Cell News

Newsletter of the German Society for Cell Biology

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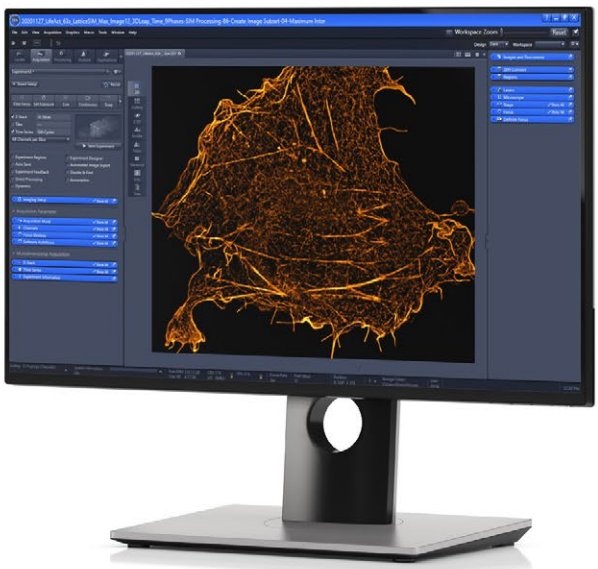
01/2024

DGZ

Reveal the vibrant sub-organelle network of life



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CONTENT

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Cover Image:

DGZ Awards 2024: The calls for the Walther Flemming Award, the Nikon Young Scientist Award, the BINDER Innovation Prize and the DGZ Master Thesis Awards can be found in this issue on pages 5-6.

Dear members of the DGZ,

Nearly half a year has passed since our last newsletter but looking at recent challenges for life sciences in Germany it seems that time has not really progressed:

- The amendment of the "Wissenschaftszeitvertragsgesetz" has now been decided by the federal cabinet and the implemented 4-year restrictions on Postdoc contracts - while not as catastrophic as the originally intended 2-3 year limit – will strongly impact scientific careers in Germany. Without the actual creation of new permanent positions in the academic sector the new limits can only mean that even more scientists will leave the country in their most productive period - or will orient themselves outside of academia. For more on this see our joint statement with other life science societies on pages 45-46.
- While an initiative by animal right activists to end all animal experiments and testing in Europe was barely averted in 2023 we are now faced with a novel initiative from the Federal Ministry of Food and Agriculture to increase penalties on "unnecessary" killing of animals. While mainly aimed at agriculture, the lack of a dedicated framework that specifically addresses regulations for lab animals in Germany makes the new regulations equally applicable to all scientific work involving vertebrate model systems. If implemented, the threat of mandatory imprisonment for the responsible scientists - combined with the legally vague term of a "vernünftiger Grund" for killing animals - can only lead to an immediate stop of all animal experimentation in Germany.

Looking at the different challenges impacting Science in Germany in recent years, the frequent disconnect between well-intentioned legislative measures and the far-reaching and potentially irreversible negative effects of said measures on the life sciences is alarming. But rather than complaining about initiatives at a stage in the process where no more than slight modifications are still possible, scientists need to organize better and proactively bring their interests to the attention of politics and politicians. At a time of wide-ranging global challenges that often rely on scientific input for solutions we otherwise risk to restrict our capabilities to respond to these challenges.

At the level of our own organization there are a couple of announcements that we want to bring to your attention:

- We are about to finalize an agreement of affiliation with the **European Journal of Cell Biology** (EJCB), which will provide all our members a 30 % discount on open access fees for all publications within the journal. The EJCB has a strong editorial board of German and European cell biologists and covers many of the core topics of our society. It therefore provides an optimal platform for publications by our members.
- **Master's thesis awards**: Starting in 2024 we will award yearly prizes to two students for their Master thesis projects in cell biology. Awardees must be members of the DGZ and be nominated by their supervisors. The first call for this award can be found in this issue of Cell News (page 6).

The two awardees for 2024 will be presented together with the Nikon Young Scientist Award, the Walther Flemming Award and the BINDER Innovation Prize during our online **DGZ Award Ceremony** on October 7th.

- The year 2025 will mark the **50th anniversary of the DGZ**. We will commemorate this important date with a historical conference in Heidelberg – the "birthplace" of the DGZ - from Oct 26th-29th, 2025. More details on this conference will be distributed soon.
- Our upcoming **members meeting** including elections of the next board will be a little earlier this year, on Oct 11th. The virtual meeting will again be held via zoom.

As always, we welcome your feedback and suggestions on how we can continue to improve our society.

