processes in vascular and parenchymal lung diseases, are currently underway.

Werner Seeger chairs the following **large Research Consortia**:

The **Excellence Cluster CPI** (together with S. Dimmeler and T. Braun): *7.130 Mio €/year*

The **DZL site UGMLC**: *6.280 Mio €/year*

The **Institute for Lung Health (ILH)**: *6.000 Mio €/year*

The Worldwide Pulmonary Hypertension **Meta-Registry GoDeep:** *0.314 Mio €/year*

Third Party funds of the Research Group Leaders Pullamsetti and Savai are given in the subsequent sections

PULLAMSETTI GROUP

Molecular Mechanisms of Pulmonary Vascular Diseases

PH is characterized by a progressive and sustained increase in pulmonary vascular resistance and mean pulmonary arterial pressure, leading to increased right ventricular afterload and reduced cardiac output, which culminates in right ventricular failure and premature death. When PH primarily develops in the pulmonary arterioles, this condition is termed pulmonary arterial hypertension (PAH). The pathophysiological abnormalities involved in PAH include severe vascular remodeling, concentric or eccentric laminar lesion development, neointima development, and complex “plexiform lesion” formation that ultimately leads to the occlusion and loss of precapillary arteries. Pulmonary vascular remodeling is a critical pathological hallmark of PAH and is the result of phenotypic alterations in vascular cells, including hyperproliferative, antiapoptotic, proinflammatory, promigratory, and profibrotic vascular cell responses. We and other researchers have observed that vascular cells [endothelial cells (PAECs), smooth muscle cells (PASMCs), and adventitial fibroblasts (PAAFs)] cultured from hypertensive lung vessels manifest and maintain this phenotype when transferred outside the vascular microenvironment, thus demonstrating a persistent/sustained phenotypic change. However, the molecular regulatory mechanisms that drive the normal vascular cell components to acquire and sustain the disease phenotype remain elusive.

Among the various epigenetic mechanisms, histone modifications such as lysine acetylation and methylation are crucial for the regulation of chromatin structure and DNA accessibility, which correlate with the state of gene transcription. Histone modifications provide binding platforms for diverse DNA/chromatin-modifying enzymes, chromatin remodelers, and transcription factors (TFs), thereby playing important roles in gene expression and DNA replication and repair. Misregulation of epigenetic modifications has been identified in the development of cancer and cardiovascular diseases causing pathological transcriptional changes, thus emphasizing the requirement for genome-wide profiling of the epigenomic and transcriptomic landscape in cardiovascular diseases such as PH.

Our research group combines basic science approaches and translational research to elucidate the pathogenic sequelae underlying PH and right heart dysfunction and to elaborate novel treatment concepts. We are pursuing an integrated concept to understand the common pathophysiological processes and molecular mechanisms that underlie structural vascular abnormalities in PH. Particular emphasis is placed on the contribution of TFs and epigenetic mechanisms to pulmonary vascular remodeling. Our aim is to reverse remodeling events and to regain physiological lung vascular and parenchymal structure and function and to develop TF- and epigenetics-focused treatment concepts for PH currently not available.

Noncanonical Hippo/ MST signaling in PH pathogenesis

The MST (mammalian Ste20-like kinase) 1 and 2(STK4/3) form the catalytic core of HIPPO, an evolutionally conserved growth-suppressor cassette. In adult somatic cells, MST1 and 2 act as growth suppressors and protect against cancer, fibrosis, and the proliferation-driven remodeling of the systemic vasculature. MST1/2 are activated by cleavage and auto-phosphorylation at T180/183 and inhibit cell proliferation and induce apoptosis via a broad range of mechanisms, the majority of which include phosphorylation of the large tumor suppressors (LATS)1/2 and concomitant inhibition of the transcriptional co-activators YAP (Yes-associated protein)/TAZ (WWTR1), nuclear retention and activation of forkhead homeobox type O (FOXO) TFs, and inhibition of Akt-mTOR. However, the roles and mechanisms of the action of MST1 and MST2 in PH and how they regulate the FOXO TFs previously shown to play an important role in PH pathogenesis are unknown.

Using early-passage pulmonary vascular cells from human PAH and non-diseased lungs and mice with smooth muscle-specific tamoxifen-inducible Mst1/2 knockdown, we found that, in contrast to canonical

antiproliferative/ proapoptotic roles, MST1/2 act as proproliferative/prosurvival molecules in human PAH PASMCs and PAAFs and support established pulmonary vascular remodeling and pulmonary hypertension in mice with SU5416/hypoxia-induced pulmonary hypertension. By using unbiased proteomic analysis, gain- and loss-of function approaches, and pharmacological inhibition of MST1/2 kinase activity by XMU-MP-1, we next evaluated mechanisms of regulation and function of MST1/2 in PAH pulmonary vascular cells.

It revealed that MST1 and MST2 form different interactomes in PAH and nondiseased PASMC/ PAAFs and identified 13 proteins that interact with MST1 exclusively in PAH, but not control PASMC. Out of these 13 proteins, only BUB3 (Mitotic Checkpoint Protein) and 60S ribosomal protein L22-like 1 (RPL22L1) also interacted with MST1/2 exclusively in PAH PASMCs and PSMC6 in PAH PAAFs. In PAH PASMCs, MST1/2 acted via forming a disease-specific interaction with BUB3 and supported ECM (extracellular matrix)- and USP10 (Ubiquitin Specific Peptidase 10)- dependent BUB3 accumulation, upregulation of Akt-mTORC1, cell proliferation, and survival. Supporting our in vitro observations, smooth muscle-specific Mst1/2 knockdown halted upregulation of Akt-mTORC1 in small muscular PAs of mice with SU5416/hypoxia-induced PH (Fig. 1).

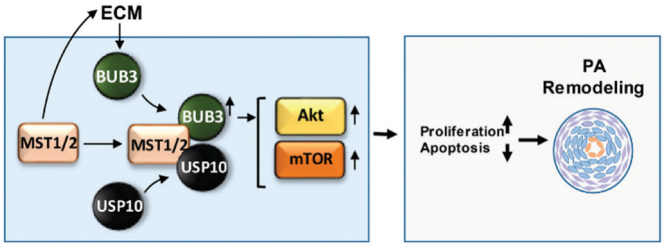

Fig. 1. Schematic representation of proposed function of MST1-2/BUB3 signaling in PAVSMC in PAH. PA indicates pulmonary artery (Kudryashova, Dabral et al., Circ Res., 2022).

On the other hand, we found that, in PAH PAAFs, the proproliferative function of MST1/2 is caused by IL-6-dependent MST1/2 overexpression, which induces PSMC6 (Proteasome 26S Subunit, ATPase 6)-dependent downregulation of the FoxO isoform FoxO3 and hyperproliferation. Importantly, FOXO3 interacted with the proteasome complex in the presence of MST1/2, leading to its degradation, and ultimately contributing to the hyperproliferative and prosurvival phenotype of PAH PAAF (Fig. 2).

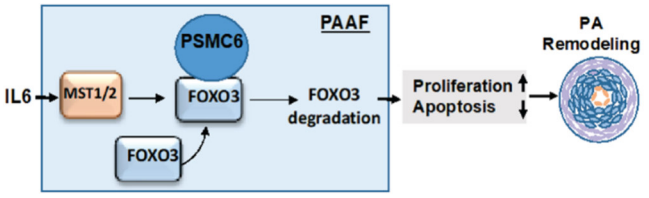

Fig. 2. Schematic representation of proposed function of MST1-2/FOXO3 signaling in PAAF in PAH. PA indicates pulmonary artery (Kudryashova, Dabral et al., Circ Res., 2022).

Together, these studies describe a novel proproliferative/prosurvival role of MST1/2 in the PAH pulmonary vasculature, provides a novel mechanistic link from MST1/2 via BUB3 and FoxO3 to the abnormal proliferation and survival of PASMCs and PAAFs, remodeling and pulmonary hypertension, and suggests new target pathways for therapeutic intervention.

lncRNAs and PH

Human genome analysis has shown that 80% of the human genome is transcribed into noncoding RNAs, of which the majority are long noncoding.

RNAs (lncRNAs), with transcripts >200 nucleotides in length. lncRNAs, which lack protein coding ability, are expressed in a wider diversity of species than other RNAs such as mRNA, microRNA, or small nucleolar RNA. Despite their poor species conservation and low abundance, lncRNAs play significant roles in various biological processes, including X chromosome inactivation, genomic imprinting, cell differentiation, and developmental patterning. They regulate various molecular pathways by, for example, acting as an RNA decoy, microRNA sponge, or ribonucleoprotein component, or by the recruitment of chromatin modifiers, inhibition of translation, or splicing. lncRNAs have been shown to have roles in various diseases and have been identified as potential therapeutic targets. However, the role of lncRNAs in the pathogenesis of PAH remains largely unknown.

Using RNA sequencing data, the lncRNA TYKRIL was identified to be consistently upregulated in pericytes and PASMCs exposed to hypoxia and derived from patients with idiopathic PAH. TYKRIL knockdown reversed the pro-proliferative and anti-apoptotic phenotype induced under hypoxic and idiopathic PAH conditions. Owing to the poor species conservation of TYKRIL, ex vivo studies were performed in precision-cut lung slices from patients with

PAH. Knockdown of TYKRIL using LNA GapmeR technology in these human lung slices decreased the vascular remodeling. The number of proliferating cells in the vessels was decreased and the number of apoptotic cells in the vessels was increased in the TYKRIL GapmeR-treated group compared with control.

Expression of PDGFRß, a key player in PAH, was found to strongly correlate with TYKRIL expression in the patient samples, and TYKRIL knockdown decreased PDGFRß expression. From the TF–screening array, it was observed that TYKRIL knockdown increased the p53 activity, a known repressor of PDGFRß. RNA immunoprecipitation using various p53 mutants demonstrated that TYKRIL binds to the N-terminal of p53 (an important region for p300 interaction with p53). The proximity ligation assay revealed that TYKRIL interferes with the p53–p300 interaction and regulates p53 nuclear translocation.

The findings of this study identified, for the first time, that TYKRIL is a checkpoint molecule in p53/PDGFRß signaling with functional relevance in both hyperproliferating PASMCs and pericytes, suggesting that it may serve as a novel therapeutic target in PAH.

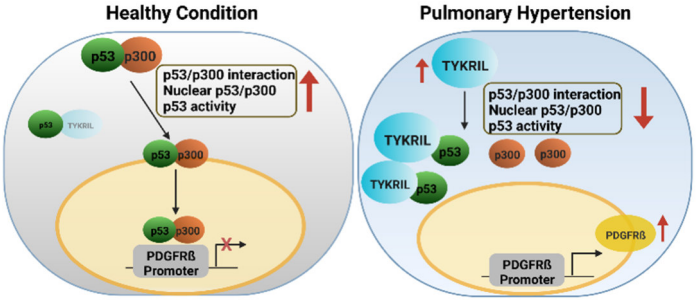

Fig. 3. A representative image summarizing the TYKRIL molecular mechanism modulating p53/PDGFRb signaling (Zehendner, Valasarajan et al., Am J Respir Crit Care Med., 2020).

Epigenetic mechanisms in PH

Phenotypic alterations in resident vascular cells contribute to the vascular remodeling process in diseases such as PAH. How the molecular interplay between transcriptional coactivators, TFs, and chromatin state alterations facilitates the maintenance of persistently activated cellular phenotypes that consequently aggravate vascular remodeling processes in PAH remains poorly explored.

RNA sequencing (RNA-seq) in PAAFs from adult human PAH and control lungs revealed 2460 differentially transcribed genes. Chromatin immunoprecipitation sequencing (ChIP-seq) revealed extensive differential distribution of transcriptionally accessible chromatin signatures, with 4152 active enhancers altered in PAH-FBs. Integrative analysis of RNA-seq and ChIP-seq data revealed that the transcriptional signatures for lung morphogenesis were epigenetically derepressed in PAH-FBs, including coexpression of T-box TF 4 (TBX4), TBX5, and SRY-box TF 9 (SOX9), which are involved in the early stages of lung development. These TFs are expressed in mouse fetuses and then repressed postnatally, but were found to be maintained in persistent PH of the newborn and reexpressed in adult PAH.

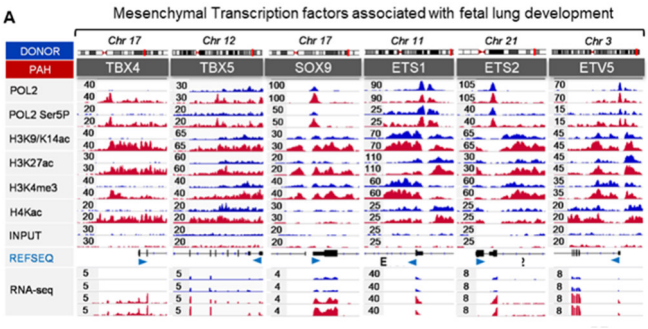

Fig. 4. Genome browser views of the genomic regions flanking the TSS of the TFs with an active chromatin state or repressed chromatin state in PAH-FBs compared to donors (Chelladurai et al., Sci Transl Med. 2022).

Silencing of TBX4, TBX5, SOX9, or E1A-associated protein P300 (EP300) by RNA interference or small-molecule compounds regressed PAH phenotypes and mesenchymal signatures in arterial FBs and smooth muscle cells. Pharmacological inhibition of the P300/CREB-binding protein complex reduced the remodeling of distal pulmonary vessels, improved hemodynamics, and reversed established PAH in three rodent models in vivo, as well as reduced vascular remodeling in precision-cut tissue slices from human PAH lungs *ex vivo*.

These data strongly support epigenetic reactivation of TFs associated with lung development in PAH, offering epigenetic interference as novel treatment concept.

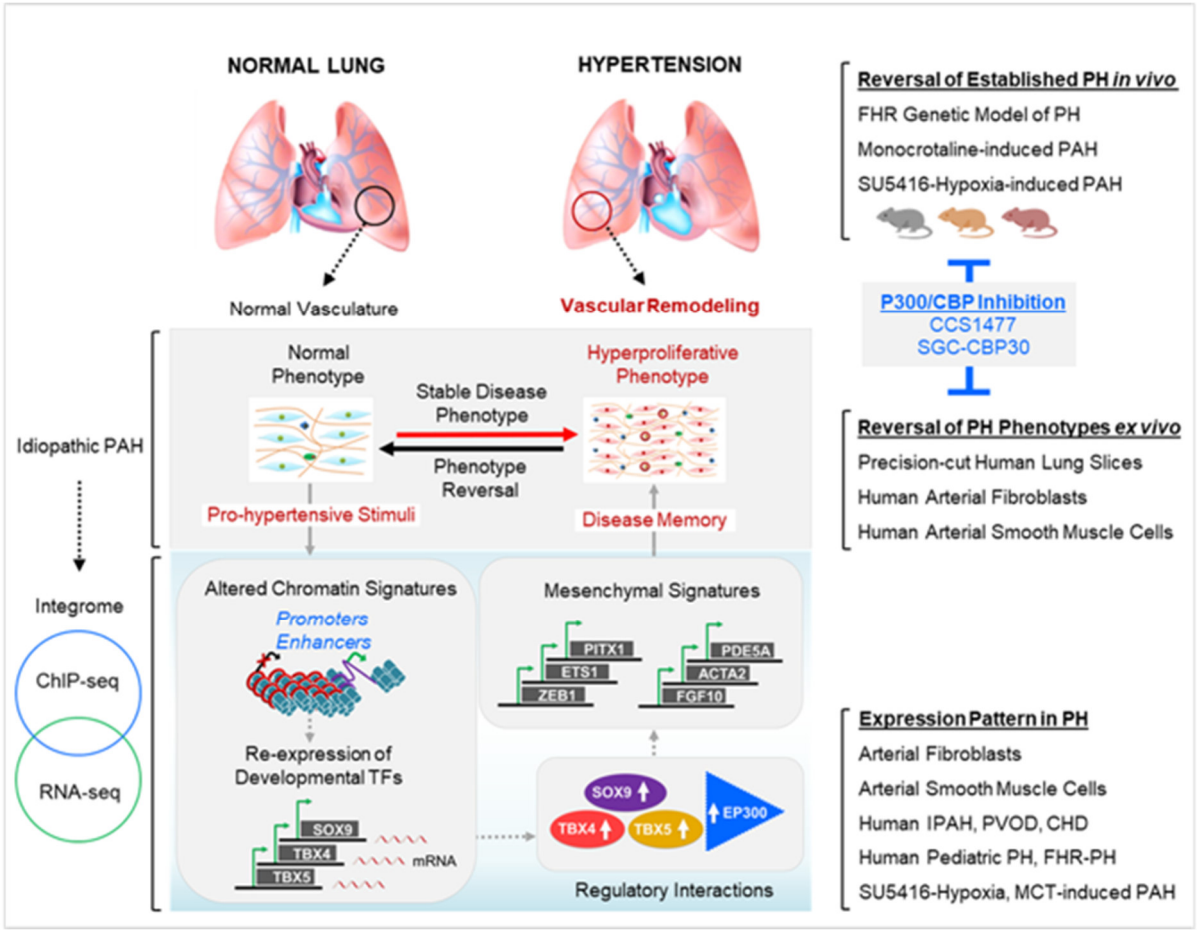

***Fig. 5.* Summary of the findings:** *This study reports the evidence of substantial genome-wide alterations in the chromatin landscape and transcriptome of cells isolated from patients with PAH. Integromic analysis uncovered the dysregulation of transcriptional coactivators (P300/CBP), developmental TFs (TBX4, TBX5, SOX9) and their transcriptional targets in PAH. Pharmacological inhibition of P300/CBP reversed established PH* ex vivo *and* in vivo *(Chelladurai et al., Sci Transl Med. 2022).*

SAVAI GROUP

Lung microenvironmental niche in cancerogenesis

Despite the heterogeneous nature of established lung tumors, they are assumed to be initiated from malignant transformation of a single cell. Tumors are, however, not a homogeneous collection of malignant cells, but they contain various different cell types in addition to the neoplastic cells, including angiogenic endothelial, infiltrating immune cells and mesenchymal/stromal cells. Such cells establish the tumor microenvironment through reciprocal communications, thereby driving tumor growth and metastasis, the hallmarks of cancer. Indeed, these central features of tumor biology and their resistance to anti-cancer therapies are assumed to be conferred by the combination of (epi-) genetic, phenotypic and functional diversity of the different neoplastic/ microenvironmental cell types. We focus on the role of immune/ inflammatory cells in the lung cancer niche in driving cancer progression and metastasis. To this end, several lung cancer mouse models were established and are employed in the various studies (transgenic lung tumor models as well as primary and metastatic lung tumor models), supported by elaborate small animal lung tumor imaging techniques. Additionally, humanized patient-derived engrafts and viable human precision-cut lung slices (PCLS), exploiting fresh human lung tumor tissue samples available via access to large clinical lung cancer cohorts, are combined with technologies for (epi-)genome-wide profiling of human and mouse microenvironmental and neoplastic cells. With this armamentarium, the following major scientific foci are addressed (A–C) (Fig. 6):

A: Spatiotemporal dynamics and signaling of the epimmunome in lung cancer

B: Interplay between genomics and the niche epimmunome as a driver of plasticity and immune dysregulation in lung cancer

C: Stromal niche remodeling and epimmunome mediated crosstalk between lung cancer and associated lung diseases.

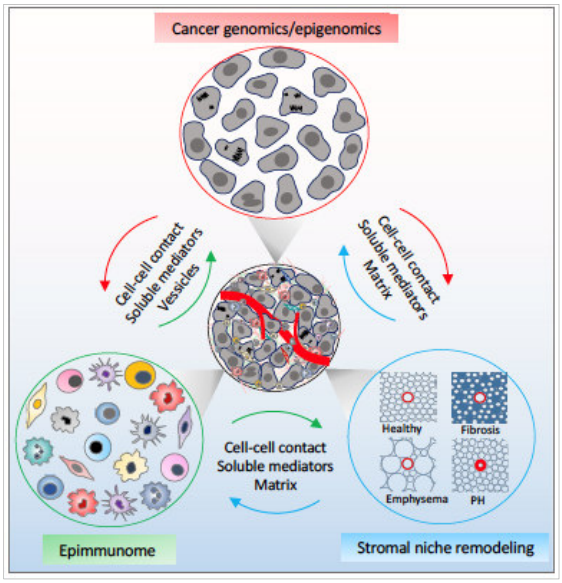

Fig. 6. Overview of the scientific focus.

Re-education/reprogramming of TAMs to become tumoricidal effectors as a novel strategy for cancer therapy

Macrophages are functionally plastic because they are induced in response to and modulated by the alteration of molecules in the TME. Therefore, manipulation of environmental stimuli to repolarize M2-like TAMs to a tumor-suppressive phenotype under pathological conditions is a potential clinical strategy for cancer therapy. Accordingly, we addressed following specific aims:

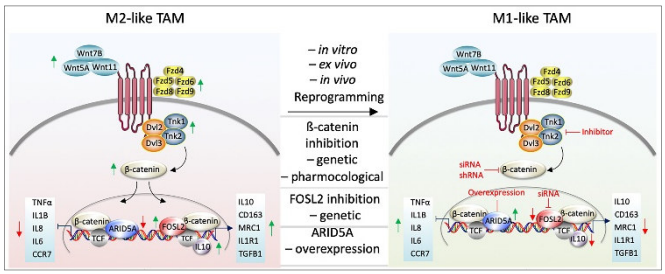

Fig. 7. Inhibition of Wnt/ß-catenin signaling and FOSL2 and activation of ARID5A cause a phenotypic switch to M1-like TAMs; correlation of ß-catenin/FOSL2/ARID5A with the survival of lung cancer patients (Sarode et al., Science, Adv 2020).

Targeting ß-catenin signaling in TAMs: Macrophage-specific ablation of β-catenin reprogrammed M2-like TAMs to M1-like TAMs both in vitro and in various in vivo models, which was linked with the suppression of primary and metastatic lung tumor growth. An in-depth analysis of the underlying signaling events revealed that β-catenin-mediated transcriptional activation of FOS-like antigen 2 (FOSL2) and repression of the AT-rich interaction domain 5A (ARID5A) drive the gene regulatory switch from M1-like TAMs to M2-like TAMs. Moreover, we found that high expressions of β-catenin and FOSL2 correlated with poor prognosis in patients with lung cancer. In conclusion, β-catenin drives a transcriptional switch in the lung tumor microenvironment, thereby promoting tumor progression and metastasis (Summarized in Fig.7).

HDAC2-SP1 orchestrated M2-like macrophage phenotype drives lung cancer growth: In this study, we demonstrate that the epigenetic regulators are key controllers of macrophage fate in the spatially heterogeneous tumor microenvironment. Spatial proximity of histone deacetylase 2 (HDAC2)–overexpressing M2-like macrophages to tumor cells was found to be significantly correlated with poor overall survival. Suppression of HDAC2 in M2 TAMs upregulates M1 marker gene expression and downregulates M2 marker genes, indicating a switch to an anti-tumor M1-like phenotype. Myeloid cell–specific suppression of HDAC2 and pharmacological inhibition of class I HDACs retards lung tumor growth. HDAC2 regulates transcription of genes such as IL10, IL16, MIF, ALOX15, IL8, IL12 via influence on acetylation of both histone and non-histone proteins to regulate TAM phenotypic plasticity, with a particular role of the HDAC2-SP1 axis. Therefore, specifically targeting HDAC2 in in lung cancer TAMs may provide a novel strategy for immunotherapy.

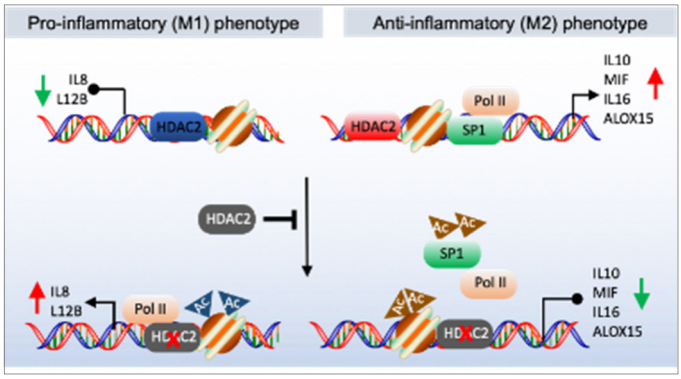

Fig. 8. HDAC2 regulation drives alterations of TAM polarization. Modulation of HDACs allows orchestrating the TAM phenotype (Zheng et al., Cancer Res., In revision).

LncRNA ADPGK-AS1 regulates macrophage mitochondrial and phenotypic state: In this study, we identified cytoplasmic lncRNA ADPGK-AS1 in M2 like TAMs, upregulated and translocated to the mitochondria. Overexpression of ADPGK-AS1 promotes its binding to mitochondrial ribosomal proteins, thereby enhancing energy production and a change in the macrophage phenotype. Subsequently, macrophage specific knockdown of ADPGK-AS1 led to elevated inflammatory signals and reduced lung tumor growth *in vitro* (tumor-cell macrophage cocultures), *ex vivo* (human lung tumor precision-cut lung slices) and *in vivo* (humanized mouse model; coinjection of macrophages and tumor cells). Our data indicate that ADPGK-AS1 regulates mitochondrial metabolism and functional transition between the macrophage phenotypes. Targeting ADPGK-AS1 could thus represent a novel approach for managing pathologies associated with macrophage deregulation (Summarized Fig.9).

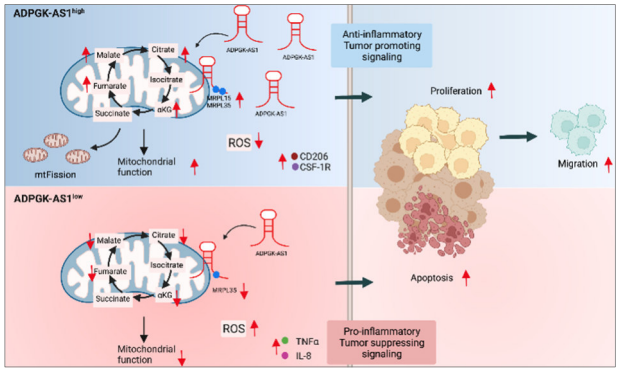

Fig. 9. *lncRNA ADPGK-AS1 regulates mitochondrial metabolism and functional transition of the macrophage phenotype (Karger et al., EMBO J, In revision).*

Bone marrow-derived fibrocytes are involved in the progression of lung carcinomas

Fibrocytes are bone marrow–derived monocytic cells implicated in wound healing processes. Here, we identify their role in lung cancer progression/metastasis. Single cell RNA sequencing of bone marrow and lung tumor CD45+ cells identified a novel fibrocyte specific marker signature (CD45+, Col1+, CD44+, CD163+, CCR5+, CD162+, CXCR3+, CCR2+). Selective manipulation of fibrocytes in syn-geneic mouse and human xenograft as well as KRas-driven transgenic mouse tumorigenesis models documented that these cells boost lung cancer niche features and enhance metastasis. Importantly, patients with lung cancer showed greater numbers of circulating fibrocytes and marked fibrocyte accumulation in the cancer niche. Using double and triple co-culture systems with human lung cancer cells, fibrocytes and macrophages, we substantiated the central features of the cancer-supporting niche: enhanced cancer cell proliferation and migration, M2-like macrophage differentiation, augmented endothelial tube formation, endothelial sprouting and fibrocyte maturation. Endothelin and its receptors were upregulated (ET1, ETA, ETB), and dual ET receptor blockade suppressed all cancer-supportive phenotypic alterations in vitro. Notably, dual ET receptor blockade inhibited lung tumor growth in the experimental models, majorly via acting on fibrocyte interaction with the cancer niche. We thus provide evidence for a hitherto unrecognized role of fibrocytes in lung cancer progression and metastasis, suggesting targets for novel treatment strategies. (Fig. 10).

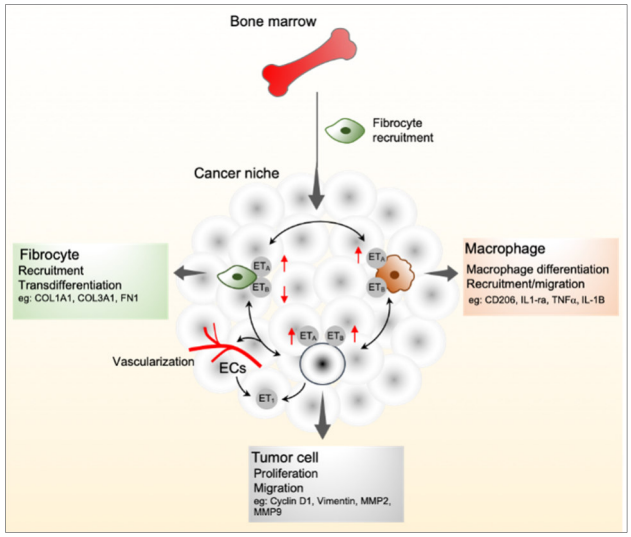

Fig. 10. *Increased fibrocyte recruitment and subsequent accumulation in the lung tissues lead to interaction with cancer cells and further tumor microenvironmental cells. Upregulation of endothelin and its receptors is involved in tumor cell growth, immune modulation and angiogenesis (Weigert et al., Nat Commun., 2022).*

Microenvironmental Th9 and Th17 lymphocytes induce metastatic spreading in lung cancer

In this project, we explored the effect of tumor-infiltrating lymphocyte subpopulations on lung cancer biology by studying in vitro cocultures, in vivo mouse models, and human lung cancer tissue. Lymphocyte conditioned media (CM) induced epithelial-mesenchymal transition (EMT) and migration in both primary human lung cancer cells and cell lines. Correspondingly, accumulation of Th9 and Th17 cells was detected in human lung cancer tissue and correlated with poor survival. Coculturing lung cancer cells with Th9/Th17 cells or exposing them to the respective CM induced EMT in cancer cells and modulated the expression profile of genes implicated in EMT and metastasis. These features were reproduced by the

signatory cytokines IL-9 and IL-17, with gene regulatory profiles evoked by these cytokines being partly overlapping and complementary. Co-injection of Th9/Th17 cells with tumor cells in WT, Rag1-/-, Il9r-/-, and Il17ra-/- mice altered tumor growth and metastasis. Accordingly, inhibition of IL-9 or IL-17 cytokines by neutralizing antibodies decreased EMT and slowed lung cancer progression. In conclusion, Th9 and Th17 lymphocytes induce lung cancer cell EMT, thereby promoting migration and metastatic spreading and offering potentially novel therapeutic strategies. (Summarized Fig. 11).

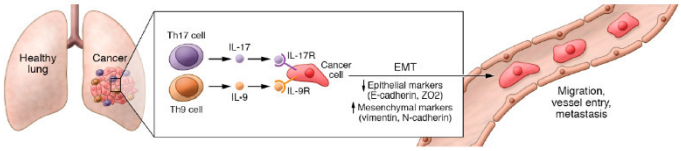

Fig. 11. *Th9 and Th17 lymphocytes induce lung cancer cell epithelial-mesenchymal transition via release of their signature cytokines, thereby promoting migration, vessel entry, and metastatic spreading (Salazar et al., J Clin Invest., 2020).*

Impact of pulmonary arterial hypertension on overall survival in lung cancer patients

To investigate the incidence and impact of PH in lung cancer, we retrospectively analyzed lung cancer patients in our center from the years 2017 to 2020. To investigate the incidence and impact of PH in lung cancer, we retrospectively analyzed lung cancer patients in our center from the years 2017 to 2020. Measurements of pulmonary artery (PA) and ascending aorta (A) diameter from the images acquired with baseline high-resolution computed tomography revealed that 293 of 670 (43.7%) lung cancer patients had a mean PA size of ≥28mm, assumed to indicate PH. We also calculated the PA/A ratio, as a stronger surrogate parameter for PH. To confirm the PA size and the PA/A Ratio as a surrogate marker for PH, a correlation analysis with echo pulmonary artery systolic pressure (ePASP) was undertaken in 132 patients. Further, we investigated the impact of PH on the survival of lung cancer patients, we evaluated the progression free survival (PFS) and overall survival (OS) using an adjusted Cox PH regression analysis. Importantly, the median PFS was significantly shorter in patients with lung cancer and PH when a cutoff PA size of ≥28mm was used, and the difference was even more prominent, when a cutoff PA/A ratio of >1 was employed. In corroboration, median OS was significantly reduced for patients with PA size ≥28 mm and PA/A ratio >1 compared to PA size <28mm and PA/A ratio ≤1, respectively (Fig.12).

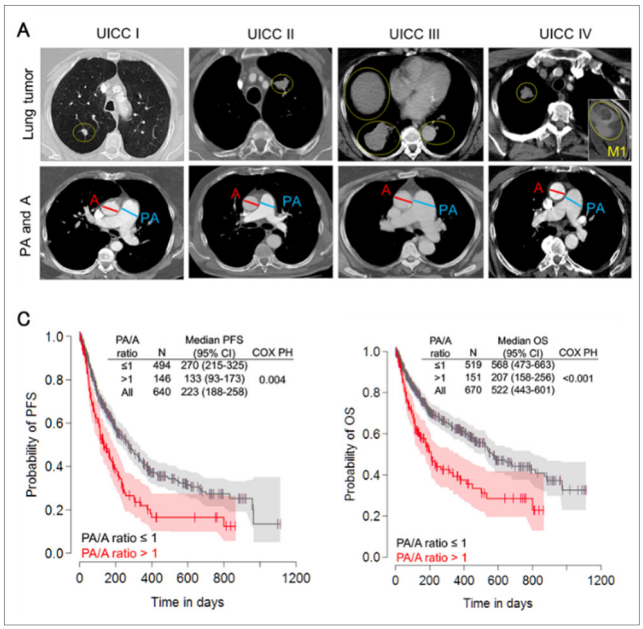

Fig. 12. *Impact of of pulmonary artery (PA) size and PA/aorta size ratio (PA/A), indicators of PH, on survival in lung cancer (Eul et al., Am J Respir Crit Care Med., 2020).*

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Awards, Achievements, and Accomplishments

2022: IMPRS graduate school, Best Poster award (Alonso)

2022: Institute for Lung Health (ILH), Start-up Grant (Seidel)

2022: Cardio-Pulmonary Institute (CPI), poster prize (Mansouri)

2022: SBMC conference Heidelberg, Best Poster award (Karger)

2022: Rene Baumgart Research Award (Chelladurai)

2022: Cardio-Pulmonary Institute (CPI), Startup Grant (Chelladurai)

2022: Institute for Lung Health (ILH), Postdoc Grant (Valasarajan)

2021: Rene Baumgart Research Award (Valasarajan)

2021: American Heart Association (AHA) Best Poster award (Nayakanti)

2021: Butrous Foundation Young Investigator Award (Chelladurai)

2021: Cardio-Pulmonary Institute (CPI), Post-doc fellowship (Sarode)

2020: NOVARTIS InCa- Research Award (Inflammation and Lung Cancer) (Sarode)

2020: German Research Foundation (DFG): Heisenberg Professor (Savai)

2020: Pulmonary Vascular Research Institute (PVRI) Best Poster award (Valasarajan)

2019: International Trainee Scholarship Award, American Thoracic Society (ATS) (Valasarajan)

Third Party Funding (Pullamsetti)

2021-2025: European Research Council Consolidator Grant (ERC-CoG) Pulmonary hypertension: "aberrant" mimicry of lung vascular morphogenesis?". *2.000.000,00 €*

2021-2024: Federal Ministry of Education and Research (BMBF): "Molecular analysis of COVID samples (Transcriptome and Epigenome)". *2.000.000,00 €*

2021-2024: Dinosaur Trust "Correction of a BMPR2 Mutation using CRISPR/Cas9-assisted Genome Editing in Familial Pulmonary Arterial Hypertension Patients". *300.000,00 €*

2020-2024: German Research Foundation (DFG, SFB 1213): "FoxO transcription factors in PH: critical integrators of multiple signaling pathways driving vascular remodeling". *487.200,00 €*

2020-2024: German Research Foundation (DFG, SFB 1213): "Regulatory network of histone modifications in human pulmonary arterial hypertension". *402.400,00 €*

2021-2024: Hessian Ministry of Science and the Arts (LOEWE): "The role of the inflammatory lung microenvironment in the transition of idiopathic pulmonary fibrosis to lung carcinoma". *252.220,00 €*

2021-2024: Hessian Ministry of Science and the Arts (LOEWE): "The role of T cell subpopulations in lung cancer associated pulmonary hypertension". *126.110,00 €*

2019-2022: Federal Ministry of Education and Research (BMBF): PoC-Initiative Helmholtz-Fraunhofer-Hochschulmedizin, "Nano-PAX PoC*". 300.000,00 €*

2019-2022: Cardiopulmonary Institute (CPI): CPIFlex Funds. "Alternative Splicing in Idiopathic Pulmonary Fibrosis – regulation by ER-stress and implications for fibrogenesis". *150.000,00 €*

2019-2022: Cardiopulmonary Institute (CPI): CPIFlex Funds. "Clonal Hematopoiesis of Indeterminant Potential in age-associated chronic lung diseases". *150.000,00 €*

Third Party Funding (Savai)

2022-2026: German Research Foundation (DFG): "Re-education of tumor promoting macrophages by RNAi based therapeutics". *382.300,00 €*

2022-2025: Federal Ministry of Education and Research (BMBF) Biological and physical optimization of particle beams: Radiation protection for the patient. *215.570,00 €*

2020-2025: German Research Foundation (DFG): Heisenberg Professorship: Lung Microenvironmental Niche in Cancerogenesis. *535.500,00 €*

2019-2023: Federal Ministry of Education and Research (BMBF) MicroRNA-574-5p as a tumor and stratification marker for prostaglandin E2-mediated tumors. *320.000.00 €*

2020-2024: German Research Foundation (DFG, SFB 1213): "Inflammatory lung microenvironment in lung cancer-associated PH – the role of macrophages". *495.200.00 €*

2020-2023: German Research Foundation (DFG): "Clinician Scientist Program in Biomedical Research". *360.000,00€*

2021-2022: Federal Ministry of Education and Research (BMBF)/ German Center for Lung Research (DZL): "Neoadjuvant anti PD-1 immunotherapy in resectable NSCLC: the NEOMUN trial". *139.150,00 €*

2021-2022: Federal Ministry of Education and Research (BMBF)/ German Center for Lung Research (DZL): "Tumor cell - immune cell cross talk in lung cancer". *675.000,00 €*

2021-2024: Hessian Ministry of Science and the Arts (LOEWE): "Imaging macrophage plasticity and heterogeneity in the lung tumor niche - integrative singlecell omics". *252.220,00 €*

2021-2024: Hessian Ministry of Science and the Arts (LOEWE): "Single cell phenotyping of the lung/tumor microenvironment". *252.220,00 €*

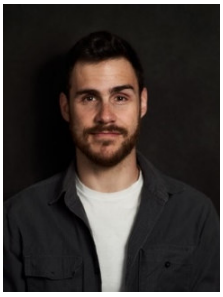
2019-2022: Cardiopulmonary Institute (CPI): CPIFlex Funds. "Microenvironmental cues as drivers of macrophage plasticity and polarization across lung diseases". *150.000,00 €*

2019-2022: Cardiopulmonary Institute (CPI): CPIFlex Funds. "Soiling the seed of lung cancer: Inter organ communication between lung and brain". *150.000,00 €*

4. Research Groups

Circadian Regulation of Cardiometabolism

Pieterjan Dierickx (group leader)



Pieterjan Dierickx, Ph.D | Independent research group leader, Max Planck Institute for Heart and Lung Research

University Education

2012-2017	PhD studies in Geijsen/van Laake lab, Hubrecht institute, Utrecht, The Netherlands
2009-2011	Master of Science in Biochemistry & Biotechnology (Major: Biomedical Biotechnology, Minor: Plant Biotechnology), Ghent University, Belgium
2006-2009	Bachelor of Science in Biochemistry & Biotechnology program, Ghent University, Belgium

Scientific career

2023-	DZHK Junior Research Group Leader
2022-	International Max Planck Institute Research School (IMPRS) lecturer
2022-	CPI independent research group leader, Max Planck Institute for Heart and Lung Research
2017-2022	Postdoctoral Fellow, Lazar Lab, Institute for Diabetes, Obesity and Metabolism, UPenn, Philadelphia, USA. Postdoc fellowships: NHI (1y) and AHA (2y).

Professional Activities

2022-	Member of the DGK (German Cardiac Society) and Young DZHK
2022-	Faculty member of the CPI (CardioPulmonary Institute)
2020-2022	Member of the AHA (American Heart Association)
2019-2022	Member of the SRBR/EBRS (Society for Research on Biological Rhythms)
2014-	Member of the (ESC) European Society of Cardiology

Group members – Dierickx lab

PhD Students
Bryce J. Carpenter
Seval Kübra Korkunç
Kai Cui

Technical Assistants
Ankita Jha
Yannick Mangold

Dierickx Lab

Research – Dierickx lab

The Dierickx lab is interested in how the circadian clock drives rhythmic processes in the heart. Circadian rhythms coordinate many aspects of behavior and physiology (e.g., fasting/feeding and body temperature cycles,) to be in synchrony with the 24-hour rotation of the earth. In humans, disruption of these rhythms is highly associated with increased risk for cardiovascular disease development. The indispensability of clock proteins in the heart is mechanistically illustrated by our recent findings that REV-ERB loss in cardiomyocytes specifically leads to dilated cardiomyopathy and premature death in mice.

Projects in the lab are centered around an integrated approach combining next-generation sequencing techniques, whole-body physiology analysis in specialized mutant mouse lines and the use of *in vitro* cardiac models. We aim to develop cardiometabolic insufficiency treatment strategies and will use data

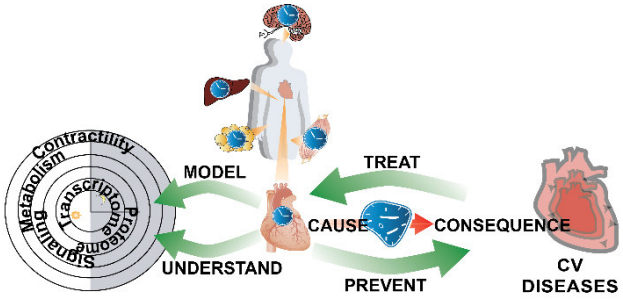


Fig. 1. Schematic describing the research interests and vision of the Dierickx lab.

from human patients to better understand how deregulation of the clock contributes to heart disease. Investigating how the circadian clock and its intricate connection to rhythmic metabolic programs can be deployed to tackle prevention, diagnosis and treatment of heart failure will be the overarching goal.

Overview research lines

RL1: Dissecting the role of circadian clock deregulation in the development of heart disease

Research line 1 (RL1, Fig. 2) focuses on understanding how disturbing the circadian clock leads to heart disease. Our newly developed cardiomyocyte-specific Rev-erbα/β DKO (CM-RevDKO) model will serve as a tool to study the etiology of dilated cardiomyopathy. While complete knockout of both *Rev-erbs* leads to dilated cardiomyopathy around 6 months of age (Fig. 2, 'developmental', Dierickx et al., 2022), inducible knockout after adulthood does not (Fig. 2, 'adult', unpublished). This indicates that clock disturbance during development has effects later at life, a phenomenon that correlates with human epidemiological studies pointing at the developmental origin of cardiovascular diseases. To identify the (epigenomic) differences that underlie the described discrepancy between developmental and adult, I will pursue my comparison of both models via a combination of RNA-seq, ChIP-seq and/or Cut&Run for histone marks. As REV-ERBs (absent in our mouse model) and E4BP4 (constitutively high in our mouse model) are both potent transcriptional repressors, I anticipate differences will be reflected by alterations in active and repressive epigenomic read outs such as H3K27ac and H3K27me3. Accordingly, our recent findings in the developmental model indicate that both transcriptional repressors play a key role in lipid oxidative metabolism and *Nampt* expression (RL2, Fig. 3a) in the adult heart. However,

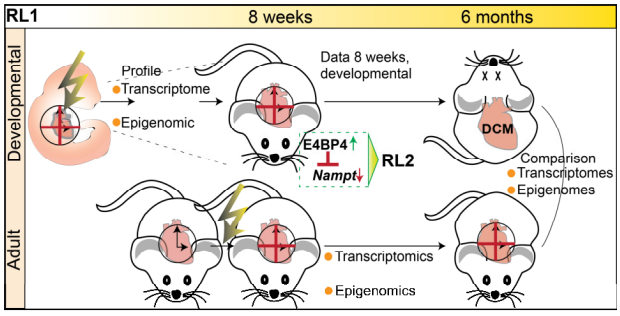


***Fig. 2** – **RL1**. Elucidating why developmental clock disturbance has consequences later in life.*

their dysregulation during development might cause additional, so far unknown, complexities that contribute to premature death in the developmental model but not the adult model. This work will lay the foundation for comparison with epidemiological data in order to focus on the most relevant targets that might prevent the development of cardiovascular diseases. Such work will be followed by studies making use of human iPSC-derived CMs at different maturation states.