Best regards,

The DGZ Board - Roland Wedlich-Söldner, Sandra Iden, Gislene Pereira and Julia Groß

WALTHER FLEMMING AWARD 2024

The German Society for Cell Biology (DGZ) and ibidi GmbH offer the "Walther Flemming Award" for excellent research in cell biology. The award consists of a financial contribution of EUR 3000 and is given to senior postdoctoral researchers and early career group leaders for recent work that defines their emerging independent research profile.

Candidates need to be members of the DGZ and can either be nominated or apply directly for the prize.

Applications should be submitted **in a single pdf file (max. 10 MB)** and consist of cover letter, CV and copies of 1-3 publications that document the relevant work of the applicant. Applications will be reviewed by a dedicated award committee of the DGZ.

Please send your application by e-mail to the DGZ office:
dgz@dkfz.de

Deadline: **July 31, 2024**

NIKON YOUNG SCIENTIST AWARD 2024

The German Society for Cell Biology (DGZ) and Nikon Deutschland (Business Unit: Healthcare) annually offer the "Nikon Young Scientist Award" for excellent research in cell biology by PhD students or young postdoctoral researchers within 3 years after graduating (an extension of up to 2 years will be granted for periods of parental leave). The awardee will receive a financial contribution of EUR 1500.

Candidates need to be members of the DGZ and can either be nominated or apply directly for the prize.

Applications should be submitted **in a single pdf file (max. 10 MB)** and consist of cover letter, CV and copies of publications that document the work of the applicant. Applications will be reviewed by a dedicated award committee of the DGZ.

Please send your application by e-mail to the DGZ office:
dgz@dkfz.de

Deadline: **July 31, 2024**

BINDER INNOVATION PRIZE 2024

BINDER
Best conditions for your success

The BINDER Innovation Prize is sponsored by BINDER GmbH in Tuttlingen since 1998 and annually awarded by the German Society for Cell Biology (DGZ). The award is given for outstanding contributions to cell biology and consists of a financial contribution of EUR 4000. It is aimed at junior investigators that have already established and developed their own research profile.

Candidates need to be members of the DGZ and can either be nominated or apply directly for the prize.

Applications should be submitted **in a single pdf file (max. 10 MB)** and consist of cover letter, CV, a research profile and copies of three selected first/last author publications. Applications will be reviewed by a dedicated award committee of the DGZ.

Please send your application by e-mail to the DGZ office: dgz@dkfz.de

Deadline: **July 31, 2024**

DGZ MASTER THESIS AWARDS 2024 NEW

Call for Nominations: DGZ Master Thesis Awards

The DGZ Board is pleased to announce the launch of the DGZ Master Thesis Awards, an exciting new opportunity to support and recognize excellent work by emerging cell biologists in Germany

Overview
Awards: We will present two awards each year, with DGZ Workgroup speakers playing a key role in selecting the awardees.
Eligibility: PIs are invited to nominate Master theses from their current or former students. The thesis must have been completed within the past 12 months, and the nominee must be a DGZ member.
Workgroup Alignment: The application should specify which DGZ Workgroup aligns with the nominee's work.

Awards Details: Each awardee will receive € 500 and the opportunity to present their work in a 10-minute slot during the virtual DGZ Award Session in autumn.

This initiative is designed to acknowledge early academic achievement in cell biology and provide a platform for the awardees to showcase their research.

We encourage you to participate in this initiative and look forward to your nominations from your lab. Please send your applications (cover letter, student CV and thesis) **as a single pdf file (max. 10 MB)** by e-mail to the DGZ office: dgz@dkfz.de
Your input will be invaluable in promoting the next generation of cell biologists in Germany.

Deadline: **July 31, 2024**

SAVE THE DATE: 26–29 OCTOBER 2025

Celebrating 50 Years of the DGZ in Heidelberg

Dear colleagues,

We are pleased to announce that the next **DGZ International Conference** will take place on October 26-29, 2025, in Heidelberg, Germany.

More detailed information and the program will be sent to you closer to the date, but please mark your calendar now.

This will be a great opportunity to interact and share your exciting data with old and new friends and celebrate the 50th anniversary of the DGZ.

We hope to see many of you in Heidelberg.