RL2: Investigation of NAD⁺ metabolism as therapeutic target for heart failure

Heart failure (HF) is a severe disease and an enormous burden on our society. Although characterized by impaired bioenergetics, focusing on treatment of HF from a metabolic angle is underexplored. Downregulated NAD⁺ metabolism (co-enzyme for many processes governing energy metabolism) is common in HF, yet little is known about the transcriptional regulation of the rate-limiting enzyme, NAMPT, generating NAD⁺. I propose to explore mechanisms by which the REV-ERB-E4BP4-NAMPT axis could be targeted to enhance/prevent bio-energetic imbalance in the failing heart.

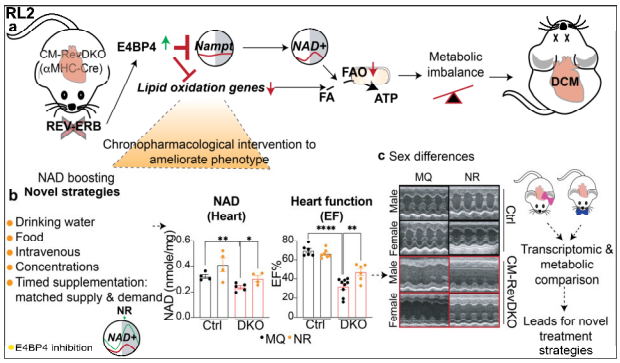


***Fig. 3** – **RL2**. Characterization of new NAD+ enhancing strategies to combat DCM.*

We have recently discovered that long-term treatment with NR (nicotinamide riboside), an NAD⁺ precursor, has the potency to increase NAD⁺ levels in multiple tissues including the heart in clock-disrupted mice enhancing heart function (Dierickx et al., 2022). Importantly, we only observed diminished adverse remodeling and expanded lifespan effects in female mice. Here, we aim to further optimize NAD⁺ supplementation using different supplementation strategies (food, drinking water, intravenous injection) in order to obtain highest cardiac delivery without burdening other organs with superphysiological NAD⁺ levels, as seen in the liver for example. Importantly, the circadian aspect of NAD⁺ related treatment has been largely overlooked but offers tremendous opportunities to test NAD⁺ supplementation in a circadian fashion so as to boost its levels when most needed (Fig. 3b). In mice, this is during the dark phase (active period). In humans (NAD⁺) metabolism is known to be different between men and women and sex differences could be part of inconsistent results of NAD⁺

boosting compounds in human subjects. Therefore, I will probe transcriptional as well as metabolic differences between male and female KO mice treated with NAD⁺ boosters (Fig. 3c). This will elucidate targetable mechanisms and pathways that can be directly linked to human physiology and potentially lead to a more uniform and streamlined treatment of dilated cardiomyopathy (DCM). Combined, these experiments will yield detailed insights into potential novel chronopharmacological approaches to ameliorate the cardiometabolic status in the failing heart.

RL3: Profiling inter/intra-organ crosstalk in a heart failure setting

RNA-sequencing of heart tissue in young 8-week-old mice that do not show a DCM phenotype yet, allowed for the identification of a set of significant changes at the transcriptional level. Pathological dilation however, is only observed around six months to get worse rapidly after that. This indicates that at least part of the alterations underlying the phenotype can be observed long before the disease phenotype manifests itself. To investigate whether CM-specific knockout of *Rev-erbs* results in remodeling of other non-CM cardiac cell type, we performed snRNA-seq (single nucleus RNA-sequencing) on 8-week-old hearts (Fig. 4a, Dierickx et al., unpublished). This led to an unbiased dataset of changes in cardiomyocytes, but interestingly also non-cardiomyocytes, such as macrophages, endothelial cells and fibroblasts, highlighting previously unidentified communication between affected CMs and immune, vascular and stromal cells. For example, *Nampt* downregulation in CM was found to correlate with *Nampt* upregulation in cardiac fibroblasts, which suggests a compensatory crosstalk between these different cell types within the heart. Here, we aim to validate these changes via co-culture experiments (direct contact vs transwell systems) as well as by treating different non-CM cell types with conditioned medium from KO vs control CMs (Fig. 4b). Gene expression of the receiving cells will be assessed and the conditioned medium will be screened via lipidomics, proteomics and metabolomics in order to explore which molecules are responsible for this critical communication between cell types. In addition, to fully characterize disease progression, we will perform additional snRNA-seq at multiple timepoints to define the dynamics of deregulated gene programs at the single cell level (Fig. 4c). We aim to overlap available single cell (and bulk) RNA-seq datasets from DCM-HF patients with my snRNA-seq datasets from non-symptomatic KO mice (Dierickx et al., unpublished) and with the new datasets from the older symptomatic (DCM) KO mice (Fig. 4b). Deregulated genes that are solely expressed in one cell-type will be overlapped with the tier 1 (druggable genome), which includes efficacy targets of approved small molecules and biotherapeutic drugs as well as candidates in clinical-phase, to investigate whether certain genes/pathways could be targeted (Fig. 4d).

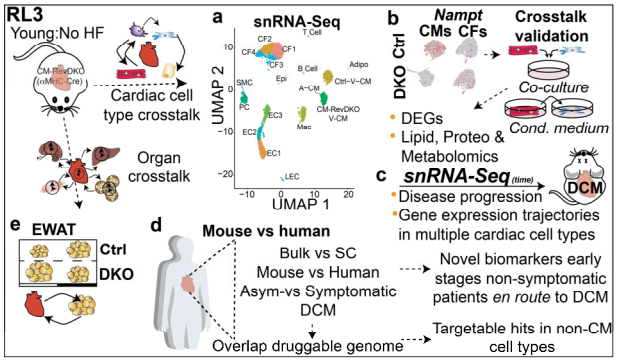


Fig. 4 – RL3. Exploring inter and intra-organ crosstalk in a DCM setting.

There are no available biomarkers for non-symptomatic patients en route to DCM, but our model could aid the discovery of such markers, potentially in a non-invasive manner. Our CM- RevDKO-based DCM model shows improper fatty acid (FA) oxidation and decreased *Cpt1a* (an important mitochondrial FA importer) levels (Dierickx et al., 2022). I hypothesize that this can lead to systemic FA overflow. Since epididymal white adipose tissue (EWAT) is a major depot for lipid storage, I will measure EWAT weight and compare to controls at different time points during one day. EWAT remodeling will be investigated at the transcriptomic level (which, if promising, can be extended with proteomics and lipidomics) (Fig. 4e) to assess whether their endogenous clock and/or other genes are affected.

In summary, the work presented in RL1-3 will elucidate previously unappreciated fundamental mechanisms by which the circadian clock regulates cardiometabolic gene regulation and NAD⁺ metabolism. Exploring NAD⁺-boosting strategies to enhance cardiac bio-energetics in the failing heart in a circadian manner will aid to pave the way for therapy and preventive medicine.

Progress and embedding

During our first weeks we were able to secure funding for the projects revolving around optimizing therapeutic NR strategies and human disease progression modelling from the DZHK (DZHK Junior Group Leader grant for the next 6 years). With this funding I appointed one TA (now fully FELASA trained) and one bio-informatics PhD student. In

the first months the PhD student was able to analyze our first pilot single nuclei RNA-seq dataset. For this project we established a collaboration with the group of Dr. Mario Looso (CPI). Together with his group as well as Dr. Dumbovic (GU) we secured additional CPI funding for a single nuclei RNA-seq project in which we want to assess cardiac cell type-specific circadian rhythms as well as how they influence eachother.

A second PhD student started in September on the developmental project (also fully FELASA trained now). Together we managed to establish 4 mouse lines in the lab (shipped from US) and perform the first experiments on these animals. We are currently in the process of finishing up a number of animal protocols (TVAs) allowing us to perform the highly specialized experiments proposed. For his project in which we want to compare the developmental and adult models, we already established collaborations with Dr. Stefan Gunther and his core facility (MPI-HLR) and submitted 50 samples for bulk mRNA-seq. This PhD student and I also published our first MPI-affiliated review article (Carpenter & Dierickx, 2022) as preparation for the NR-project.

With our second TA we are currently optimizing human iPS-CM differentiation protocols to generate 2D/3D cardiac organoid models. The TA is learning the techniques in Frankfurt from the group of Dr. Jaya Krishnan, an expert in cardiac differentiation models. Once this technique is fully mastered, we are planning on generating preliminary data on hiPSC-CMs that we will engineer with circadian reporters. A recently purchased bioluminescent microscope (LV200) will allow for assessing the development of circadian rhythms during directed differentiation/maturation as well as aging. These data will be used to write an ERC starting grant revolving around the non-canonical roles of circadian clock facturs in proliferation, differentiation, maturation, aging, and reprogramming. For this project we also started collaborating with Dr. Johnny Kim (UG, CPI), an expert in the field of regeneration and reprogamming in the vasculature.

In addition, I established a 'Chronobiology' course, which I am teaching to the IMPRS PhD students

Publications (2019-2022)

Carpenter BJ & **Dierickx P#** (2022) Circadian NAD^+ metabolism, from transcriptional regulation to healthy aging. *Am J Physiol-cell Phys* 323: C1168–C1176

Dierickx P#, Carpenter BJ, Celwyn I, Kelly DP, Baur JA & Lazar MA# (2022) Nicotinamide riboside Improves cardiac function and prolongs survival after disruption of the cardiomyocyte clock. *Front Mol Med* 2: 887733

Dierickx P#, Zhu K, Carpenter BJ, Jiang C, Vermunt MW, Xiao Y, Luongo TS, Yamamoto T, Martí-Pàmies Í, Mia S, Latimer M, Diwan A, Zhao J, Hauck AK, Krusen B, Nguyen HCB, Blobel GA, Kelly DP, Pei L, Baur JA, Young ME & Lazar MA# (2021) Circadian REV-ERBs repress E4bp4 to activate NAMPT-dependent NAD^+ biosynthesis and sustain cardiac function. *Nat Cardiovasc Res* 1: 45–58

Chirico N, van Laake LW, Sluijter JPG, van Mil A & **Dierickx P#** (2020) Cardiac circadian rhythms in time and space: The future is in 4D. *Curr Opin Pharmacol* 57: 49–59 (Article featured on cover)

Dhillon P, Park J, Hurtado Del Pozo C, Li L, Doke T, Huang S, Zhao J, Kang HM, Shrestra R, Balzer MS, Chatterjee S, Prado P, Han SY, Liu H, Sheng X, **Dierickx P**, Batmanov K, Romero JP, Prósper F, Li M, Pei L, Kim J, Montserrat N, Susztak K (2021) The Nuclear Receptor ESRRA Protects from Kidney Disease by Coupling Metabolism and Differentiation. *Cell Metab* 33: 379-394.e86.

Guan D, Xiong Y, Trinh TM, Xiao Y, Hu W, Jiang C, **Dierickx P**, Jang C, Rabinowitz JD & Lazar MA (2020) The hepatocyte clock and feeding control chronophysiology of multiple liver cell types. *Science* 369: 1388–1394

Dierickx P, Emmett MJ, Jiang C, Uehara K, Liu M, Adlanmerini M & Lazar MA (2019) SR9009 has REV-ERB-independent effects on cell proliferation and metabolism. *Proc Natl Acad Sci USA* 3: 201904226

Hu W, Jiang C, Guan D, **Dierickx P**, Zhang R, Moscati A, Nadkarni GN, Steger DJ, Loos RJF, Hu C, Jia W, Soccio RE & Lazar MA (2019) Patient Adipose Stem Cell-Derived Adipocytes Reveal Genetic Variation that Predicts Antidiabetic Drug Response. *Cell Stem Cell* 24: 299-308.e6

#Corresponding author

External Funding and Awards (2019-2022)

Cardiopulmonary Institute (CPI) excellence cluster Area 4: collaborative research grant (2023): €30,000

DZHK: Junior Research Group Grant (2023-2029): €1,675,000

Cardiopulmonary Institute (CPI) excellence cluster: Junior Research Group Grant (2022-2026): €1,500,000

American Heart Association (AHA): Postdoctoral Fellowship (2019-2021): US$150,000

EBRS meeting: Best poster award (2022): 500 CHF

The company of Biologist: Travel Grant (2022): £400

Epigenetics

Lei Gu (group leader)

Dr. Lei Gu

Work Experience

since 04/2021	Independent Research Group Leader at Max Planck Institute for Heart and Lung Research, Germany
01/2019-03/2020	Instructor in Pediatrics at Division of Newborn Medicine, Boston Children's Hospital and Cell Biology Department, Harvard Medical School *(Parental leave: 06/2016--06/2017)*
03/2014-12/2018	Postdoctoral Research Fellow at Division of Newborn Medicine, Boston Children's Hospital; Cell Biology Department, Harvard Medical School; Broad Institute of MIT and Harvard Mentor: Dr. Yang Shi
10/2008-09/2010	Research Assistant at Department of Bioinformatics, Fraunhofer Institute for Algorithms and Scientific Computing (SCAI), Schloss Birlinghoven, Sankt Augustin, Germany Mentor: Dr. Martin Hofmann-Apitius
07/2006-09/2008	Junior Staff Scientist at the CAS - MPG Partner Institute for Computational Biology (PICB), Shanghai, China. Mentors: Dr. Li Jin and Dr. Andres Dress

Education

2013	PhD (magna cum laude) in Bioinformatics at the German Cancer Research Centre, Heidelberg University Mentor: Dr. Roland Eils

Group members – Gu lab

Phd Students:

Ilaria Venturelli
Zhixin Niu
Tengjia Jiang
Ruoxi Liu
Yi He
Hongbiao Huang

Introduction

Our lab combines bioinformatics, epigenomics cancer biology, fly genetics and mass spectrometry to identify and investigate roles of novel epigenetic modifications and their regulating enzymes in aging, development and diseases and novel mechanisms in transgenerational epigenetic inheritance (Fig. 1).

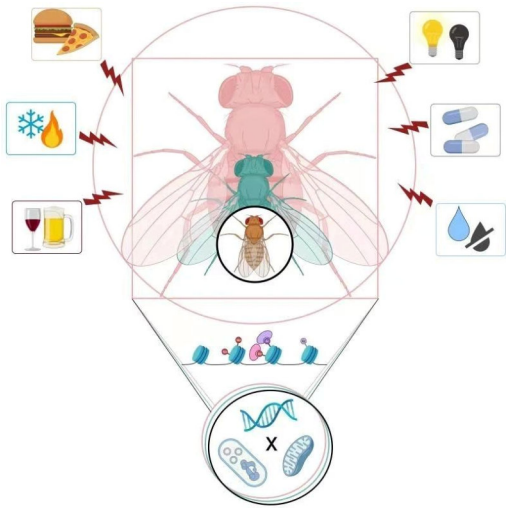


***Fig. 1.** Schematic working model and research interests of the Gu lab.*

Projects

Cancer Epigenetics

Genome-wide sequencing technologies have led to an unprecedented discovery of somatic mutations in “epigenetic modifiers” in human cancers. We have applied multidisciplinary and integrative approaches to understand how mutated epigenes function towards disrupting epigenetic landscapes that, in turn, lead to cancer progression in various cancers. Specifically:

(a) Deciphering a lethal CpG Island Mediated Phenotype (CIMP) in Ependymomas of Infancy. (*Nature*, 2014)
(b) Identifying a chromatin “reader” that influenced prostate cancer progression. (*Nature Genetics*, 2015)
(c) Identifying DNA methylation dynamics during B cell maturation in chronic lymphocytic leukemia (CLL). (*Nature Genetics*, 2016)
(d) Identifying recurrent mutations in the cohesion complex component STAG2 or STAG3 involved in resistance to BRAF inhibition in melanoma and the impact on 3D cancer genome upon STAG2 deactivation. (*Nature Medicine*, 2016; *Nature Communications*, 2022) (Fig. 2)

These findings are expected to reveal novel epigenetic mechanisms underlying cancers and pave new avenues towards cancer therapeutics to help patients afflicted by these devastating cancers.

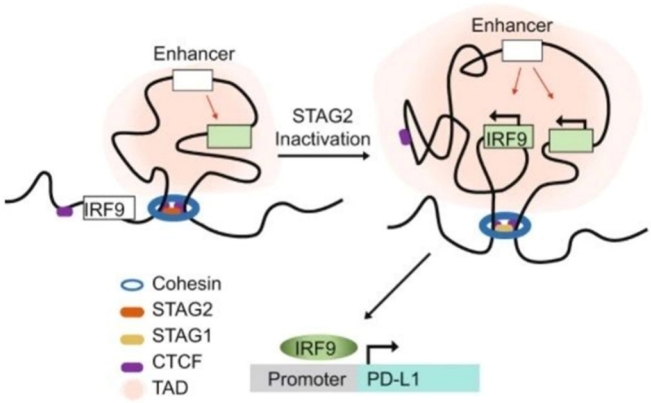


Fig. 2. A schematic model depicting the regulation of IRF9 and PD-L1 expression by STAG2 via modulating 3D genome organization at the IRF9 locus.

Epigenetic Interitance

Increasing evidence has emerged to suggest that environmental exposure during the prenatal period can increase the risk to develop diseases later in life. Prenatal exposure to tobacco smoke was described as a risk factor for a multitude of different diseases in children, including lung diseases, obesity, and cancer. However, the data supporting the relationship between environment, epigenetics, and transgenerational inheritance is still lacking due to difficulties in sample collection and lack of proper tools to dissect the complex data. We studied genome-wide, environmentally induced epigenetic changes and their functional relevance for disease risks later in life within a longitudinal mother-child birth cohort. By integrated analysis of longitudinal whole genome bisulfite sequencing, ChIP-sequencing and RNA sequencing, we unraveled a genome-wide epigenetic stable reprogramming converging upon transcriptional enhancers.

We found a number of disease-related pathways deregulated, among them, the Wnt signaling, which is involved in the airway inflammatory response to cigarette smoke in smoking mothers as well as their newborn children. This shows an association between epigenetic reprogramming of genes within the Wnt signaling pathway already at time of birth and the development of impaired lung function later in the children’s lives. (Molecular systems biology, 2016, Cover Article, featured in *Science*)

In addition, we computationally identified a novel DNA modification involved in transgenerational epigenetic inheritance in C.elegans, and CG14906 (mettl4) as U2 snRNA m6A methyltransferase, which was previously reported as a DNA m6A methyltransferase. This study answered a long-lasting question regarding the enzymatic activity of mettl4, and thus paved the way for further investigaton on the functions of mettl4 in different biological settings. (*Cell*, 2015; *Cell Research*, 2020; *Cell Discovery*, 2020; *Nature Machine Intelligence*, 2020)

Understanding how the environment impacts our epigenome and passes the information on to next generations would have massive implications on how we could approach and solve current complex diseases dominated by the interaction between environment and (epi)genetic composition.

Epigenetic Mechanisms in Regenerative Medicine

Regenerative medicine is aiming at the development of methods to regrow, repair and replace damaged or diseased cells, organs or tissues. This for example includes investigating as to why, given the same genome, the neonatal mouse heart has the ability to regenerate up to 7 days after birth.

Our lab is interested in understanding what epigenetic mechanisms contributed to the process of regeneration and, moreover, how the regenerative ability is lost during evolution. A key question is to understand when and how a differentiated cell cannot reactive the de-differentiation process.

We showed that chromatin accessibility plays a key role in enforcing cell fate decisions. This discovery could aid strategies to manipulate this process during the process of regeneration. (*Cell Reports*, 2021 (Fig. 3).

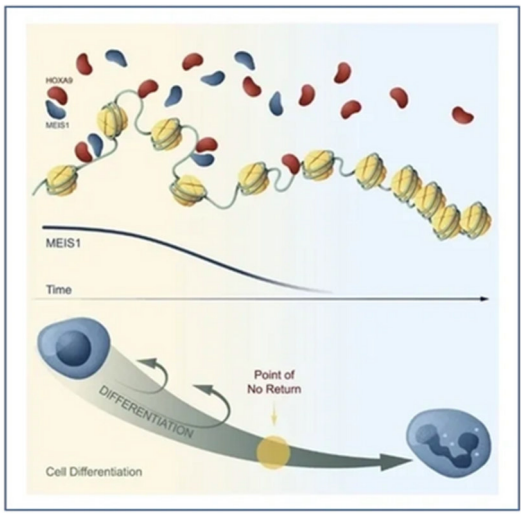


Fig. 3. A schematic model depicting the balance between self-renewal and differentiation and the mechanism of irreversible cell fate commitment.

Computational Tool Development and Protocol Improvement

Epigenetic modifications such as carbon 5 methylation of cytosine base in a CpG dinucleotide context are involved in the onset and progression of human diseases. A comprehensive understanding of the role of genome-wide DNA methylation patterns, the methylome, requires quantitative determination of the methylation states of all CpG sites in a genome. Analyses of the complete methylome by whole-genome bisulfite sequencing (WGBS) are rare because of the required large DNA quantities, substantial bioinformatics resources and high sequencing costs.

We improved the WGBS protocol, which allows investigating the methylome at single base resolution by using 5 ng of input DNA compared to 3-5 ug required for traditional WGBS. Hence, the improved protocol allows the comprehensive methylome analysis of limited amounts of DNA isolated from precious biological specimens. (*Nature Protocols*, 2013)

Circular layout is an efficient way for the visualization of huge amounts of genomic information. We developed a computational package named “circlize”, which provides an implementation of circular layout generation in R as well as an enhancement of available software. The flexibility of this package is based on the usage of low-level graphics functions such that self-defined high-level graphics can be easily implemented by users for specific purposes. Together with the seamless connection between the powerful computational and visual environment in R, circlize gives users more convenience and freedom to design figures for better understanding genomic patterns behind multi-dimensional data. (*Bioinformatics*, 2014)

We also develop a novel computational approach for better diagnosis in eye disease and characterization of the hypusine sites, which is a unique modification on lysine residues in eukaryotic translation initiation factor 5A (Eif5a). (*Scientific Reports*, 2021; *Current Proteomics*, 2018).

Publications (2019-2022)

Hongyun Zhao; Teng Da; Lifeng Yang; Xincheng Xu; Jiajia Chen; Tengjia Jiang; Austin Feng; Yaqing Zhang; Dennie T Frederick; **Lei Gu**; Li Cai; John M Asara; Marina Pasca di Magliano; Genevieve M Boland; Keith T Flaherty; Kenneth D Swanson; David Liu; Joshua D Rabinowitz; Bin Zheng (2022) Myeloid-derived Itaconate Suppresses Cytotoxic CD8+ T Cells and Promotes Tumor Growth. Nature Metabolism (accepted)

Zhaowei Chu*, **Lei Gu*,#,** Yeguang Hu, Xiaoyang Zhang, Man Li, Jiajia Chen, Da Teng, Man Huang, Che-Hung Shen, Li Cai, Toshimi Yoshida, Yifeng Qi, Zhixin Niu, Austin Feng, Songmei Geng, Dennie T. Frederick, Emma Specht, Adriano Piris, Ryan J. Sullivan, Keith T. Flaherty, Genevieve M. Boland, Katia Georgopoulos, David Liu, Yang Shi, Bin Zheng# (2022) STAG2 regulates interferon signaling in melanoma via enhancer loop reprogramming. Nature Communications, 13, 1859

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Dongxiao Fan, Chenshu Liu, Zeling Guo, Kan Huang, Meixiu Peng, Na Li, Hengli Luo, Tengyao Wang, Zhipeng Cen, Weikang Cai, **Lei Gu**, Sifan Chen and Zilun Li (2021) Resveratrol Promotes Angiogenesis in a FoxO1-Dependent Manner in Hind Limb Ischemia in Mice. Molecules, 26(24), 7528

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External Funding (2019-2022)

2021-2026: DFG/EXC 2026, Cardio-Pulmonary Institute (CPI), Project ID 390649896

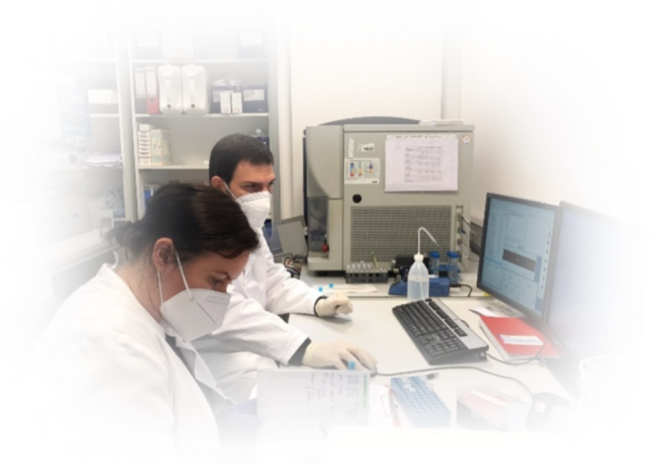
Tengjia Jiang: 2021-2025: Jingying PhD scholarship

Ruoxi Liu: 2021-2025: China Scholarship Council (CSC) PhD scholarship

Hongbiao Huang: 2022-2023: China Scholarship Council (CSC) PhD scholarship

Betül Beyza Güneş: 2021-2022: Erasmus scholar

5. Scientific Service Groups



Flow Cytometry and Cell Sorting

- **Khrievono Kikhi (Group Leader)**

  Kerstin Richter (Technical Assistant)
  Giuseppe Esposito (Flow Cytometrist)

Introduction

The Flow Cytometry and Cell Sorting Service Group was established in 2015 to consolidate and expand the existing flow cytometry and cell sorting procedures. The Facility is a service oriented group and strives to meet researchers' need through a tailored one-to-one user training session, offer expert advice with experimental design, post-acquisition data analysis and interpretation, and dedicated support in the use of our flow cytometers and cell sorters.

At present, we support over 70 researchers, both internal and external covering a diverse range of applications that require cell sorting and flow cytometric analysis for their projects. These applications relate to samples which are sourced from various tissues from mouse and zebrafish. The main focus is cell sorting as it facilitates the isolation of single cells with high purity and viability that are required for a comprehensive range of downstrea assays such as single cell RNA sequencing, bulk RNA sequencing, clonal expansion of CRIPS-Cas9 genome edited cells to name a few.

Instruments

Flow Cytometers

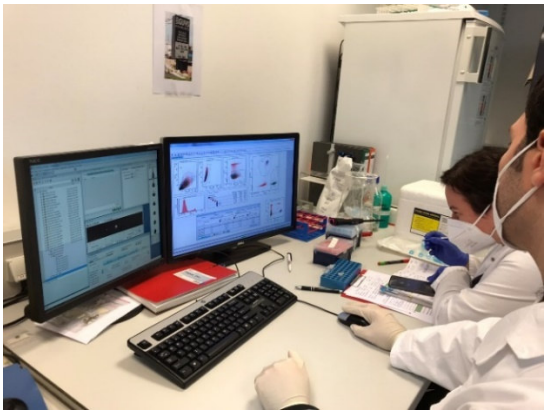
BD LSRFortessa™ Special Order Research Product (SORP)

The BD LSRFortessa™ SORP cell analyser was added to the core facility in 2016. It is equipped with 5 lasers (355 nm, 405 nm, 488 nm, 561 nm and 640 nm) and allows for simultaneous detection of upto 18 parameters plus 2 scatter parameters. It provides all the options required for optimisation of new experiments, implementation of new applications and defining complex multi parametric analysis.

Cell Sorters

BD FACSAria™ III Cell Sorter

The multicolour FACSAria™ III cell sorter was purchased in 2011 and is fitted with 4 lasers (405 nm, 488 nm, 561 nm, and 633 nm) and 14 detectors to measure 12 fluorescence parameters and 2 scatter parameters. It is the workhorse of the facility as most cell sorting is done on this instrument owing to its more available features and versatility. Particle size ranging from 200 nm to 20 μM can be detected on this instrument.



BioRad S3e Cell Sorter

The S3e cell sorter was purchased in 2017 to complement the Aria III cell sorter and meet the high demand for cell sorting in the facility. It has 2 lasers (488 nm and 561 nm), 4 detectors and 2 scatter channels. Experiments that are fairly simpler and requires 1-4 fluorescence parameters are well suited for sorting with this instrument.

Union Biometrica BioSorter®

Union Biometrica's Large Particle Sorter allows for the sorting of objects that do not fit within the parameters of conventional cell sorting because of large particle size. Since its purchase in late 2016, this instrument has enabled sorting of particles larger than 40μM such as cardiomyoctes from adult mice, adipocytes, megakaryocytes, zebrafish hearts, and whole organisms e.g. zebrafish larvae and *C.elegans*. As a result, new protocols have been developed and established in the facility to include large particle cell sorting and add to the growing repertoire of our applications to meet the specific needs and demands of a researcher's project.

Invitrogen Bigfoot Spectral Cell Sorter

In order to maintain a competitive flow cytometry platform and keep abreast of cutting edge technology in the field, a spectral cell sorter was purchased and installed in 2020-2022, during the times of pandemic.

The Bigfoot Spectral Cell Sorter is equipped with 5 lasers (349 nm, 405 nm, 488 nm, 561 nm and 640 nm) and has 60 detectors - 55 fluorescent parameters and 5 scatter channels. Unlike conventional cytometer, where a fluorophore is assigned to one detector, a spectral cytometer has the unique capability of measuring the entire emission spectrum of a fluorophore when excited. Spectral flow cytometry also has the advantage of allowing operators to work with expansive panels, and defining autofluorescence signal (Fig.1) which enables effective resolution of cell population of interest in complex samples exhibiting a high background, and non specific signal intensity.

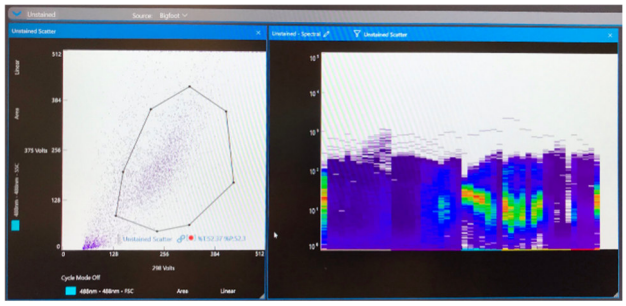


Fig. 1. *Defining autofluorescence signal with unstained sample.*

The Bigfoot has the option to function either as a conventional or a spectral cell sorter, and can sort upto 6 different populations. Every new technology and instrument bring its own unique set of challenges that are application specific which needs to be optimised. With the skill set and expertise of facility staff members, the enormous potential of the new technology, we seek to develop and establish innovative methods that will explore the full use of this interface of technology and biology.

Applications

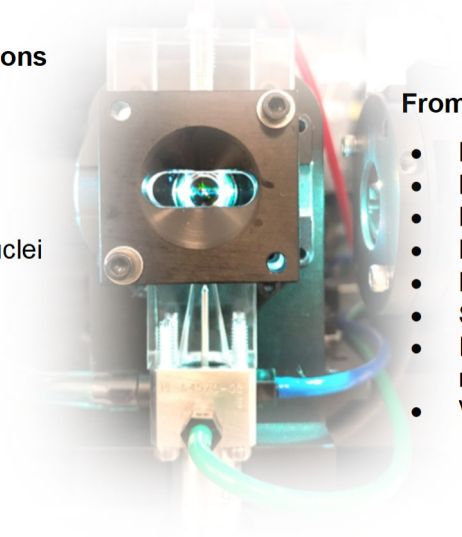
Flow Cytometry Analysis

- Multiparametric immunophenotyping of cells from heart, lung, blood and bone marrow
- DNA content analyses of various primary cells and cell lines
- Cell proliferation and Apoptosis Assays
- Gene expression frequencies in primary cells ans cell lines

Cell Sorting Applications

From zebrafish

- Cardiomyocytes
- Endothelial cells
- Epicardial cells
- Cardiomyocyte nuclei



From Mouse

- Muscle Stem cells
- Mesenchymal Stem cells
- Epithelial cells from lungs
- Pericytes from lung and brain
- Nuclei from cardiomyocytes
- Smooth muscle cells from lung
- Immune cells from heart, lung and bone marrow
- Various cell lines.

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Small Animal Imaging

- **Astrid Wietelmann, PhD (Service group leader)**

  Ursula Hofmann (Technical Assistant, MRI)

Astrid Wietelmann Ph.D.

since 2003	Research Associate, Max-Planck-Institute for Heart and Lung Research, Bad Nauheim
1998-2003	Research Associate, Kerckhoff-Klinik, Bad Nauheim
1987-1997	Research Associate, Gmelin-Institut für Anorganische Chemie und Grenzgebiete der Max-Planck-Gesellschaft, Frankfurt
1983-1986	Doctoral Thesis, Ludwig-Maximilians-Universität, Institut für Anorganische Chemie, München (1983-1986)
1977-1986	Studies in Chemistry, Ludwig-Maximilians-Universität, München
1982-1983	Diploma Thesis, Ludwig-Maximilians-Universität, Institut für Klinische Chemie, München

Introduction

Noninvasive, high-resolution small animal imaging systems have emerged as important tools for laboratory animal research and a variety of different modalities are available for researchers.

Our facility comprises Small Animal Magnetic Resonance Imaging (MRI) and Micro X-ray Computed Tomography (µ-CT).

Magnetic Resonance Imaging (MRI)

Facilities and Equipment

MRI is a method suitable for studying *in vivo* and noninvasively inner organs and tissues using magnetic fields and radiofrequency pulses. MRI is a versatile technique with a variety of new applications in biomedicine. MR not only allows visualization of structure of plants, animals, and humans and their organs, but also provides dynamic information about function and metabolism down to molecular level. Because of the proportionally large natural abundance of the hydrogen atom (^{1}H) in biological systems as well as the associated large magnetic moment, most of the MR imaging occurring in biological research is centered on a signal from hydrogen. Other nuclei, less abundant, used in magnetic resonance imaging and spectroscopy are e.g. ^{13}C or ^{31}P.

We work with a horizontal 7.05 T Bruker PharmaScan, operating at 300,51 MHz for ^{1}H and a gradient field strength of 760mT/m, which we use to investigate organs (heart, vessels, brain, lung, etc.) and extremities of small animals like rats, mice, newts and zebrafish. The nominal bore size of the magnet is 16 cm, with inserted gradient system and rf (radio frequency, RF) coils, the maximal useable bore size amounts to 6 cm. Using Paravision 6 for imaging and TopSpin 3.1 for high resolution spectroscopy, we are able to use a ^{1}H array cryoprobe as well as a ^{3}He surface cryoprobe with an integrated ^{1}H resonator. Both coils and preamplifiers are helium-cooled: Working at low temperatures of about 20 K results in an increase of sensitivity up to a factor of 2.5, a higher spatial resolution and shorter scan times. We have three planar surface resonators (receive-only) with an inner diameter of 10, 20 and 30 mm and a transmit-only volume resonator (72 mm) for different applications with small and well-defined regions of interest. Additional volume resonators with an inner diameter of 1.3, 2.5, 3.5, and 6.0 cm are available; our resonators are partly designed and constructed at our institute. Preclinical MRI techniques are increasingly used to perform longitudinal studies.

Cardiac Imaging

MRI is now a well-established modality for imaging of the cardiovascular system in rat and mice, Fig. 1.

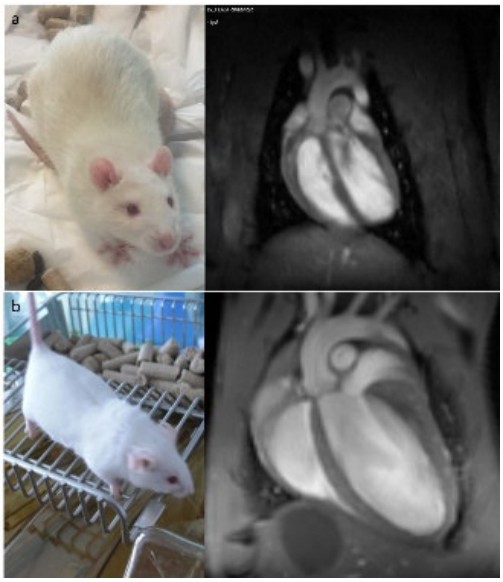

Fig. 1. *Rat and mouse heart MRI. 4-chamber views of a rat (a, upper panel) and mouse heart (b, lower panel) are displayed.*

Beside determination of tissue, organ or tumor sizes, our main focus lies on monitoring changes over time after treatment of transgenic mice. One of the best-known forms of dynamic MRI experiments is functional MRI, typically monitoring changes in blood flow. In recent years, MRI has become the standard for the quantitative evaluation of cardiac function, mass, and infarct size. Wall motion and strain analysis are used to display myocardial dysfunction. To obtain information on the morphology and functional parameters of rat and transgenic mouse hearts, we established standard protocols that enable us to rapidly acquire high quality images. Physiological gating is required to minimize motional influence of the beating heart and respiration on the MR experiment but also to synchronize the imaging sequence to the cardiac cycle. Currently, we need less than 1 hour to carry out all experiments necessary for the determination of the functional parameters of rat or mouse ventricles, like end-diastolic, end-systolic, and stroke volumes, ejection fraction, cardiac output, ventricular mass, wall-thickness and strain (Fig. 2).

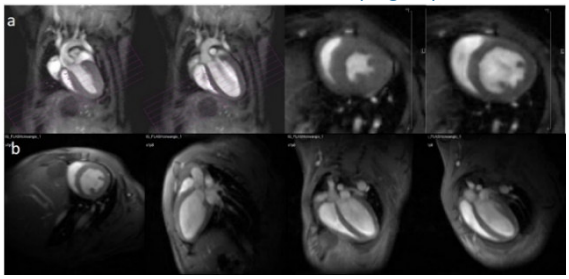

Fig. 2. *Mouse heart MRI. (a) endsystolic and enddiastolic phase of mouse heart in 4-chamber-view and midventricular short axis 2-chamber view; (b) 2-, 3- and 4-chamber views for segmentation of myocardial strain and hemodynamic forces.*

This is also due to the fact, that we use retrospective gating technique: cardiac and respiratory cycles are detected by a navigator signal, and therefore no triggering hardware is required. The so-called "self-gating" Intragate™ Tool (Bruker BioSpin, Ettlingen, Germany) provides a steady state condition which avoids the flashing effects common to conventional ECG triggering and respiratory gating. For cine cardiac MR imaging of living adult zebrafish or newts (Fig. 3), the retrospective gating technique (Intragate™ Tool) is mandatory.

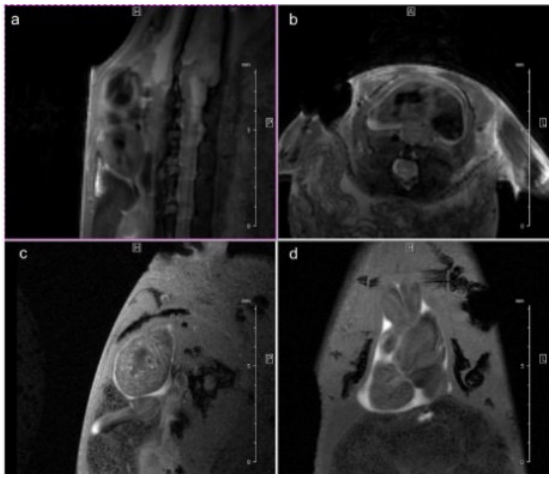

Fig. 3. *Newt heart MRI. In the upper panel two localizer. In the lower panel an image of the ventricle in axial (c) and coronal (d) orientation is presented.*

The small size of the zebrafish heart and the fact that we deal with a fish, makes MRI quite challenging. The biggest challenge is the supply of the fish with oxygen during the measurements. Momentarily, we are using two cradle set-ups: a chamber with water flow (Fig. 4a) or without floating water (Fig. 4b).

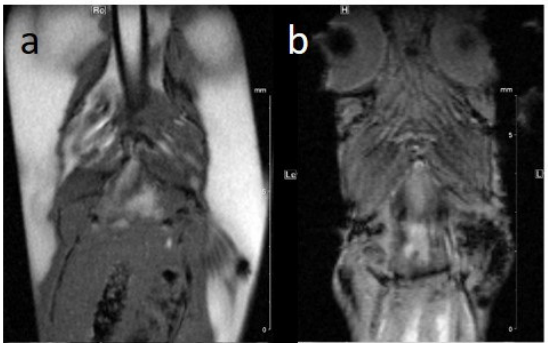

Fig. 4. *Zebrafish heart MRI. 3-chamber views of zebrafish heart derived from a cine IG Flash.*

In utero MRI

Investigating perinatal lethality in the mouse model, the determination of the fetal heart rate *in utero* can yield crucial information. Due to the high number of fetuses in the uterus, the correlation of a fetus to its corresponding heart signal is challenging. Employing the IntraGate™ self-gated cine FLASH sequence,

which was modified to provide a spatially encoded navigator (PolyGate), it is possible to monitor cardiac rate and to reconstruct retrospectively cines of the fetal hearts at E17.5, Fig. 5.

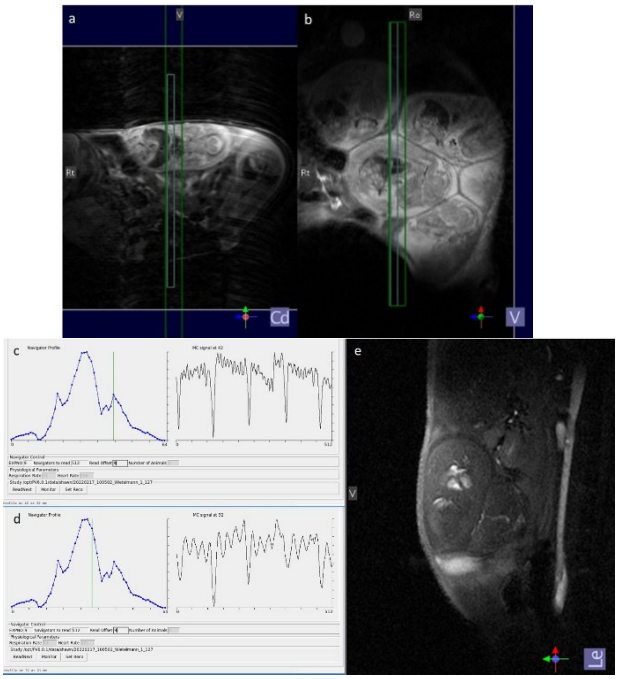

Fig. 5. In utero mouse fetuses MRI. 2D respiration gated RARE in (a) axial, (b) coronal orientation. Mother´s (c) and fetus´ (d) navigator profile; MR image of fetus´ heart by PolyGate (e).

Triggered T1 mapping

Quantitative mapping of the longitudinal relaxation time T1 by MRI is an important tool to study changes in the myocardium, eg. after myocardial infarction or quantification of the myocardial extracellular volume. Exact T1 values may be derived with 3D MR experiments, which are very time-consuming. For 2D MR experiments, the T1Gator tool by Arno Nauerth (IntraGators.com) provides apparent T1 values which differ systematically to the exact T1 values. This enables us to reliable distinguish tissues of different T1 values which permits a robust diagnosis Fig. 6.

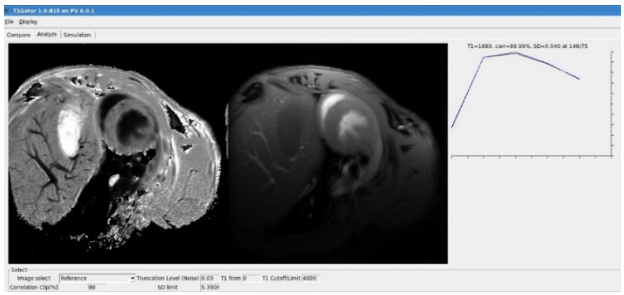

Fig. 6. Triggered T1 mapping. Exemplary T1 image from a flip angle varied gradient echo experiment (IntraGate FLASH) is shown on the left, the original MR image in the middle and the corresponding T1 value on the right.