Walther Flemming Award
Deciphering Autophagosome Formation by correlative cryo-electron tomography

Original publications: Wilfling et al., Mol. Cell 2020 | Bieber et al., PNAS 2022 | Li et al., PNAS 2023

Introduction

Autophagy, a conserved cellular housekeeping mechanism, has long intrigued scientists due to its role in maintaining cellular health and in defending the cell against pathogens. During autophagy, autophagosomes form de novo via a cup-shaped intermediate structure called the phagophore, which expands to engulf cytosolic material. The engulfed material undergoes lysosomal degradation, enabling cellular components to be recycled[1]. In yeast the phagophore is thought to originate from vesicles that fuse to form an initial double membrane disc. The captured cargo ranges from dysfunctional organelles to protein aggregates and pathogens, highlighting the critical role of autophagy in cellular maintenance. However, the importance of autophagy extends beyond cellular cleanup: it is pivotal in aging, immunity, and disease pathogenesis, including cancer and neurodegeneration[2]. Autophagosome biogenesis is a key step in this process, yet its underlying membrane transformations have remained elusive.

Cryo-electron tomography (cryo-ET) is an advanced imaging technique that allows visualization of complex biological structures at near-atomic resolution in their native, hydrated state[3,4]. Unlike traditional electron microscopy imaging methods that require samples to be fixed or stained, cryo-ET preserves specimens in a near-native state by rapidly freezing them, thus avoiding the formation of ice crystals that can damage cellular structures. This flash-freezing process is known as vitrification. However, to be sufficiently electron transparent for high-resolution imaging in a transmission electron microscope (TEM), the vitrified cells must be locally thinned down to a thickness of around 200 nm. This is achieved by focused ion beam milling in a FIB/SEM microscope, where most of the cellular material is ablated to leave behind a thin lamella. This low throughput method is often limited by the likelihood of capturing rare biological events within the lamella volume. A solution to this problem is correlative cryo-ET: the combination of fluorescence microscopy and cryo-ET. Here, the vitrified cells are first imaged in a cryo-confocal microscope and the 3D information is used to guide lamella milling and TEM data acquisition to the area of interest[5].

Characterizing autophagic cargo by correlative cryo-ET

We first applied this technique to the field of autophagy by characterizing the ultrastructure of a previously undescribed autophagic cargo. In this study, we described a selective autophagy pathway for the degradation of phase-separated endocytic protein deposits, specifically within the context of clathrin-mediated endocytosis (CME) in S. cerevisiae (yeast)[6]. Typically, CME machinery proteins assemble and disassemble at the plasma membrane in a well-orchestrated fashion to transport various cargo molecules from the cell surface to the interior[7]. Genetic perturbations lead to aberrant accumulations of endocytic machinery proteins at the plasma membrane[8]. We found that the early endocytic protein Ede1 acts as a selective autophagy receptor that directly recruits Atg8, a ubiquitin-like protein essential for autophagosome formation, to the aberrant CME protein assemblies, referred to as ENDs (Ede1-dependent endocytic protein deposits)[6]. These ENDs form through liquid-liquid phase separation (LLPS) and exhibit properties distinct from regular endocytic patches, including stable and persistent accumulation of Ede1 and other early-phase CME proteins. Furthermore, we demonstrate that Ede1's ability to bind Atg[8] and mediate phase separation into condensates is crucial for the autophagic degradation pathway of ENDs, which is distinct from normal endocytosis and does not involve other known selective autophagy receptors. Correlative cryo-ET provided crucial insights into the structural properties of the phase-separated compartments (ENDs) at various stages of the selective autophagy process. First, it revealed distinct Ede1-positive drop-like condensates that excluded other cytosolic components, such as ribosomes, and localized to the plasma membrane (PM), supporting a model in which END formation involves phase separation driven by Ede1 and other CME proteins (Figure 1a). Second, direct visualization of Ede1-containing assemblies within autophagic bodies strongly supported the degradation of these protein condensates by autophagy (Figure 1b). This provided a proof of concept that correlative cryo-ET can be used to study the process of autophagy directly in cells at high resolution.

How autophagosome membranes deform during biogenesis

Autophagosome formation involves distinct morphological transitions, from initial membrane nucleation to the growth of a cup-shaped phagophore, and finally closure into a spherical autophagosome. Having demonstrated the feasibility of the correlative cryo-ET approach, we then used it, followed by comprehensive data analysis, to elucidate the ultrastructural organization and transformation of phagophore membranes during autophagosome biogenesis[9]. This allowed us to capture and study the sequential steps of autophagosome formation in yeast in unprecedented detail (Figure 2a and 2b). This approach not only allowed us to visualize the process in its native cellular context, but also to quantify the morphological characteristics of autophagosome membranes at different stages of the biogenesis pathway.