Further selected projects

MRI may be used to quantitatively measure lung tumor burden and to follow up e.g. tumor growth. Fat, muscle, tissue-free fluids, and bones generate different signals in response to various radio frequency pulses at distinct magnetic fields, due to their different relaxation properties. We perform MRI studies on mouse using T1-weighted multi-slice-multi-echo (MSME) pulse sequences and analyze the volume of muscle and fat in a well-defined and comparable area of the mouse.

Selected cooperations

At present researchers from all four departments and the independent research groups benefit from the service we provide at our MRI facility. Regarding support in elaboration of new examination protocols and programming of sequences we have a close cooperation with Clemens Müller (Department of Radiology, Kerckhoff-Klinik GmbH, Bad Nauheim). Regarding the T1 Gator Tool for T1 mapping and the PolyGate Tool we work together with Arno Nauerth (IntraGator.com, Germany).

Micro X-ray Computed Tomography (µ-CT)

This facility was established in May 2017 with the purchase of the Bruker SkyScan 1276, an *in vivo* and *ex vivo* X-ray microtomograph. Our µ-CT offers high spatial resolution, the ability to differentiate between different tissue densities and it allows three-dimensional visual reconstructions of tissue. The SkyScan 1276 is suitable for analysis of bones, vasculature, lung, heart, body fat and tumors of living animals like rat, mice and smaller species, but also qualifies for *ex vivo* analyses. At the moment one scientist from each department is in charge of measurements for their relevant department. Below, images of selected projects are presented. *Ex vivo* µ-CT is used routinely to quantify skeletal tissue mass in small animal

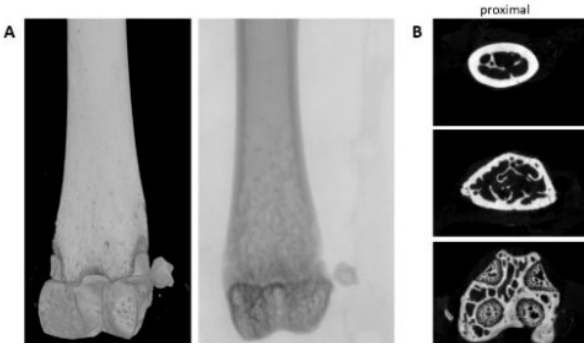

Fig. 7. µ-CT of a mouse distal femur. (A) longitudinal view, (B) transaxial view from distal to proximal. Pictures provided by S. Sapski, Dept. II.

models. Fig. 7 depicts the femur of a mouse. Longitudinal studies on *in vivo* rat or mouse lung using prospective respiratory gating is possible as well; two different methods of detector motion, -step and shoot and continuous-, may be used and we are able to achieve with new detector motion method "Continuous" excellent pictures with shorter scan time and less X-ray burden (Fig. 8). In Fig. 9 the heart of an adult zebrafish fixed with paraformaldehyde and stained with 2.5% phosphomolybdic acid (PMA) contrast agent is shown.

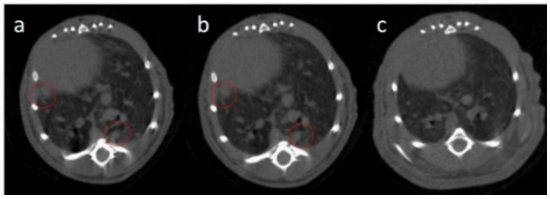

Fig. 8. *µCT of a mouse lung with tumor burden (a,b), without (c), step and shoot (a) and contiuous (b,c) method. Pictures provided by K. Turkowski & Y. Knepper, Dept. IV.*

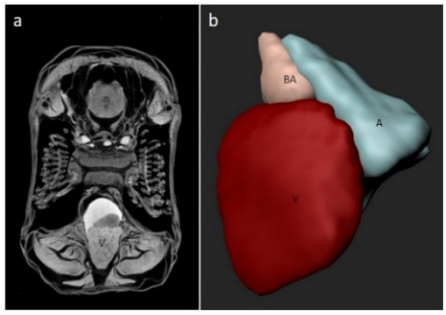

Fig. 9. *µCT of ex vivo zebrafish heart (a) and 3D model of the heart (b). Pictures provided by A. Bensimon-Brito, Dept. III.*

Publications (2019-2022)

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Bioinformatics

- **Dr. Mario Looso (group leader)**

Members of the IT / Bioinformatics Service Group

Lab Members – since 2019
(Current / Past)

Postdocs
Dr. Carsten Kuenne
Dr. Sweta Talyan
Dr. Jens Preussner
Dr. Guilherme Valente

Students (PhD)
Mette Bentsen
Vanessa Heger
Hendrik Schultheis
Jasmin Walter
Aditya Bhagwat

Students (MSc)
Kristina Mueller
Yousef Alayoubi
Leon Metzger
Jan Detleffsen
Philipp Goymann
Marina Kiweler
Arsenij Ustjanzew
Sebastian Lieske
Micha Kessler
Moritz Hobein

Visiting Scientists
Dr. Salma Dilai

Staff
Kathrin Klee
Noah Knoppik
Peter Hofmann
Vincent Heinz
Nina Meixner
Rene Wiegandt
Franz Ziegengeist
Hosro Lotfikah
Frank Voss
Daniel Spothelfer

Trainee
Adrian Hirsemann

The unit is organized as a combined group of classical computational group with a focus on infrastructure and IT support, as well as a scientific support group with a focus on applied bioinformatics. The group structure and its funding sources are depicted the organigram above. The combined setup of the group facilitates planning, design and implementation of tailored IT solutions for scientific needs, the group composition brings together all career levels from undergraduate students, bachelor and master degrees, as well as “Fachinformatiker”, PhD students and Postdocs.

Ongoing Projects

The epigenetics toolbox

The study of epigenetic regulation of gene expression is crucial to understand the mechanisms underlying differentiation of complex tissues. In this context, transcription factors (TFs) play important roles in targeting particular genes. However, while recent advancements of high-throughput sequencing methods generate massive amounts of data on epigenetics and TF binding, there is an increasing need for robust bioinformatics tools to study, interpret and integrate the data. Thus, we develop an epigenetics toolbox to perform bioinformatics analysis in the context of TF binding networks.

In order to identify potential TF binding sites in rare cell types, we developed TOBIAS (Transcription factor Occupancy prediction By Investigation of ATAC-seq Signal; Bentsen et al., *Nat. Commun.,* 2020), which utilizes TF footprinting to predict TF binding

(Fig. 1A). Based on ATAC-seq or SC-ATAC-seq data, TOBIAS finds genomic positions where chromatin is protected by Tn5 cutting due to binding of TFs - positions known as footprints. These footprints are then correlated with known TF sequence preference in a genome-wide manner.

In conclusion, our toolbox significantly extends standard analysis on ATAC/ChIP data and thereby helps to unravel the influence of TF binding within a diverse set of biological conditions. It is open to a multitude of omics data formats and comes with robust pipelines making them applicable to a variety of cells, tissues and organisms.

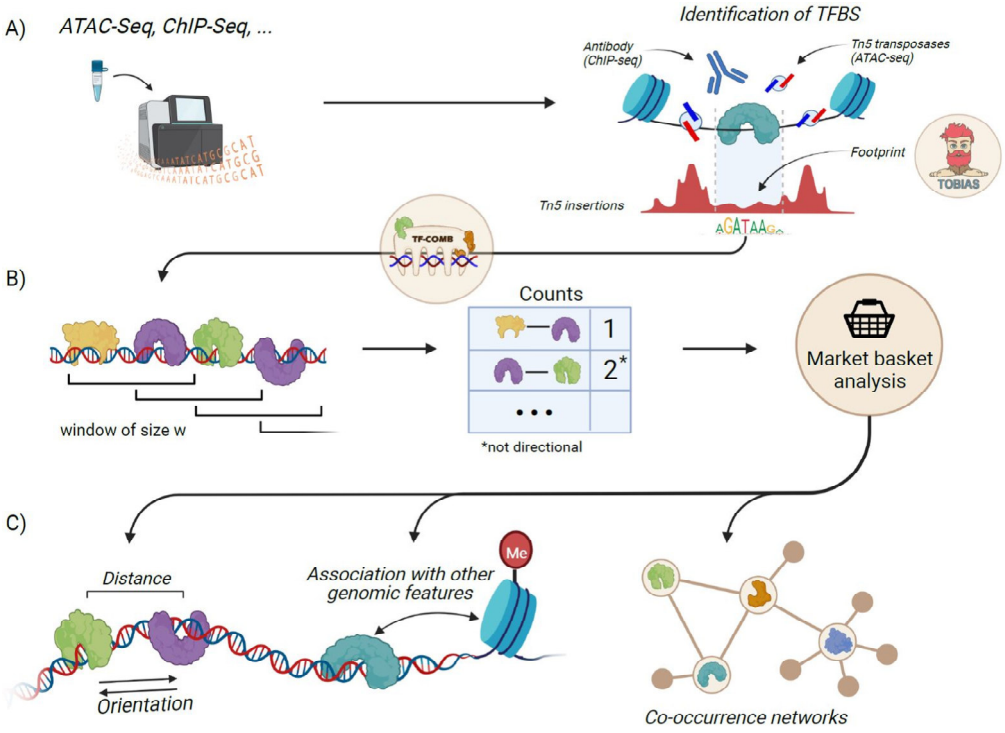

Fig. 1. Overview of the epigenetics toolbox. A) *TFBS can be extracted from different epigenetics assays e.g. ATAC-seq and ChIP-seq, but there are no limitations on the input source (top left). Using TOBIAS, a software from our toolbox, TFBS can be identified from (sc)ATAC-seq as bound TFs create footprints in ATAC-seq insertions.* ***B)*** *The identified TFBS act as input for TF-COMB, a tool for unraveling TF co-occurrences by counting the TFs within specific windows followed by an adapted market basket analysis.* ***C)*** *To discover TF binding grammar TF-COMB offers a variety of further analyses including preferred distance and orientation, associations with genomic features and network analysis of TF pairs.*

From TOBIAS analyzes in multiple cell types derived from SC ATAC datasets, it became apparent that distinct TFs often act together in the same biological conditions - a concept known as *TF co-occurrence*. In order to detect co-occurring TF binding sites, we further developed the TF-COMB (Transcription Factor Co-Occurrence using Market Basket analysis; Bentsen et al., *CSBJ,* 2022) framework, which utilizes a market basket analysis (MBA). MBA has classically been applied to investigate shopping habits such as “if the customer buys cereal, they are likely to buy milk”, however, this approach can be applied to TF co-occurrence analysis like “if TF1 binds, it is also likely that TF2 binds” as well (Fig. 1B). Our co-occurrence analysis on TF binding sites uncovers an organizes network of TF-TF co-occurrences, which also exhibits a particular syntax in terms of distance, binding site orientation and relative location in open chromatin. Interestingly, this observation of *binding grammar* also extends to the co-occurrence of TFs with known histone modifications and genomic elements, as we uncovered groupings of TFs specifically co-localizing within enhancers, promoters and even closed chromatin (Fig. 1C).

multiCRISPR² and EXCI-SEq

The CRISPR-Cas technique has revolutionized the way of editing genomes. CRISPR is based on a guide RNA (gRNA) and a nuclease, which is guided to the desired site in the genome by appropriate pairing of the gRNAs with the target DNA. By deleting, inserting or altering the genome on target locations, diverse gene modifications are attainable.Together with the Kaulich group in Frankfurt, we established EXCI-SEq, a new method intended to utilize a defined pair of gRNAs for excising specific region in genome (Fig. 2A). In order to accomplished the aim of designing the gRNAs, we developed a computational tool called multicrispr (Bhagwad et al., *LSA*, 2020) and now successively multicrispr², which is an R package harboring the functionality to design single gRNAs and paired gRNAs libraries for large scale screenings.

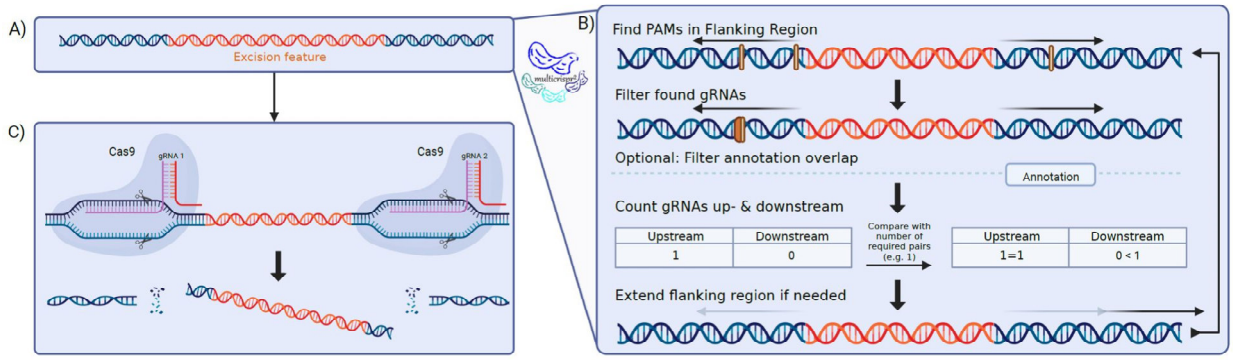

Fig. 2. Overview of EXCI-SEq and multiCRISPR² for designing pairs of gRNAs. A) *Defining feature regions to be excised.* ***B)*** *Flowchart on how multiCRISPR² designs gRNA pairs around the defined excision feature.* ***C)*** *Using EXCI-SEq to excise the feature region.*

There are a number of parameters to consider for designing gRNA libraries. A large part of the work is to evaluate the gRNAs found according to their potential target activity. This applies to both the on-target region and the off-target region. With the goal of maximizing on-target activity and minimizing the number of off-targets for each gRNA, multicrispr² applies a series of filters to detect gRNA pairs for features of interest (Fig. 2B). Notably, multicripsr² allows for an additional filter by giving regions in which no gRNA should be located, an often requested feature in context of large scale library designs. The multicrispr² package is used within a standardized framework, which allows for reproducible and tailored library designs (Fig. 2C).

The BCU repository

Omics data and data analysis are characterized by large quantities, high dimensionality, and complex workflows. This frequently leads to multiple competing interpretations of the raw data that highly benefits from interactive exploration based on advanced IT infrastructure and software. To address this obstacle, we develop the BCU repository, a combination of a i) web-based front-end for interactive data access, ii) Kubernetes cloud frameworks and iii) docker based web-applications. The repository utilizes iv) an internally developed offline operator for Kubernetes called MAMPOK, which keeps track of deployments, communication and data transfer to and from the cloud, as well as further management tasks. In this context, typical projects are defined as an experiment with a specific experimental setup, the generation of raw omics data, a versioned interpretation of the data, and finally a running application that presents the interpretation of the data (Fig. 3).

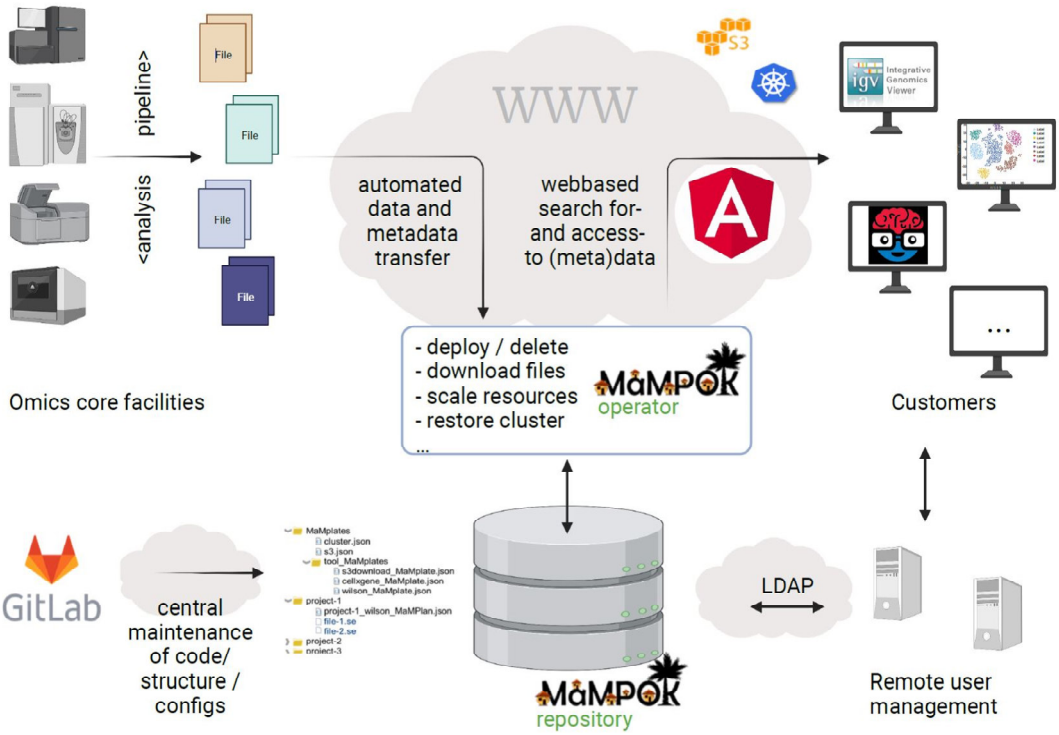

Fig. 3. *Overview on repository infrastructure.*

The web front end provides access for non computational scientists to the repository and offers integration of standard user management systems such as MS Active Directory, which allows for the utilization of existing organizational structures such as departments, research groups, or functional groups. The interface also allows the user to deploy working environments such as R Studio or IGV in a user specific context. In addition to providing interactive access to individual scientific projects, the BCU repository holds a standardized metadata interface for the generation and structured storage of experimental setups, following the FAIR principle for handling experimental data. Therefore, we developed a dynamic and hierarchical structure to describe and store metadata of biological experiments in a maschine and human readable format.

Highly automated and standardized analysis workflows

Due to improvements in method development, single-cell sequencing experiments are gaining popularity for uncovering individual cell populations in heterogeneous mixtures of cells. However, whereas analysis of bulk sequencing experiments can be analyzed in an automatized fashion, single-cell experiments require more hands-on tuning of parameters for each dataset. In order to streamline such analysis, we are developing a standardized set of workflows for the analysis of single cell experiments, in particular scRNA-seq, scATAC-seq and the multi-omics assay 10X Multiome (ATAC + RNA). These workflows consist of a number of sequential jupyter notebooks, which are pre-loaded with all necessary analysis steps, and the options to adjust parameters accordingly. Additionally, the results of the analysis are written out in a standardized manner, which can easily be shared with collaborators for input on the progression of the analysis.

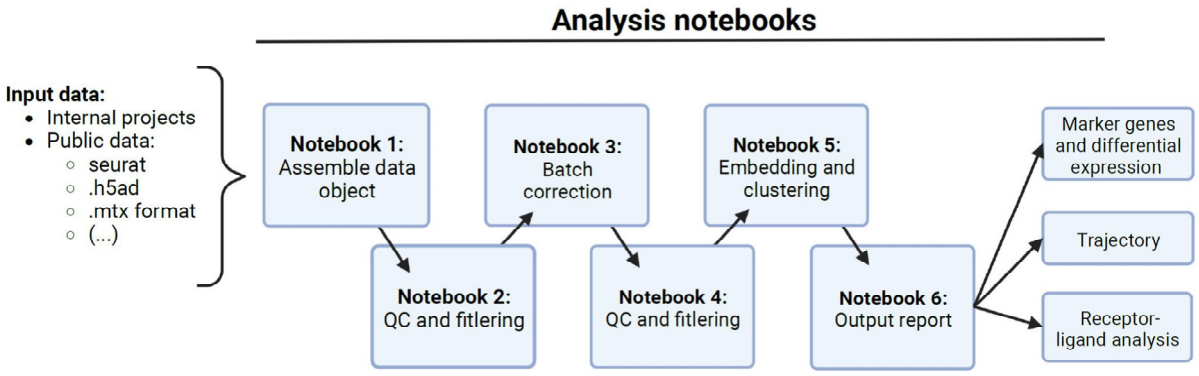

Fig. 4. Overview of a standardized scRNA-seq workflow. *Data from various sources are analyzed in an automated fashion through a series of notebooks, which always read the output of the previous notebook. After the standard steps, a number of downstream analyses are available including more specialized trajectory and receptor-ligand analyses.*

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Service Unit Animal Facility

- **Dr. Nouha Ritschel (head of the facility, responsible veterinarian)**
- **Dr. Petra Prückl (veterinarian, animal welfare officer)**

Animal caretakers:
Faris Al-Hassoni
Mohamed Al-Suliman
Christina Borchardt
Felix Brecher
Oliver Buchhold
Lisa Buchholz
André Cannon
Meike Diefenthäler
Petra Eichenauer
Tanja Enders
Carina Frank
Michael Gajda
Lukas Giese
Julia Grala
Jennifer Heller
Karin Jung
Martin Laszczyk
Annette Löffler
Felicienne Monsi-Agboka
Monika Müller-Boche
Romina Passarelli
Michelle Pauels
Rita Retzloff
Fabienne Schilling
Diana Schmidt
Bärbel Schmück
Nina Schneider
Annika Welzel
Kevin Wilson
Ana-Maria Wojtaschek
Yuliya Ziab

2 **external** staff members

1 **trainee**

Cage cleaning unit:
Abdenur Adhen
Jochen Fabacsowics
Marcus Heller
Manuel Kuprat
Ralph Mannert
Eric Schmück

Transgenic Service:
Susanne Kreutzer
Daniel Heil

General Facility Structure and Organization

Despite all efforts to use alternative methods, animals continue playing a central role in biomedical research dealing with complex organ and systemic functions. At the Max Planck Institute, the following species are currently kept: mice, rats, zebrafish, medaka and drosophila.

The animal facility is currently located at two sites and provides husbandry appropriate for individual species in accordance with legal requirements as well as scientific demands. This is achieved by 1) the proper technical equipment, 2) an optimized service-oriented management, and 3) qualified and professional staff members.