This quantitative morphological analysis yielded several important insights, such as a remarkably thin intermembrane distance between the two bilayers of the autophagic membrane which hints to a large contribution of lipid transfer as opposed to vesicle fusion during biogenesis (Figure 2c). Moreover, we found that growing phagophores become thinner with a more highly curved rim as they mature, providing a structural basis for understanding the biophysical forces shaping this unique cup-shaped structure (Figure 2d). Together, these data suggest a model in which vesicles primarily contribute to early phagophore growth, whereas direct lipid transfer from the ER becomes predominant in later stages.

Since cryo-ET provides contextual information directly in situ, we next analyzed the contacts of growing phagophores with other organelles, such as the vacuole and the endoplasmic reticulum (ER) (Figure 2e). These interactions showed a polarized organization, suggesting a spatial coordination in the recruitment of membrane sources and possibly a segregation of functional domains during phagophore expansion (Figure 2f). For example, the ER interacted almost exclusively with the phagophore rim. The distance between the two membranes at the ER/phagophore contact site and the appearance of rod like densities is consistent with the idea of lipid transfer between the ER and the phagophore by the lipid transfer protein Atg2.

Taken together, our second study sheds light on the complex orchestration of membrane transformations in autophagosome biogenesis. This research has not only enriched our knowledge of a fundamental cellular process, but also exemplifies the synergy between cutting-edge imaging technologies and biological research, paving the way for future discoveries in cell biology.

A glimps into mammalian selective autophagy

In our latest study, we investigated the elimination of large intracellular pathogens in a process termed xenophagy inside human cells[10]. Salmonella are intracellular, gram-negative bacteria, which manipulate cells to take them up within membrane-bound vacuoles called Salmonella-containing vacuoles (SCVs)[11]. Correlative cryo-ET revealed that rupture of the SCV prompts the formation of multiple phagophores around the bacteria (Figure 3a). These structures, ranging from disk-shaped to expanded cup-shaped, utilize the SCV, Salmonella's outer membrane, or existing phagophores as templates for growth. They show similar physical features as phagophores in yeast but their swelling of the rim is often more pronounced (Figure 3b).

Similar to yeast, we observed contacts between the ER and the phagophore rim. Again, rod-like densities were observed spanning the distance between the two membranes with dimensions within the size range of the in vitro determined Atg2 structure (Figure 3c). Notably, also omegasomes, a specific early autophagic structure of higher eukaryotes marked by Double-FYVE domain-Containing Protein 1 (DFCP1), showed association with the ER without apparent membrane continuity (Figure 3d). These observations underscore the role of the ER in phagophore expansion.

Conclusion

Overall, the combination of cryo-ET and cell biology enriches our understanding of the autophagic process, and sets the stage for future research aimed at unravelling the molecular and biophysical mechanisms of autophagy. Furthermore, we envisage that in the next few years it will be possible to map and determine the structure of specific components of the autophagy machinery in situ using sub-tomogram averaging and template matching. The application of cutting-edge imaging techniques such as cryo-ET will undoubtedly continue to illuminate the complex yet fascinating world of autophagy, opening up new avenues for scientific discovery. But even beyond autophagy, this series of studies highlights the ability of correlative cryo-ET to directly target rare biological events in situ. This reveals contextual information about protein structures in cells, allowing structural information to be linked to their functional spatial organization within cells.

Acknowledgements

I would like to thank all the co-authors and collaborators who have contributed to the work presented, as well as all the people with whom we have discussed these projects. In particular, I would like to thank Anna Bieber, Cristina Capitanio, and Meijing Li for their enthusiasm and dedication to the projects described. I would also like to thank my supervisors Wolfgang Baumeister and Brenda Schulman for their full support and for giving me the opportunity to explore autophagosome biogenesis using cryo-electron tomography with a fantastic team and now to continue it in my own group. I would also like to thank all my previous supervisors, mentors and collaborators for their support and scientific guidance over the years. I thank the DGZ and ibidi for honouring me with this award, as well as the fund-

ing agencies that have supported my past and present research, including ERC, DFG, EMBO, BIF. Finally, I would like to thank my family for their constant support and encouragement throughout my career.

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Florian Wilfling received his Bachelor's and Master's degree in biochemistry from the Technical University of Munich. His subsequent doctoral work at the LMU Munich and the Yale School of Medicine, supervised by Prof. Tobias Walther, was focused on the translocation mechanisms of ER-resident transmembrane proteins to lipid droplets and their impact on lipid droplet growth.His postdoctoral research at the Max Planck Institute of Biochemistry in Planegg, Germany, with Prof. Stefan Jentsch focused on selective autophagy pathways and the role of intrinsic receptors for the targeted degradation of complex macromolecular machines. As a project leader in the group of Prof. Wolfgang Baumeister and Prof. Brenda Schulman, Florian Wilfling then proceeded to use cryo-electron tomography for the study of autophagosome biogenesis in situ. In 2021, Florian Wilfling established his own research group at the Max Planck Institute of Biophysics in Frankfurt am Main, Germany. His team is investigating how cargo properties influence autophagosome biogenesis and how this process occurs at the structural level on the cargo surface.

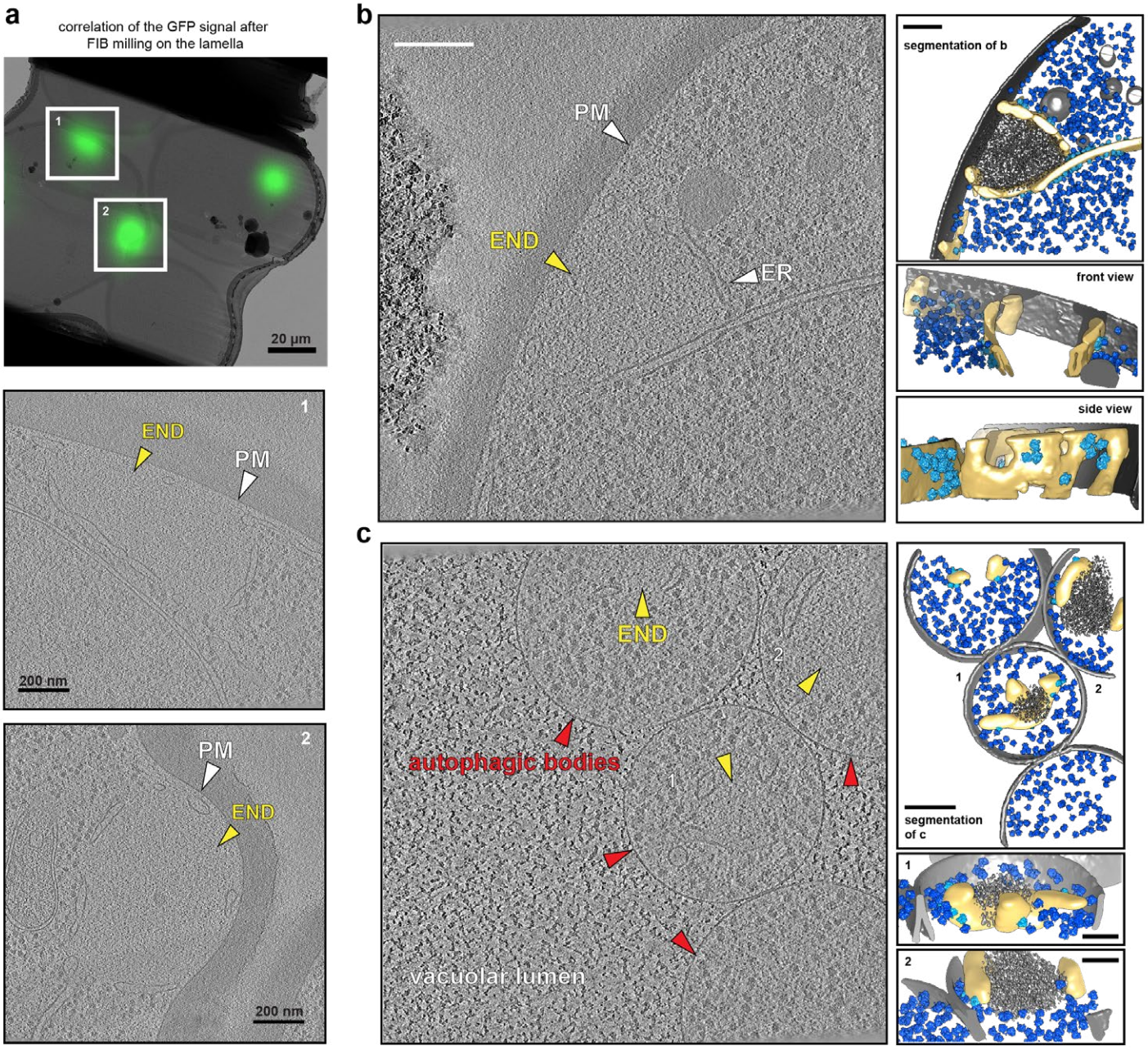

Figure 1: Ultrastructural dissection of END. (a) After cryopreservation, potential sites of interest are located by fluorescence light microscopy (FLM) and scanning electron microscopy (SEM). Correlative beads are both located in FLM and the ion beam mode of a dual beam focused ion beam (FIB) SEM to obtain a 3D correlation between both imaging modes. Predicted points of interests are then calculated from the FLM 3D stack data and promising sites are selected and cut using the cryo-FIB, resulting in thin lamellas. Finally, 2D correlation can be performed between beads in the FLM and transmission electron microscope images to obtain points of interest on the lamellas (a, top image), which are then analyzed in detail by high-resolution cryo-TEM. Shown are two examples of tomograms recorded at sites of correlated signal. Both examples show areas of Ede1 protein accumulations, which are surrounded by endoplasmic reticulum. The interior of the amorphous protein structure is clearly devoid of all ribosomes and originates directly from the plasma membrane. Other organelles in the vicinity, such as the nuclear membrane or mitochondria appear unchanged. (b) Tomogram slice and corresponding segmentation of an END formed in a clathrin-mediated endocytosis assembly mutant. (C) Tomogram slice and corresponding segmentation of an END within an autophagic body in the vacuolar lumen. Degradation of autophagic bodies in the vacuolar lumen is stalled by deletion of the lipase Atg15. Adapted from [6].