The design of the animal facility allows the partitioning into specific pathogen free (SPF) areas. Within the facility, we also have laboratories accessible to scientists. This allows experimental procedures to be performed without losing the hygienic status of the affected animals.

In order to avoid fatal infections of the entire mouse population, incoming animals have to pass veterinary checks while remaining quarantined in the isolation ward for a defined period of time. Once animals have passed the mandatory diagnostic tests and the quarantine period, they can be moved into the facility.

Microbiological and serological samples are routinely taken in all rooms of the animal house to control the hygienic status of the facility. As we are dedicated to applying the 3Rs wherever possible, we take those samples from resident animals, which allows us to reduce the number of required animals. It is guaranteed that the National Animal Protection Law, Directive 2010/63/EU of the European Parliament and

of the Council of 22 September 2010 on the protection of animals used for scientific purposes, the Convention on International Trade in Endangered Species of Wild Fauna and Flora as well as National Epidemical Regulations are incorporated into all regulations concerning housing and maintenance. The two responsible veterinarians ensure optimal medical surveillance at all times, especially regarding the animals that are part of experimental procedures and schemes. The continuous surveillance of animals in ongoing experiments by our Animal Welfare Officers allows for transparent communication between scientists and regulatory authorities.

The institute is an educational institution, and the animal facility is therefore constantly training new recruits. During their three years of apprenticeship, the trainees work in each section of the facility and also take part in theory lessons.

Each room of the facility is supervised by one member of the animal care staff who is, again, supported by another colleague to make sure that scientists always have a certain staff member in charge of their issues. This is mandatory for a full-service-oriented animal facility. Every staff member does both service works (listed below) and general husbandry work.

Husbandry

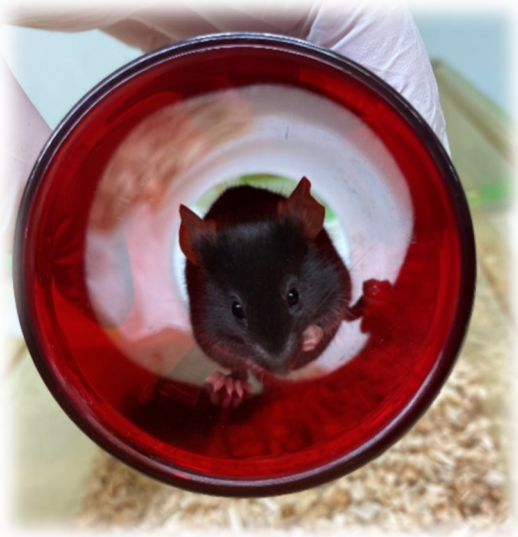
Husbandry of Mice and Rats
All mice are housed in Individually Ventilated Cages. This system allows us to control ventilation and temperature in order to provide best possible climatic conditions for housing and in order to avoid infections. All animals are provided with enrichment material according to legal requirements.

Rats are housed in conventional caging-systems, the common management for this species.
As measures to reduce, avoid and refine any form of suffering of the animals from their birth to their death the SOPs are permanently reviewed and optimized. As a result, the new method of cupping/tunnel handling to transfer animals during everyday work is currently being implemented.

As a further effort to put the non-required animals to a sensible use, rodents are now given to a bird of prey station as feed animals.

Husbandry of Fish
The aquarium configuration for fish (Danio rerio and Oryzias latipes) is a high-tech system. To enable an optimized food supply according to the different stages of development feeding robots are utilized.
To guarantee the best possible water quality, large water treatment plants equipped with mechanical and biological filters as well as regulated fresh water supply work constantly. In addition, continuous controls are run to monitor various parameters.

Husbandry of Drosophila
Drosophila melanogaster stocks are maintained in vials with culture media at 25°C on a 1.5 week generation cycle when necessary for experiments. Back up are maintained at 19°C on a 4-5 week generation cycle. Both back up maintenance room and experimental lab are combined to allow short distances for optimal working conditions.



Services

The facility offers a full service husbandry of our genetically modified mice. Valid care of the animals is ensured at all times including intensified care and professional assistance while the animals are in an experiment. Service tasks also include tissue biopsies, blood sampling and colony management supported by a database system as well as the arrangement of national and international animal shipments.

Transgenic Service

Another service implemented in the facility are so-called “assisted reproduction techniques”, which are provided by two specially-trained technical assistants. This way, it is possible to overcome breeding problems or prevent dissemination of infectious agents. Mouse lines not actively used in projects are often “kept on ice” and resuscitated later on when necessary, thus reducing costs and mouse numbers.

The murine germplasm is archived by the cryopreservation of spermatozoa, embryos or ovaries. Embryos are produced *in vivo* or by *in vitro* fertilization using fresh or cryopreserved spermatozoa. Blastocyst and pronuclear injections are performed routinely. The rederivation of mouse lines can be performed by transferring embryos and ovaries to suitable recipients.

Publications (2019-2022)

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Imaging Platform

- **Kenny Mattonet (service group leader and specialist for light microscopy)**
  Tobias Rasse (former service group leader and specialist for light microscopy, 2019 - 2021)

  Janett Piesker (application specialist for electron microscopy))
  Sebastién Gauvrit (former imaging specialist, 2019-2020)

Overview

The imaging platform provides centralized light- and electron microscopy services, supports the planning and optimization of imaging experiments and helps scientists analyze and interpret imaging data. Additional services and responsibilities include user training, maintenance and basic repairs, consultation, and establishment of new methods.

Transmission electron microscopy (TEM) is offered as a full service, which includes preparations, data acquisition and support during data analysis. In the past three years, the facility generated 2814 images of ultrastructures in support of 24 main projects. The available techniques include standard Epon embedding, specialized embedding for fragile embryonic tissues and negative staining. Further techniques such as progressive lowering temperature (PLT) embedding and immunogold labelling for tissues are being established.

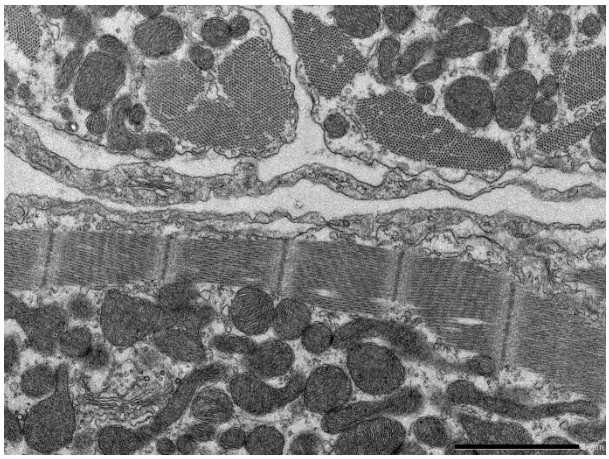

Fig. 1. ***Transmission electron microscopy of heart tissue at 6.000x magnification.*** *Sarcomeres cut lengthwise and crosswise, mitochondria and Golgi apparatus. Scale bar: 2 µm.*

Basic widefield and confocal laser scanning microscopy (CLSM) is mainly offered as self-service for users after introductions and hands-on training on the systems. In the past three years, 71 researchers and students actively used the four available light microscopes. Availability and hygienic access to the instruments was ensured throughout the pandemic.

Table 1: ***Current and future instrumentation in the facility.*** *Asterisks mark equipment that will be installed in late 2022/early2023. CLSM: confocal laser scanning microscope.*

Company	Microscope	Description
Jeol	JEM1400 Plus	Transmission electron microscope (TEM)
Leica	SP8-MP	CLSM, 2-photon setup, live cell imager
Leica	SP8	CLSM, live cell imager
Nikon	Ti2-Eclipse	Widefield with automatic slideloader
Zeiss	LSM880	CLSM, widefield, Airyscan 2 detector
Leica*	Thunder-Imager*	Widefield, FRAP-scanner, live cell imager
Leica*	LMD7*	Laser dissection microscope

The automated or semi-automated scanning of microscopy slides as well as the analysis of the data is offered as both self- and full service depending on user needs.

In addition, the facility is engaged in teaching and public outreach. We organize visits for high school students and host workshops for IMPRS and the CPI. The former coordinator of the facility, Dr. Tobias Rasse, played an essential role establishing the Max Planck Bioimaging Core Unit Network (MaxBI) that improves the collaboration between imaging core facilities throughout the Max Planck

Society. Furthermore, he helped with the development of guidelines for improving quality assessment and reproducibility in light microscopy (Nelson et al., 2021) and recommendations for operating imaging core facilities during the SARS-Cov-2 pandemic.

Projects & Services

The imaging platform constantly expands the available services and techniques. Between 2019 and 2021, Dr. Rasse developed new techniques for the automated acquisition and analysis of large datasets. His automatic sample recognition and segmentation algorithms improved the throughput of the Nikon Ti2-Eclipse slide loader. In addition, he developed the open source platform OpSeF for image segmentation by deep learning (Rasse et al., 2020).

Building on these improved slide scanning and segmentation protocols, we recently upgraded the slide loader and also developed new semi-automatic approaches for slide scanning in collaboration with Nikon. The improved protocols simplify the setup of small or medium sized screens in brightfield and fluorescence and make it feasible to use this instrument as a self-service.

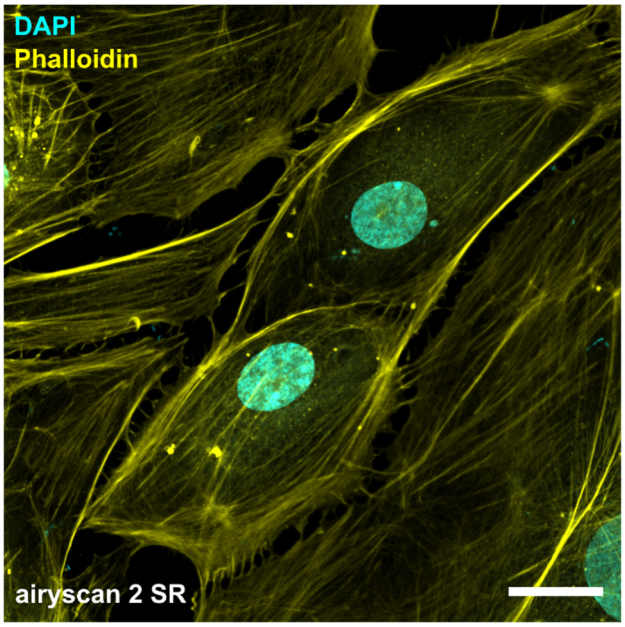

Fig. 2. ***HDLECs stained for Phalloidin and DAPI.*** *With a maximum axial resolution around 120 nm at 488nm, the Airyscan2 detector of the LSM880 is the best instrument for resolving intracellular structures in the imaging facility. Scale bar: 20 µm.*

Making use of the new high throughput scanning capabilities, we recently started a project aimed at the automation of lung morphometry. The goal is to feed in large high-resolution scans of mouse lung lobes and generate data that characterize the tissue such as the mean linear intercept (MLI), alveolar septal thickness and alveolar area.

To expand the available TEM services, we are establishing PLT embedding and immunogold labelling in collaboration with the electron microscopy core facility at the EMBL in Heidelberg. We identified several projects that would currently benefit from such labelling, optimized the sample preparation for these samples and will prepare the first embeddings and stainings in Q1 2023.

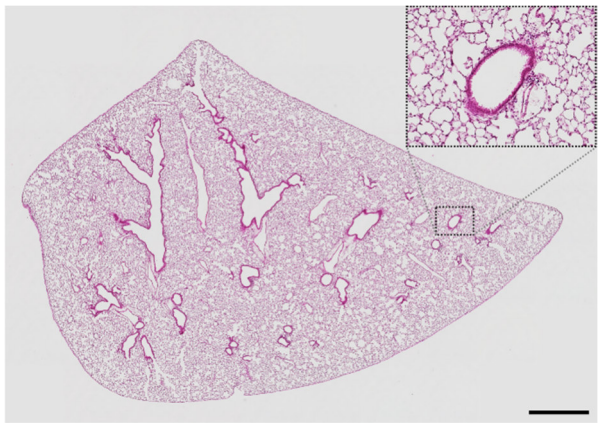

Fig. 3. ***Automated scan of HE-stained adult mouse lung lobe.*** *The object was scanned with a semi-automatic approach with cellular resolution and required less than 2 min of setup time. Focus, channels, shading corrections and artefact-free stitching do not require any user intervention, giving researchers access to high throughput microscopy as a convenient self-service. Scale bar: 1000 µm.*

Infrastructure Development

During the past years, the overarching included increased demand for live cell imaging as well as the scanning and analysis of large tissue sections and multi-well plates. We adapted the existing infrastructure to meet these objectives in multiple ways: We upgraded the SP8 and SP8-MP microscopes with assay design software and inserts for multiwell plates. The Zeiss LSM880 was equipped with additional fluorescence filters that allow the use of this microscope as a fully functional widefield setup for the fast scanning of tissue sections. We also upgraded the Nikon Ti2-Eclipse slide loader with new

software and highly customized user protocols that allow a convenient semi-automated or fully automated scanning of 1 to 120 microscopy slides in various resolutions. To handle the larger quantities of data, also upgraded existing image processing workstations and virtual machines. This includes modifications to make these workstations mobile and services that bring them to the offices of scientists that need them.

In addition to the upgrades of existing systems, we applied for and purchased two instruments:

1. A state-of-the art live cell widefield microscope with FRAP scanner (delivery in December 2022) that will enable the fast acquisition of multiwell plates in high resolution, long term live imaging experiments and high resolution scans of large cell culture and tissue samples.
2. A LMD7 laser micro dissection microscope (delivery in Q1 2023) for the fast dissection of tissues, living cells and for downstream applications such as cell culture, proteomics and transcriptomics.

To make space for the additional equipment, the imaging platform now includes an additional lab.

Most improvements in the electron microscopy facility in recent years aim to increase sample throughput. Old and defect equipment for ultramicrotomy was replaced with a new UC7 ultramicrotome and an EM Rapid trimming device. In addition, we invested in an embedding automaton for standard Epon embedding.

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Next Generation Sequencing Platform

- **Stefan Günther, Ph.D. (service group leader)**

  Samina Mahmood (technical assistance)

Overview

The Next Generation Sequencing (NGS) platform at the Max Planck Institute for Heart and Lung Research was established in 2013 to enable direct usage of a state-of-the-art sequencing technology for scientists of the institute.
The platform had started with an Ion-Torrent sequencing platform (Thermo Fisher “Proton”) that allowed only processing of smaller projects. However, the platform has developed continuously during the last years adopting the increasing demand of sequencing experiments generated within the institute. Milestones in the past years were the establishment of the Illumina sequencing platform by NextSeq500 instrument (2015) and upgrade to newest Illumina Nextseq system Nextseq2000 in 2021. Furthermore, the development of robotics-supported library preparation methods and establishment of high throughput methods were consistently pursued to enable faster handling of samples and realization of larger experiments at the same time.

More recently, the second generation of liquid handlers in terms of size and capacity have been commenced after an upgrade in 2017 (Perkin-Elmer “Zephyr” and “NGS Express”). Automated solution handling was moreover successfully established for more specialized demands, like small volume/sample processing or complex protocols (Takara Bio “SMARTer Apollo System”). To enable high throughput, walk-away solutions a new Beckman Biomek i7 Robot was acquired in Q2/2022. Additionally, we efficiently adopted the platform periphery for higher throughput by acquisition of supporting technologies, like plate-based QC for starting material (RNA, Agilent “Fragment Analyzer”, Perkin Elmer “Labchip GX Touch”) or final library preparations (DNA, Perkin-Elmer “Labchip GX Touch”).

These steps enabled the facility to reduce the turnover time for processing samples to two to three weeks in average with a weekly output of 100 - 200 samples, corresponding to approximately 10-20 experiments per week. Automatization of library preparation steps, optimization of the sequencing setup and loading as well as comparisons of available library preparation solutions and kits, could reduce the costs per sample significantly within the last years.

The major technique used in the institute is RNA-seq, followed by DNA sequencing approaches like ChIP-seq, ATAC-seq and Exome-seq. To minimize delays in data analysis we established, in collaboration with the bioinformatic core facility, fully automatized pipelines for those major applications. These start with non-pre-processed raw data and produce user-friendly results and publication quality figures (see Bioinformatic core MPI Bad Nauheim). Additionally, the data is compatible to further analysis with the GUI-based analysis software (Wilson), which had been developed in house.

A second branch within the core is the provision of single cell sequencing approaches for scientists at the institute. We first focused on single cell transcriptomics (scRNA-seq) but established also single cell epigenomics solutions. Currently, we develop and establish additional methods, like single cell ChIP, methylation or multi-omic approaches. To enable the usage of many different cell types, sizes and numbers we tried not to focus just on one single technology. Instead, we acquired a whole set of instruments to support experiments with a few hundred to 100.000s of cells, ranging from single nuclei to large differentiated primary cells with > 100µm in size (Fluidigm “C1”, Takara Bio “ICELL8”, 10x Genomics “Chromium Controller”, Silicon Biosystems “DEPArray”). To enable unbiased results and optimize the quality of cell suspensions used for single cell approaches we also implemented methods and strategies to discriminate live and death cells by application of levitation technology (LevitasBio “LeviCell”) or laminar washing to avoid centrifugation steps (Curiox “Laminar Wash Mini”). To reduce the costs of single cell experiments we also successfully established multiplexing strategies (cell hashing) for most of the single cell approaches.

Importance and usage of the facility is also represented in tremendous numbers of publications (> 40 in highly ranked journals) that are based or benefit from data or analyses achieved within the NGS core. To preserve this situation, the aim of the next years will be to maintain the quality and reliability to enable achievement of convenient data for all upcoming sci-

entific questions. Moreover, we will continuously focus on the development of new techniques as well as establishment of public available methods in order to keep the facility at respective state-of-the-art level in the future.

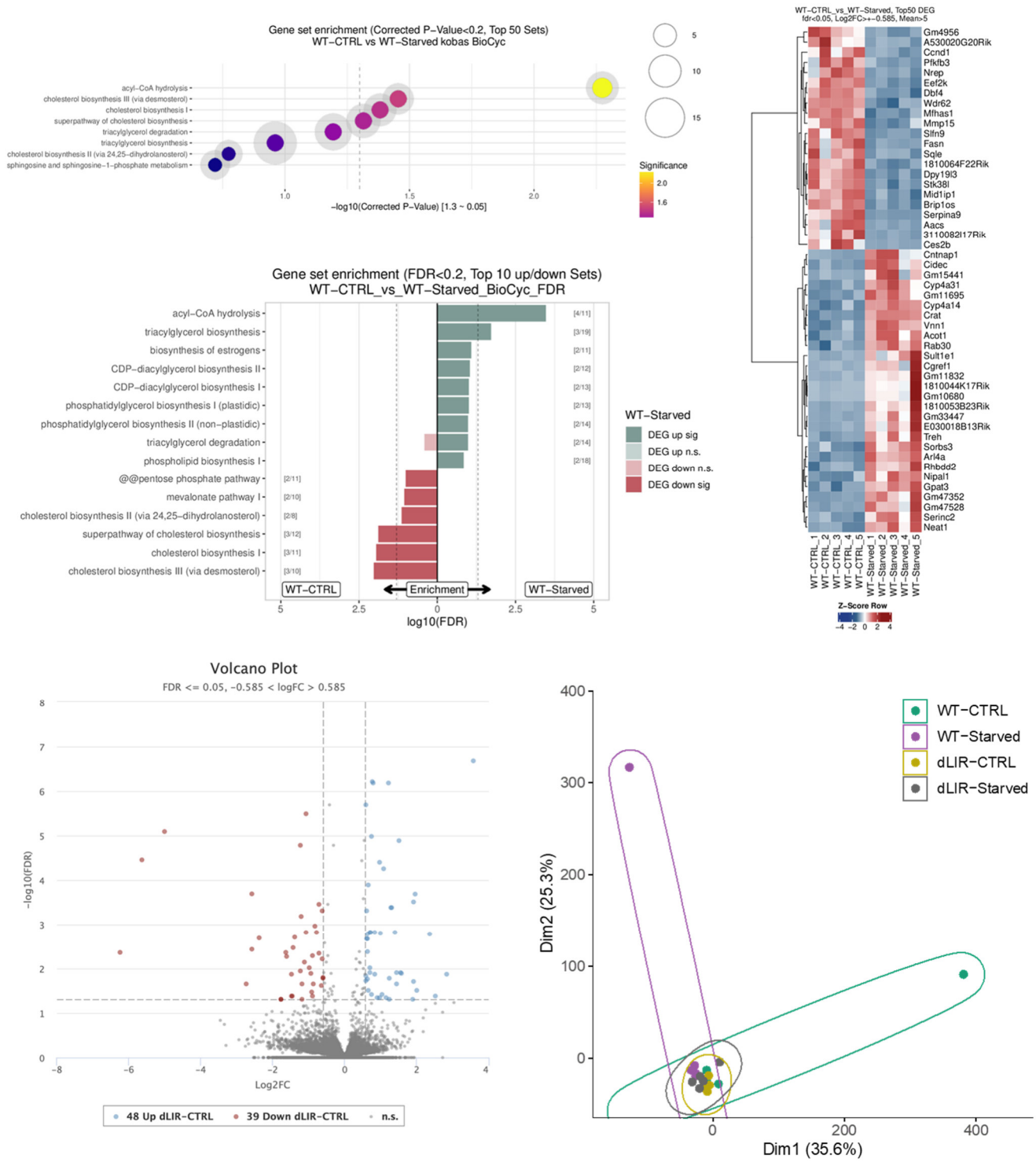

Fig. 1. *Exemplary plots for Analysis pipeline used for standard approaches like bulk RNA-seq, ChIP-seq or ATAC-seq.*

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Biomolecular Mass Spectrometry

- **Miloslav Sanda (Group Leader)**

  Diana Campos (Postdoctoral Researcher)
  Jonas Hart (Technician)

General overview

Introduction

Mass spectrometry (MS) is a very powerful analytical technique known to and used by most researchers in the natural and life sciences. Specifically in life sciences, MS has been more frequently recruited to reveal protein characteristics/identity by detecting its amino acid sequences.