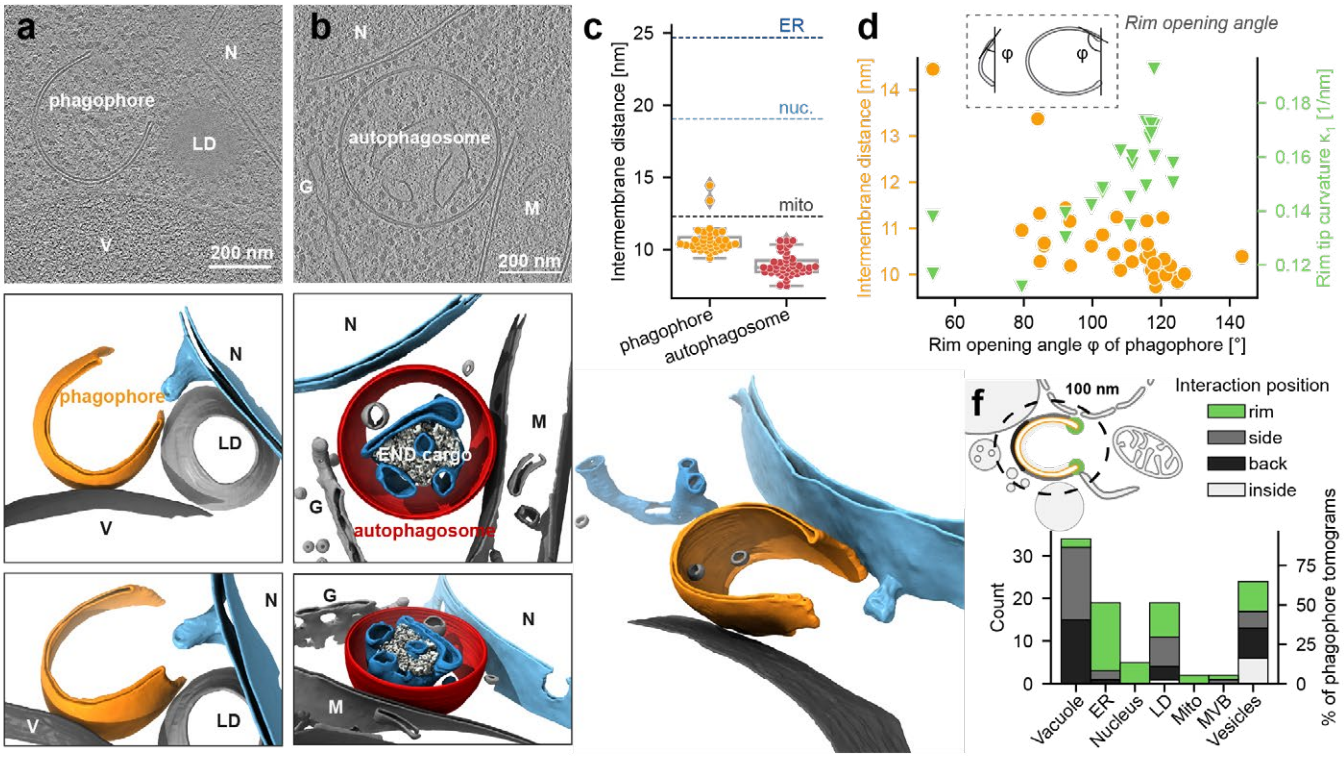

Figure 2: Insights into yeast autophagy from cryo-ET. (a-b) Tomogram slices (top) and renderings of a phagophore (a) and an autophagosome (b) in their native cellular environments in starving yeast cells. V, vacuole, LD, lipid droplet, N, nucleus, G, Golgi, M, mitochondrion. (c) Intermembrane distances of phagophores and autophagosomes; each dot indicates the mean distance for one individual structure. Mann-Whitney-U test: p = 4.5e-11. Dashed lines indicate means of mean intermembrane distances of mitochondria, the nuclear membrane (nuc.) and ER sheets. (d) Mean intermembrane distances (orange dots, left y axis) and first principal curvature at the rim tip (green triangles, right y axis) of phagophores and their rims, plotted against the rim opening angle φ as a measure for phagophore completeness. The rim opening angle φ is measured between the membrane at the rim region and a plane fit through the rim tip, as indicated in the scheme. The intermembrane distance decreases significantly with φ (Spearman's ρ=-0.67, p=9.6e-6), while the rim tip curvature increases (Spearman's ρ=0.78, p=2.9e-6). (e) Rendering of a phagophore making contacts to the nuclear membrane via its rim, and to the vacuole with its side (dashed circle). (f) Observation frequency of different phagophore-organelle contacts (cutoff distance 100 nm), with colors indicating the contact position. Adapted from[9].

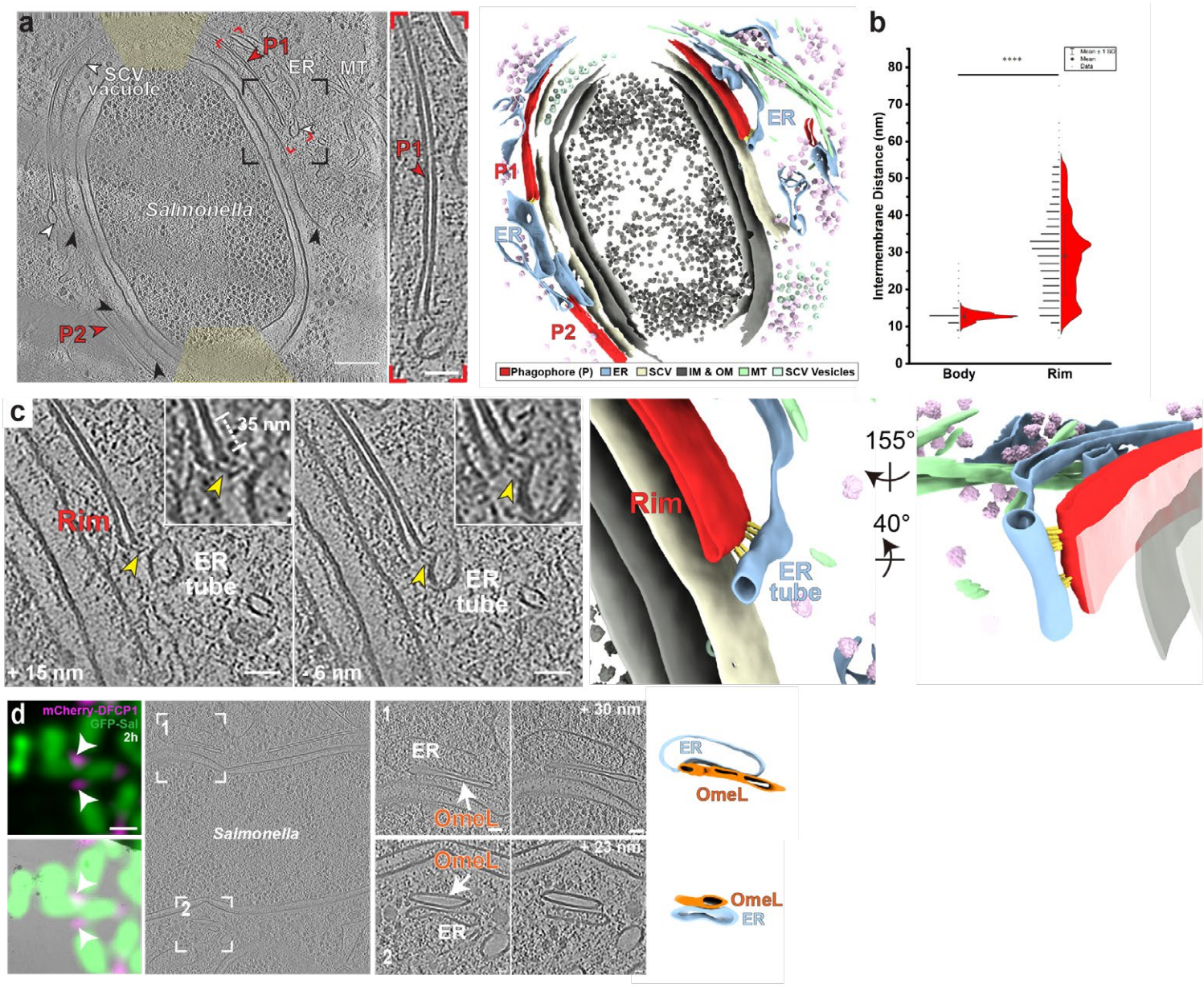

Figure 3: Characterization of xenophagy by correlative cryo-ET. (a) Tomographic slice of a damaged SCV targeted by two expanded phagophores (P1 and P2). Black arrowheads indicate the ruptured fragments of the SCV vacuole. (b) Violin plot showing the intermembrane distances of the phagophore body and rim region. 75 rims of 53 expanded phagophores were analyzed. The average distances are 12.6 ± 1.2 nm and 29.0 ± 10.9 nm, respectively. Distances were calculated from membrane middle to membrane middle. (c) Tomographic slices marked by the black box in (a) show that the phagophore rim connects with an ER tube via stick-shaped proteins (indicated by yellow arrowheads). On the right is shown a corresponding 3D rendering model. (d) Cryo-fluorescent image and the corresponding lamella overlay show two positions with mCherry-DFCP1 signal around Salmonella. The mCherry-DFCP1 expressing HeLa cells were imaged at 2 hours post infection. White arrowheads indicate the targeted DFCP1 signals. The tomographic slice of shows two disc-shaped omegasome-like structures around Salmonella with corresponding 3D rendering. Adapted from 10.

Nikon Young Scientist Award
The role of intestinal Th17 cells in neuroinflammation

Alexandra Schnell [1,2,3]

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[3] Current address: Whitehead Institute for Biomedical Research, Cambridge, MA, USA

Introduction

The concept of the "gut-brain axis" was established more than 200 years ago, when doctors observed a connection between the gastrointestinal health and the mental state of patients (1). Afterwards many studies in neuroimmunology and gastroenterology supported this concept and identified cell types involved in the communication between the gut and brain during health and disease. One of these cell types were T cells that constitute a potent effector compartment of the immune system