The Biomolecular Mass Spectrometry Service Group at Max Planck Institute for Heart and Lung Research currently hosts, operates, and maintains 3 high resolution mass spectrometers one Orbitrap Q-exactive and two Orbitrap Q-exactive HF instrument. The team offers a collaborative support to the experimental design, execution and analysis of projects based on the mass spectrometry analysis of biomolecules.

Services

Our current portfolio of methodlogy includes a special emphasis on bottom-up proteomics approaches. We offer services such as: Protein identification by MS from SDS PAGE or in solution samples, Protein separation by HPLC/FPLC (RP/SEX/IEX) chromatography technigues, Protein separation by electrophoretic techniques, Peptide separation techniques such us Low pH and High pH reverse phase chromatography and HILIC-based chromatography.

Analysis of protein networks is also one of the pillars of our group. This encompasses Middle down proteomics services. We can do perform label-free untargeted quantitative analysis, quantitative proteomics using stable isotome labelling and quantification on the MS or MS/MS level.

In addition, we recently developed a highly sensitive and reproducible data independent label-free quantitative DIA/MS methods.

Rountinelly, we perform the identification of specifically enriched proteins in (immuno) affinity purification experiments as well as expression–proteomic comparison of mutant versus wild type or treatment versus control groups.

We also offer bioinformatics preprocessing of shotgun proteomics data

For better reproducibility and higher sensitivity, we have implemented SRM/PRM based methodology, which reduces the number of missing values and substantially increases sensitivity and specificity of mass spectrometry quantification from tandem mass spectra.

In adition to qualitative and quantitative proteomics analysis, we offer comprehensive analysis of postranslation modifications such as Phophorylation and Acetylation. In case of phosphorylation, we have implemented PRM methodology to increase sensitivity and reproducibility of the measurement.

Besides the proteomic analysis, we have implemented methods that focus on the quantitative analysis of RNA and DNA modification such as methylation etc. We developed new nanochromatography methodology which allow us to analyse modified nucleosides with high sensitivity and very low sample imput (less than 20 µg of DNA/RNA).

In collaboration with epigenetic group in 2022, we established methodology focused on methylation and acetylation of histones. Another case is the implementation methodlogy is qualitative and quantitative site-specific analysis of N- and O- glycosylation analysis with special focus on resolution of isobaric structure motifs.

Our Mission

We aim to empower collaborators in interpreting data derived from their experiments and provide reporting surpassing the plain provisioning of raw identification/quantitation data derived from mass spectra. Using a common denominator set of analytical approaches, our reporting combines documentation of sample preparation and mass spectrometric analysis with a first pass bioinformatic analysis. We are always looking to support exciting science with our expertise in proteomics and protein biochemistry in general. We strive to achieve the highest possible level of excellence in view of our resources. Our goal is to build bridges of collaboration and help you find the answers to each and every scientific questsion.

Project Highlights

Phospoproteomic Analysis

We participated in a study focused on the analysis of changes in post-translational modifications (PTMs) of phosphoproteomics. In this study, we aimed to identify arachidonic-acid-regulating signal transduction pathways modulating macrophage functions and their implications for ovarian cancer.

Our analysis helped identify the ASK1 - p38δ/α (MAPK13/14) axis as a central constituent of signal transduction pathways triggered by non-metabolized Arachidonic Acid (AA). This pathway was induced by the Ca^{2+}-triggered activation of calmodulin kinase II, and to a minor extent by ROS generation in a subset of donors. Activated p38 in turn was linked to a transcriptional stress response associated with a poor relapse-free survival. Consistent with the phosphorylation of the p38 substrate HSP27 and the (de)phosphorylation of multiple regulators of Rho family GTPases, AA impaired actin filament organization and inhibited actin-driven macropinocytosis. AA also affected the phosphorylation of proteins regulating vesicle biogenesis, and consistently, AA enhanced the release of tetraspanin-containing exosome-like vesicles. Finally, we identified phospholipase A2 group 2A (PLA2G2A) as the clinically most relevant enzyme producing extracellular AA, providing further potentially theranostic options.

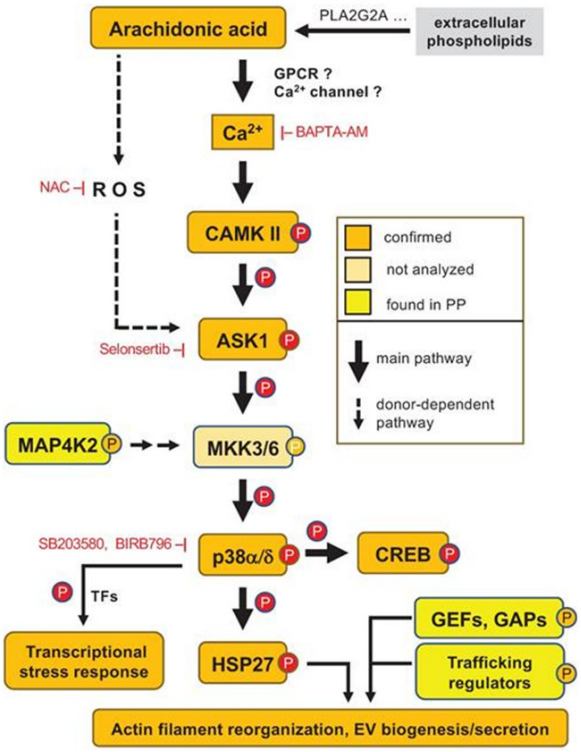

Fig. 1. ***Model of AA-regulated signal transduction pathways.*** *An AA-triggered Ca2+- ASK1 - p38 pathway induces a transcriptional stress response and mediates HSP27 phosphorylation, thereby contributing to actin filament reorganization in concert with AA-regulated RhoGEFs and RhoGAPs. Other protein kinases phosphorylated in response to AA, such MAP4K2, may also impinge on this pathway. ROS produced by different mechanisms may contribute to p38 phosphorylation to a minor extent in a subset of donors. Orange: phosphosites identified by MS and confirmed by immunoblotting; yellow: phosphosites identified by MS. EX: extracellular space; PM: plasma membrane; CYT: cytosol; NUC: nucleus. AA is most likely derived from extracellular phospholipids with PLA2G2A possibly playing a crucial role.*

Proteomics Analysis

Also, we collaborated with a team on a study focused on the identification of cytoplasmic sterol-binding proteins in *Saccharomyces cerevisiae*. Ergosterol is a prominent component of the yeast plasma membrane and essential for yeast cell viability. It is synthesized in the endoplasmic reticulum and transported to the plasma membrane by non-vesicular mechanisms requiring carrier proteins. Oxysterol-binding protein homologues and yeast StARkin proteins have been proposed to function as sterol carriers. Although many of these proteins are capable of transporting sterols between synthetic lipid vesicles in vitro, they are not essential for ergosterol transport in cells, indicating that they may be functionally redundant with each other or with additional—as yet unidentified—sterol carriers. To address this point, we hypothesized that sterol transport proteins are also sterol-binding proteins (SBPs), and used an in vitro chemoproteomic strategy to identify all cytosolic SBPs. We generated a cytosol fraction enriched in SBPs and captured the proteins with a photoreactive clickable cholesterol analogue. Quantitative proteomics of the captured proteins identified 342 putative SBPs. Analysis of these identified proteins based on their annotated function, reported drug phenotypes, interactions with proteins regulating lipid metabolism, gene ontology, and presence of mammalian orthologues revealed a subset of 62 characterized and nine uncharacterized candidates. Five of the uncharacterized proteins play a role in maintaining plasma membrane integrity as their absence affects the ability of cells to grow in the presence of nystatin or myriocin. We anticipate that the dataset reported here will be a comprehensive resource for functional analysis of sterol-binding/transport proteins and provide insights into novel aspects of non-vesicular sterol trafficking.

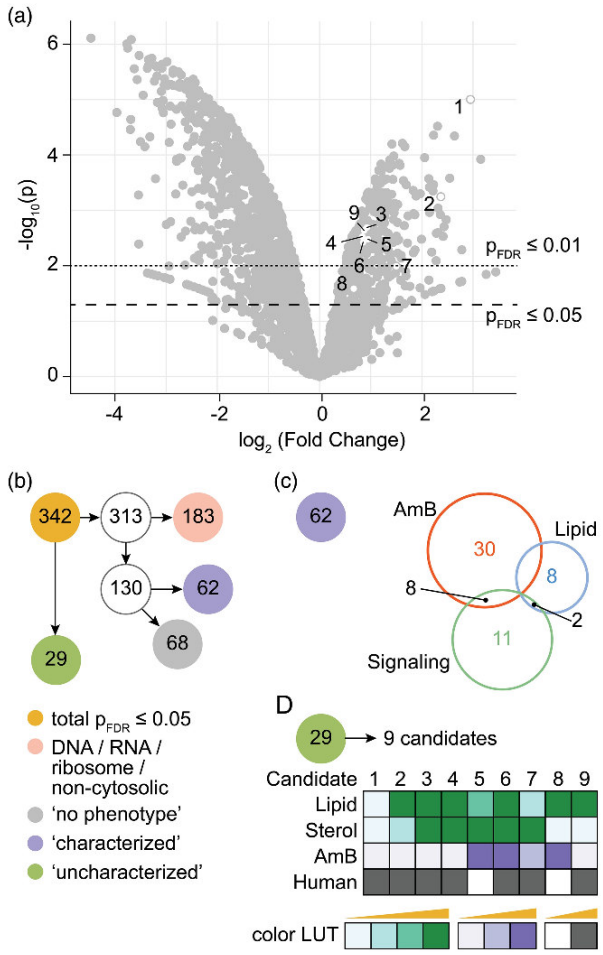

Fig. 2. ***Identification of candidate sterol-binding proteins by quantitative proteomics.*** *(a) Volcano plot of the enrichment analysis is shown. The x axis is the coefficient of the statisti-cal analysis (n = 4) and corresponds to log2-transformed normalized MaxQuant ratios of sample over input. The y axis is negative log10-transformed p values from the enrichment analysis. The right quadrant of the plot shows proteins that are enriched in the sample. The dashed and dotted lines represent nominal significance cut-offs of p = .05 and .01, respectively. (b) Flow chart depicting the prioritization of 342 candidate sterol-binding proteins that were significantly and reproducibly enriched in the sample.*

Development of Software Tools

Besides Proteomics services, we also contribute to method development. In the context of publishing data sets acquired by mass spectrometry or works based on such molecular screens, metadata documenting the instrument settings are of central importance to the evaluation and reproduction of results. A single experiment may be linked to hundreds of data acquisitions, which are frequently stored in proprietary file formats. Together with community-, repository-, as well as publisher-specific reporting standards, this state of affairs frequently leads to manual —and thus error prone— metadata extraction and formatting. Data extracted from a single file also often stand in for an entire file set, implying a risk for unreported parameter divergence. To support quality control and data reporting, the C# application MARMoSET extracts and reduces publication relevant metadata from Thermo Fischer Scientific RAW files. It is integrated with an R package for easy reporting. The tool is expected to be particularly useful to high throughput environments such as service facilities with large project numbers and/or sizes.

Fig. 3. ***Scheme of information extraction from MS raw files.***

Quantitative Analysis of RNA/DNA Modifications

In adition to mass spectrometry proteomics analysis we recently implemented nano LC-MS-PRM methodology with collaboration with Dr. Gu. We have optimized nanochromatography separation connected to nano electrospray to aim reasonable sensitivity for quantitative mass spectrometry analysis of modified nucleosides. The combination of nanoseparation and nanospray ionisation allowed us to analyse modification of limited (<50µg) amout of RNA/DNA. (Manuscript in preparation)

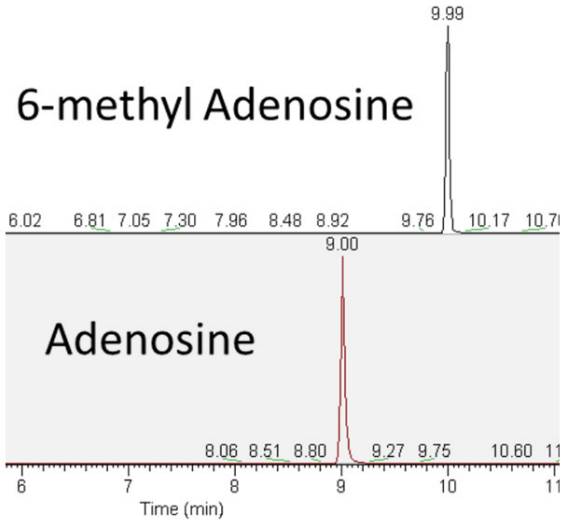

Fig. 4. ***Example of adenosine methylation analysis of 10ng of digested RNA injected on capillary column.***

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6. International Max Planck Research School for Molecular Organ Biology (IMPRS-MOB)

▪ **Dr. Bilge Reischauer (IMPRS Coordinator)**

To promote young and talented scientists, the International Max Planck Research School for Heart and Lung Research was established in 2008 as a close cooperative effort between the Max Planck Institute for Heart and Lung Research in Bad Nauheim and the two neighboring universities, Justus Liebig University Gießen and Goethe University Frankfurt. The mission of the IMPRS-HLR is to provide state-of-the-art training in biomedicine and in basic molecular mechanisms that underlie cardiovascular and lung diseases and to translate this knowledge into new individualized therapies. The IMPRS-HLR was positively evaluated in 2012, and funding was extended in 2014 for another 6 years until 2020.

Within these two funding periods, the Max Planck Institute for Heart and Lung Research (MPI-HLR) moved through a phase of dynamic expansion and growth with now 4 departments and several independent research groups and scientific service units. The research projects conducted by the doctoral students during this time covered a wide scope reflecting the research focus of the institute and the university partners, which include cell biology, cardiovascular and lung physiology, development and remodeling, but also related areas such as metabolism, immunology and tumor biology.

Based on the achievements of the last decade and new scientific developments within the MPI-HLR and the collaborating universities, a new name of the graduate school was proposed for the prolongation of IMPRS-HLR: International Max Planck Research School for Molecular Organ Biology.

IMPRS
Molecular
Organ Biology

The broadening of the thematic orientation of the IMPRS from a focus on the cardiovascular system and the lung to a more holistic view on organ biology reflects the scientific development of the MPI-HLR and its partner universities.
After the successful evaluation in November 2019, the IMPRS-MOB started in October 2020. Based on the well-established core curriculum of the graduate school since 2008, the program has evolved within the new scope of IMPRS-MOB.

Fig.1. *IMPRS-MOB class of 2022.*

Recruitment and admission

Key to a successful school is the recruitment of talented and enthusiastic graduate students. Candidates for the IMPRS-MOB program are recruited yearly through a public and transparent recruitment process. Our annual call is advertised in international journals, social media and science platforms and we receive around 300 applications per call. After careful evaluation by the recruiting committee, about 10% of the applicants are shortlisted and proceed with the application process.

In the last two years, we have held the recruitment symposium online to be able to cope with the pandemic situation but also to enable more applicants to present as we are not limited by budget or time. During the entire recruitment process, the applicants talk with faculty member, meet the PhD committee and fellow IMPRS students and receive an overview of the graduate program by the IMPRS coordinator.

At the end of the recruitment process, successful applicants are assigned to their matching research group, and the new PhD students start latest in October of the same year. Besides external applicants, IMPRS-MOB also accepts internal candidates who already started their PhD at the MPI or collaborating universities. Both tracks have to apply the same way and pass the recruitment stages for admission to IMPRS-MOB.

Graduate program

The graduate school is centrally located at the MPI-HLR in Bad Nauheim and is well-connected by public transportation with the collaborating universities. Students not only benefit from the scientific exchange with these universities but also from the multi-disciplinary offerings of the affiliated graduate programs in Gießen and Frankfurt and the excellence cluster Cardio Pulmonary Institute (CPI).

IMPRS-MOB has established excellent training opportunities and provides guidance and support to young scientists in various stages of their career:

- To foster the base for reliable research, the students attend workshops on good scientific practice right at the beginning of their PhD.
- The core of our educational program consists of 4 lecture blocks that provide a condensed recapitulation of basic molecular biology. While giving an introduction into the research topics, the lectures bring students with heterogeneous educational background on the same level.
- In methods seminars and workshops, the doctoral students gain new insights into state-of-the-art technologies and methods.
- An important part of the mentoring program of each student is the thesis advisory committee (TAC). The main goals of the individual TAC are to mentor and advise students, to monitor the progress of the research project, and to assist students in all aspects of career planning and networking.
- The annual IMPRS retreat is one of the scientific highlights where IMPRS students meet international experts, present and discuss their research projects and establish networks.
- The IMPRS students can attend career development workshops designed for different career stages such as academic writing and presentation skills and receive financial support for scientific conferences by IMPRS.

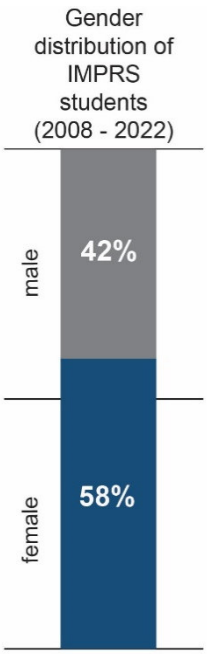
Students who graduate IMPRS after three years are invited to join all IMPRS-MOB events and activities. Especially the monthly progress report series benefits from the experience and expertise of our IMPRS alumni.

Fig.2. Graduation of IMPRS Class 2019.

Statistics and Awards

Since 2008, the IMPRS has accepted 210 students from all over the world with 58% being female scientists. Amongst these students, 165 students came from abroad (78%) to start their PhD at IMPRS-MOB. The largest group of international students is from China (41 students), followed closely by India (32 students) and Italy (16 students). Again, this reflects the diversity and international character of our graduate school.

Country of origin	Amount of students
Germany	45
China	41
India	32
Italy	16
Iran	11
Turkey	5
Egypt, Spain, Portugal	4 per country
France, Britain, Singapore, Romania	3 per country
Venezuela, Belarus, Brazil, Ghana, Mexico, Morocco, South Korea	2 per country
other countries	1 per country



Our doctoral students are successful in publishing their research in peer-reviewed journals and submit at least one first-author manuscript within their PhD. We want to highlight the following three IMPRS alumni who received awards for their outstanding PhD research in the last two years:

1) Karla Rubio received the research award of the German Society for Pneumology and Respiratory Medicine in 2020. 2) The Rudi Busse doctoral award for the best scientific doctorate in the faculty of medicine at Goethe University has been awarded to Jorge Carvalho in 2020. 3) Mohamed El-Brolosy received 2021 the Otto Hahn Medal and the Peter Hans Hofschneider Prize for ground-breaking discovery of molecular mechanisms underlying the phenomenon of transcriptional adaptation.

Overall, the graduate program provides fundamental support in scientific topics and beyond. The wide scope of our offerings enables students to connect with topics outside their own research field, foster interdisciplinary collaboration and innovative thinking and build a great foundation for a career in life sciences.

7. Third Party Funding and International Cooperations

Excellence Initiative (BMBF, DFG)

- The Cluster of Excellence Cardio Pulmonary Institute (Bad Nauheim, Frankfurt, Giessen)

Collaborative Research Centers (DFG)

- The Transregional Collaborative Research Center 81 “Chromatin Changes in Differentiation and Malignancies” (Bad Nauheim, Giessen, Marburg, Rotterdam)
- The Transregional Collaborative Research Center 128 “Initiating/effector versus regulatory mechanisms in Multiple Sclerosis – progress towards tackling the disease” (Bad Nauheim, Frankfurt, Bochum, Mainz, Munich, Munster)
- The Transregional Collaborative Research Center 267 “Nichtkodierende RNA im kardiovaskularen System” (Bad Nauheim, Munich, Frankfurt, Hannover)
- The Collaborative Research Center 834 “Endothelial Signalling and Vascular Repair” (Bad Nauheim, Frankfurt)
- The Transregional Collaborative Research Center 1039 “Liquid Signalling Diseases” (Bad Nauheim, Frankfurt)
- The Collaborative Research Center 1213 “Pulmonale Hypertension and Cor Pulmonale” (Bad Nauheim, Giessen)
- The Collaborative Research Center 1366 “Vascular Control of Organ Function” (Berlin, Giessen, Munich, Mannheim, Heidelberg, Frankfurt)
- The Collaborative Research Center 1526 “Pathomechanisms of Antibody-mediated Autoimmunity (PANTAU) – Insights from Pemphigoid Dise” (Bad Nauheim, Lübeck, Kiel, Erlangen-Nuremberg, Würzburg)
- The Collaborative Research Centre 1531 “Damage control by the stroma-vascular compartment” (Bad Nauheim, Frankfurt, Mainz, Heidelberg, Berlin)

(Clinical) Research Groups (DFG, BMBF)

- DFG Clinical Research Group “Virus-induced Lung Injury: Pathobiology and Novel Therapeutic Strategies” (Giesen, Marburg, Bad Nauheim)
- BMBF Clinical Research Group “Neoadjuvant anti PD-1 Immunotherapy in resectable NSCLC: The NEONUM Trial” (Heidelberg, Bad Nauheim)
- BMBF Clinical Research Group “Monitoring von Patienten mit NSCLC – epigenetische Analysen von Liquid biopsies sowie RNA-Analysen von Atemluftkondensaten (EMoLung)” (Bad Nauheim)

BMBF Networks

- German Center for Lung Research (DZL) (Bad Nauheim, Giessen)
- German Center for Cardiovascular Research (DZHK) (Bad Nauheim, Frankfurt, Mainz)
- DZHK Shared Expertise “Temporal and spatial dynamics of immune cell infiltration, accumulation and resolution following myocardial infarction / cardiac injury” (Bad Nauheim, Essen)
- DZHK Shared Expertise “Coronary heterogeneity in heart regeneration: finding common traits using a comparative approach” (Bad Nauheim, Mannheim)
- DZHK Shared Expertise “Microvascular shear stress-mediated NF-.B activation and its role in activity-induced hyperemia” (Bad Nauheim, Lübeck)
- DZHK Shared Expertise “Identification of noncoding regulatory variants in coronary artery diseasel” (Bad Nauheim, Heidelberg)
- DZHK Shared Expertise “Proteomic screening for neutrophil-derived mediators promoting myocardial infarction repair” (Bad Nauheim, Frankfurt, Munich)
- DZHK Shared Expertise “Identifying a new gene therapy target for cardiac regeneration” (Bad Nauheim, Goettingen)
- DZHK Shared Expertise “Epigenomic Dynamics of synthetic Heart regeneration” (Bad Nauheim, Goettingen)
- DZHK Shared Expertise “Leveraging mechanisms from zebrafish to promote engraftment of transplanted human cardiomyocytes” (Bad Nauheim, Hamburg)
- DZHK Shared Expertise “AAVs for genetic manipulation of cardiovascular phenotypes” (Bad Nauheim, Frankfurt, Kiel)
- ERA-CVD Verbund IMPHLeXIONS: Inflammation and metabolism in pulmonary hypertension associated with displaced inactivation of X chromosomes: New therapeutic options” (Germany, Canada, Austria)

LOEWE Networks

- LOEWE GLUE: Focus on G protein-coupled receptor Ligands for Underexplored Epitopes (Bad Nauheim, Marburg, Giessen, Frankfurt, Darmstadt)
- LOEWE iCANx: Focus on Cancer-Lung (Disease) Crosstalk: Tumor and Organ Microenvironemment (Bad Nauheim, Giessen, Marburg)
- LOEWE Zentrum fur Translationale Medizin & Pharmakologie (Bad Nauheim, Frankfurt)
- LOEWE Center: Frankfurt Cancer Institute (Bad Nauheim, Frankfurt, Langen)
- LOEWE Ubiquitin-Netzwerke (Bad Nauheim, Frankfurt, Darmstadt)

EU Framework Programs

- TAaGC – ERC Advanced Grant "Transcriptional Adaptation and Genetic Compensation" (Coordinating Function)
- EMERGE – ERC Consolidator Grant "Epigenetic and metabolic regulation of endothelial heterogeneity" (Coordinating Function)
- ZMOD – ERC Advanced Grant "Blood Vessel Development and Homeostasis: Identification and Functional Analysis of Genetic Modifiers" (Coordinating Function)
- V.A. Cure – Innovative Training Networks: "A multidisciplinary approach towards sustainable improvement in rare diseases care uniting Europe's top class vascular research to find new treatment strategies for vascular anomalies" (Belgium, Sweden, Germany, France, Finnland)

Graduate Research Programs (DFG)

- Graduate Training Research Group 2355 "Regulatory network in the mRNA life cycle: from coding to non-codin RNAs" (Bad Nauheim, Marburg, Giessen)
- Graduate Training Research Group 2213 "Membrane Plasticity in Tissue Development and Remodeling" (Bad Nauheim, Marburg)

International Collaborations

- Foundation Leducq Grant no. 18CVD03 "Transcription factor KLF2 and cardiovascular disease" (Great Britain, Germany, USA)
- Foundation Leducq Grant no. 15CVD03 "Eliciting Heart Regeneration Trough Cardiojmyocyte Division" (Germany, Australia, Israel, USA)
- German-Chinese Cooperation "Phatological linkage Study of SARS-CoV-2N protein targeting Cox-2 overexpression in transgenic mouse model (Germany, China)
- Novo Nordisk Fonden "Novel Receptor Targets in the Prevention and Treatment of Diabetes and Obesity" (Denmark, France Germany)
- Sturge Weber Foundation "Investigating Sturge-Weber Syndrome vascular defects in Zebrafish" (Bad Nauheim)
- Cuorips Inc. "The mode of action of iPS cell-derived cardiomyocyte sheets" (Bad Nauheim, Tokyo (Japan))
- Croucher Foundation "Elucidating the cellular and molecular mechanisms underlying cardiomyocyte binucleation" (Bad Nauheim)
- Bayer Foundation "Investigating the molecular mechanisms underlying genetic compensation to mutations" (Bad Nauheim)
- Japan Society for Promotion of Science. "Grant in Aid for Scientific Research" (Bad Nauheim, Showa (Japan))

BMBF ad personam

- DZHK "Regulation of cardiac lymphatics in heart disease" (Bad Nauheim)
- DZHK "Manipulating the AP-1 Response to Promote Cardiac Regeneration in the Adult Mammalian Heart" (Bad Nauheim)
- DZHK "Investigating cardiac troponin T-related cardiomyopathies using conditional degron strategies" (Bad Nauheim)
- DZHK "Role of Slit/Robo signaling in cardiomyocyte cytokinesis and regeneration" (Bad Nauheim)
- DZHK "Sympathetic reinnervation during zebrafish heart regeneration" (Bad Nauheim)

DFG ad personam

- Sachbeihifle Nr. 2676/2-1 "Entwicklung von 3Cs fixed-pair für gepoolteDNA-Exzisionen mit Einzelzell-Transkriptom Auflösung" (Bad Nauheim)
- Sachbeihifle Nr. 2891/2-1 "Modulation of neuroinflammation by novel endothelial GPCRs" (Bad Nauheim)
- Sachbeihilfe Nr. 4036/4-1 "Anti-fibrotische Effekte des Mirlet7/NuRD-Ribonukleoproteinkomplexes in idiopathischer pulmonaler fibrose" (Bad Nauheim)
- Sachbeihilfe Nr. 1195/4-1 "Analyse von PAR-3 bei stromungsinduzierter planarer Endothelzellpolaritat" (Bad Nauheim)

Scientific exchange

- DAAD scientific Exchange “Deciphering the role of E2F7 and E2F8 in cardiomyocyte cytokinesis, polyploidy, and cardiac regeneration” (Bad Nauheim, India)
- DAAD scientific Exchange “The role of OTX2 and HMGA2 in pituitary disorders, using zebrafish as an in vivo model” (Bad Nauheim, Argentina)
- Alexander von Humboldt Stiftung “Mechanical control of ventricular chamber development” (Bad Nauheim, India)
- Alexander von Humboldt Stiftung “Signalling and mechanotransduction in the cardiovascular system” (Bad Nauheim, China)
- Alexander von Humboldt Stiftung “Endothelial adrenomedullin/Gs signaling in type-2 diabetes” (Bad Nauheim, South Korea)

International Cooperations

8. Seminars at the MPI-HLR (2019-2022)

20 March 2019 (MPI seminar series)
Makoto Furutani-Seiki, Ph.D.
Dept. of Systems Biochemistry, Yamaguchi University Graduate School of Medicine, Japan
"Mechanical force mediated 3D organogenesis withstanding gravity controlled by YAP"

3 May 2019 (MPI seminar series)
Yusuke Ono, PhD
Department of Muscle Development and Regeneration, Institute of Molecular Embryology and Genetics (IMEG), University Kumamoto, Japan
"Positional memory governs adult muscle regeneration in a region-specific manner "

20 May 2019 (MPI seminar series)
Mridula Balakrishnan
Dept. of Developmental Biology, Memorial Sloan Kettering Cancer Centre, New York
"Twinstar and the Drosophila body wall muscle: insights into sarcomere biology and nemaline myopathy."

22 May 2019 (MPI seminar series)
Dr. Manuel Kaulich
Head of the Frankfurt CRISPR/Cas9 Screening Center (FCSC), Goethe University, Frankfurt "High-fidelity single and combinatorial 3Cs CRISPR screening"

31 May 2019 (MPI seminar series)
Joaquin Navajas Acedo
Piotrowski Lab, Stowers Institute for Medical Research, Kansas, USA
"A Tale of Two Prims: Dissecting the role of the Wnt and PCP pathways during establishment of hair cell orientation in zebrafish."

19 June 2019 (MPI seminar series)
Dr. Janna Nawroth
Associate Director, R&D and Emulate, Boston, Massachusettes, USA
"Phenotypic profiling and Lung-on-chip models for investigating airway epithelial disease"

8 July 2019 (MPI seminar series)
Dr. Minchul Kim
Max Delbrück Center Berlin, Germany
"The biology of syncytials cells – functional heterogeneity among myonuclei."

9 August 2019 (MPI seminar series)
Ophir Klein, MD, PhD
Professor of Orofacial Sciences & Pediatrics, Chief, Division of Medical Genetics, University of California, San Francisco
"Epithelial renewal and regeneration: plasticity and complexity."

9 September 2019 (MPI seminar series)
Leif Ludwig, PhD:
Broad Institute of MIT and Harvard, Regev and Sankaran Labs, Cambridge, USA
"Clonal and lineage tracing in humans enabled by single cell genomics."

9 September 2019 (MPI seminar series)
Marco Osterwalder, PhD
Department of Environmental Genomics and Systems Biology, Lawrence Berkeley National Laboratory, Berkeley, USA
"Deciphering the regulatory basis of mammalian development."

8 November 2019 (MPI seminar series)
Prof. Dr. Joan Heller Brown
The University of California San Diego (UCSD), La Jolla, CA, USA
"Igniting the flame of inflammation through cardiomyocyte CaMKII and inflammasome activation."

4 December 2019 (MPI seminar series)
Prof. Dr. Roger Foo
National University Heart Centre and Cardiovascular Research Centre, Genome Institute of Singapore (GIS)
"Chromatin wiring and rewiring in the heart"

2 December 2019 (MPI seminar series)
Jonathan Taylor, Ph.D.
School of Physics and Astronomy, University of Glasgow, Scotland
"Optical-computational techniques for timelapse imaging of heart structure and function in vivo"

15 April 2020 (CRC366 Vascular Mini Sympsium)
Kristy Red-Horse, Stanford University, USA
Eckard Lammert, HHU Düsseldorf
Maike Frye, UKE Hamburg
Roxana Ola, ECAS, Medical Faculty Mannheim
Hadil El-Sammak, MPI Bad Nauheim
Giulia Boezio, MPI Bad Nauheim
Moritz Jakab, ECAS, Medical Faculty Mannheim
Julian Wagner, Goethe University, Frankfurt
Elene Cano, Max Delbrück Center, Berlin
Isidora Paredes-Ugarte, ECAS, Medical Faculty Mannheim

11 June 2021 (MPI seminar web series)
Dr. Alexander Mikryukov

McEwen Stem Cell Institute, UHN, Keller lab Toronto, Canada
“Generation of functional endocardial cells from human pluripotent stem cells using BMP10 signaling.”

19 July 2021 (CPI Minisymposium - Frontiers in Cardiopulmonary Research)

Dr. Arica Beisaw
MPI, Bad Nauheim
‘Leveraging mechanisms from zebrafish to promote cardiac repair and regeneration’

Dr. Johnny Kim
MPI, Bad Nauheim
‘Spatio-temporal dynamics of regeneration and cancer’

Dr. Minchul Kim
MDC, Berlin
‘The biology of syncytial cells - Skeletal muscle as a paradigm‘

Dr. André F. Rendeiro (virtual)
Institute for Computational Biomedicine, New York
‘Quantifying tissue organization patterns in the human lung with multiplexed imaging and deep learning’

30 July 2021 (MPI seminar series)
Pieterjan Dierickx, PhD
Lazar lab, University of Pennsylvania, Institute for Diabetes, Obesity and Metabolism (IDOM), Philadelphia, US
“Circadian rhythms in cardiac homeostasis and regeneration.”

15 October 2021 (MPI seminar series)
Prof. Dr. Klaus H. Kaestner, Ph.D., M.S.
University of Pennsylvania Perelman School of Medicine, USA (https://www.med.upenn.edu/kaestnerlab/)
"The FoxA transcription factors: Controlling the hepatic gene regulatory network from inception to death"

2 November 2021 (MPI seminar series)
Prof. Dr. Karina Yaniv, Ph.D.
Weizmann Institute, Rehovot, Israel (https://www.weizmann.ac.il/Biological_Regulation/Yaniv/#home)
"Vascular control of organ growth and regeneration"

2 November 2021 (MPI seminar series)
Prof. Dr. Eldad Tzahor, Ph.D.
Weizmann Institute, Rehovot, Israel (https://www.weizmann.ac.il/mcb/tzahor/#home)
"Novel strategies for cardiac repair"

8 April 2022 (MPI seminar series)
Prof. Dr. Gilbert Weidinger, Ph.D.
University of Ulm, Germany
"Zebrafish fin regeneration requires generic and regeneration-specific responses of osteoblasts to trauma"

13 April 2022 (MPI seminar series)
Prof. Dr. Jeroen Bakkers, Ph.D.
Hubrecht Institute, Leiden, Netherlands
"Interplay between Calcium cycling and cardiomyocyte maturation during regeneration"

25 April 2022 (MPI seminar series)
Jan Philipp Junker, Ph.D.
Max Delbrück Center for Molecular Medicine, Berlin (https://junkerlab.com/)
"Cellular drivers of heart regeneration in zebrafish – origin and function"

25 May 2022 (MPI seminar series)
Prof. Dr. Salim Seyfried, Ph.D.
Head of Department for Animal Physiology, University of Potsdam, Institute of Biochemistry and Biology, Potsdam, Germany
"Biomechanical signaling in zebrafish endocardial morphogenesis and vascular pathologies"

27 June 2022 (CPI Academy Seminar Series)
Prof. Dr. Steve Horvath
University of California Los Angeles, ALTOS Labs, USA
“Epigenetic Clocks and Cardiovascular Diseases”

2 September 2022 (MPI seminar series)
Scott A. Lacadie, Ph.D.
Ohler Lab, Max Delbruck Center for Molecular Medicine, Berlin, Germany
"Discovery, dynamics, and targets of vertebrate transcriptional enhancers"

26 September 2022 (MPI seminar series)
Mahendra Sonawane, Ph.D.
Tata Institute of Fundamental Research, Pune, India
"Epithelial cell size regulation and mechanical properties of the zebrafish epidermis"

4 November 2022 (MPI seminar series)
Andrea Rossi, Ph.D.
Group Leader, Genome Engineering and Model Development Lab (GEMD) IUF-Leibniz Institute, Dusseldorf
"Investigating and Modelling Human Disease: Genome Editing, Sequencing and Organoids"

30 November 2022 (MPI seminar series)
Prof. Dr. Sara Wickström
Director, Max Planck Institute for Molecular Biomedicine, Münster, Germany
“Regulation of Cell Fate and Integrity by Nuclear Mechanotransduction”

9. Equal Opportunity

Cutting-edge research thrives best when people can freely develop their talents and creativity in all their diversity, regardless of their ethnic and cultural background, gender, age or disability. The Max Planck Institute for Heart and Lung Research (MPI-HLR) therefore strives for a non-discriminatory culture, in which ethnic origin, gender, cultural or ideological attitudes (as long as they are not subversive to society and/or against the law), disability or age must not have any influence on access, promotion and qualification opportunities for employees. Based on this objective, we have not only defined gender equality as a goal in the current version of the Gender Equality Plan (available through the MPI-HLR intranet or upon request), but also place a special focus on the equal treatment of all employees regardless of their origin, ethnicity, sexual identity or orientation, religion or ideology, disability or age (§ 1 AGG). For the number and position of female and male scientists, nationalities and age distribution please see chapter **12. Annex**.

One objective is to ensure that women and men can reconcile family, care and work (§ 1 BGleiG). The MPI-HLR negotiated longer opening hours at a local childcare centre for pre-school children, allowing the parents to focus on their work. To accommodate young mothers who want to return to work as soon as possible, a room is now available at the Institute where they can breastfeed their babies (Fig. 1).

Fig. 1. *Parent and Baby Room.*

In addition, childcare expenses during work-related travel and training can be (partially) reimbursed upon request.

All employees have the opportunity for professional training and further education. The Max Planck Society offers numerous seminars for scientists at various stages of their careers.

Young female scientists are supported by a number of initiatives in the MPS, e.g. Minerva FemmeNet, a mentoring program open to all female scientists at the MPIs, AcademiaNet, and the Sign Up! Career Building Program for excellent female postdocs. The Sign Up! program aims to support women with leadership potential in their career planning and to prepare them for management positions in science. Two postdocs were accepted in this program, 1 each in 2021 and 2022.

10. Public Relations | Public Outreach and Networking

- **Matthias Heil (Head of Administration and Press Officer)**
- **Nadia Richter (Media Representative, library; from November 2022 on)**

Jenny Hinz (former Media Representative, Risk Management; – until September 2022)
Virginia Geisel (Press Officer; until April 2022)

Introduction

Our public relations and Public Outreach activities aim at several groups of stakeholders:

- Scientific guests and cooperation partners (international and national)
- university and high school students (for potential recruitments)
- journalists
- politicians
- applicants for PhD, Postdoc and other positions
- local and regional public visitors
- general public

Cardiovascular diseases including diseases of the lung are of particular relevance for Western societies. Therefore, the institute's major research foci are of particular interest for stakeholders.
The regular activities at the institute entails some potential risks which, under certain conditions, could be negatively perceived by the public. In the worst case, this could lead to loss of reputation of the institution and the Max Planck Society as a whole. Examples include animal experiments, work with genetically engineered organisms and accidents involving hazardous and toxic substances. A temporary MPG-funded project together with the MPI for Terrestrial Microbiology (Marburg) and the MPI for Brain Research (Frankfurt) aims to counter these risks proactively. During the initial phase all potential risks have been assessed and documented. Subsequently, proactive public relations strategies and activities have been developed including publishing relevant information on the website, social media activities (e.g. Instagram, twitter, linkedIn) to generate a positive basic attitude in the public.

The former president of the State of Hesse, Volker Bouffier, has become Chair of the Board of Trustees. The pictures shows him visiting the fish facility of the Stainier department.

Press Releases

Press releases represent the fundamental tool for public outreach activities. Since the circulation numbers of newspapers continuously decrease, it becomes more important to ensure retrievability of press release on our website (professional google indexing). Our press releases included information on important new findings after publishing in top journals, scientific awards or general activities.

New Homepage

The website has been relaunched using the CMS Fiona, which has been provides by the MPS. The website is inline with the corporate style guide of the MPS. The content has been updated during the relaunch phase.

Networking

Hosting events

Hosting third party events, increases visabilty of the institute. Unfortunately, due to the Covid19 pandemic, only a few events were possible, including a book fair by a Bad Nauheim publishing house and conferences organized by the German Cardiac Society.

Programs for school students

One of the goals of the public outreach activities is to make science and in particular biomedical research attractive for the young generation. We therefore cooperate with local high schools. The offered program includes seminars and guided institute tours, as well as a lecture series.

Guided tours

Since the Covid19 pandemic has been present almost through the complete reporting period, only a few tours have been conducted. Our plan is to intensify these activities and to recruit interested scientists for tours to present their research and demonstrate concrete techniques they apply.

Attending job fairs, organized by high schools, turned out to be a successful tool of public outreach.

11. Boards

The Max Planck Institute for Heart and Lung Research and the William G. Kerckhoff Foundation for Scientific Research and Education are headed by a common board of trustees.

Besides its basic function as a significant element for qualified public relations, the board of trustees has additional tasks.

As a supervisory board for the William G. Kerckhoff Foundation, the board of trustees decides on the investment of assets, the budget, the annual financial statement and formally approves action by the board of management.

Members of the Board of Trustees of the Max Planck Institute for Heart and Lung Research and the W.G. Kerckhoff Foundation

Volker Bouffier – Retired prime minister of the State of Hesse, (Chairman)

Elmar Damm – Ministerialdirigent, Hessian State Ministry for Finance, Wiesbaden

Dr. Wolfgang Gerhardt – Honorary chair of the Friedrich-Naumann Foundation, Potsdam

Sonja Kastillan – Head of Science Department, Frankfurter Allgemeine Sonntagszeitung

William Kerckhoff Young JR., Ph.D. – MRSC, Medicines Research Centre GlaxoSmithKline, Stevenage, UK

Klaus Kress – Mayor of Bad Nauheim (Vice Chairman)

Dr. Matthias Leder – CEO IHK Giessen-Friedberg, Giessen

Prof. Dr. Joachim-Felix Leonhard – retired State Secretary

Dr. Ulrike Mattig – Hessian Ministry of Science and Arts, Wiesbaden

Anne McLain – Los Angeles, CA, USA

Dr. Claudia Walther – CEO Boehringer Ingelheim Fonds

Prof. Dr. Martin Westphal – Executive Vice President & Chief Medical Officer Global Medical & Clinical Affairs, Fresenius Kabi Deutschland GmbH, Bad Homburg

Prof. Dr. Matthias Willems – President of the University of Applied Sciences Mittelhessen

Members of the Management Board of the William G. Kerckhoff Foundation

Volker Remmele – Bankvorstand i.R. (Chairman)

Prof. Dr. Stefan Offermanns – MPI for Heart and Lung Research, Bad Nauheim (Vice Chairman)

Patrick Kraulich – Ministerialrat, Hessian State Ministry of Finance, Wiesbaden

Dr. Lutz Ehnert – Bad Nauheim

Scientific Advisory Board of the Max Planck Institute for Heart and Lung Research

Prof. Dr. Shaun Coughlin – Novartis Institutes for Biomedical Research, Cambridge (MA), USA

Prof. Dr. Britta Engelhardt – Medical Faculty, University Bern, Bern, Switzerland

Prof. Dr. Axel Haverich – Klinik fur Herz-, Thorax-, Transplantations- und Gefaschirurgie, MHHHannover

Prof. Dr. Alessandra Moretti – Medical Clinic and Polyclinic, Technical University, Munich

Prof. Dr. Tatiana Petrova – Department of Oncology, University of Lausanne, Epalinges, Switzerland

Prof. Dr. Frédéric Relaix – Institut Mondor de Recherche Biomedicale, Universite Paris-Est Creteil Val de MarneCreteil, France

Prof. Dr. Lila Solnica-Krezel – Washington University School of Medicine in St. Louis, St. Louis, USA

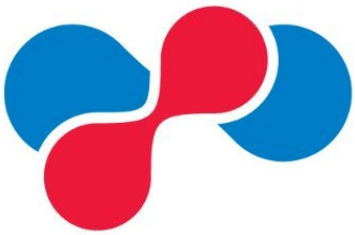
Prof. Dr. Uwe Strähle – Institute of Toxicology and Genetics, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany

Prof. Dr. Stephen J. Tapscott – Fred Hutchinson Cancer Research Center, Seattle, USA

12. Annex

SEE EXTRA FILE FOR

- Brief Status Information on Directors and PIs
- Institute Budget and Expenditure Trends
- Number of Cooperations / Third-party Funds 2019-2022
- Staff Statistics



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