making it critical that T-cell responses are strictly regulated to avoid inappropriate immune responses, such as autoimmune reactions. CD4$^+$ IL-17-producing T helper (Th17) cells were found at high numbers in the intestine where they play a major role in mucosal barrier homeostasis and host defense against extracellular pathogens (2). At the same time Th17 cells have been implicated in the induction of multiple autoimmune diseases including multiple sclerosis, psoriasis, inflammatory bowel disease, and rheumatoid arthritis (3-5). While these observations suggested the existence of multiple types of Th17 cells with distinct functions, the mechanism by which Th17 cells mediate these dichotomous functions, remained unexplored.

Characterization of Th17 heterogeneity during autoimmunity

When I joined the laboratory of Vijay Kuchroo as a PhD student, recent studies had demonstrated that germ-free mice, which are devoid of Th17 cells in the intestine, show protection from neuroinflammation, while the induction of the intestinal Th17 population led to enhancement of different types of autoimmune diseases, including neuroinflammation in the brain (6-8). In addition, in human multiple sclerosis (MS) patients, a human autoimmune disease of the central nervous system (CNS), high intestinal Th17 frequencies correlated with worse disease progression (9). I was very fascinated by these observations suggesting that intestinal Th17 cells have the ability to drive and regulate extra-intestinal autoimmune diseases and was eager to study the mechanism of this phenomenon. To shed light on the mechanism, together with my collaborators, I combined single-cell RNA- and T-cell receptor (TCR) -seq (scRNA/TCR-seq) to profile ~84,000 tissue Th17 single cells and characterized their heterogeneity, plasticity, and migration at homeostasis and during the mouse model for multiple sclerosis, called EAE (10) (**Figure 1**).

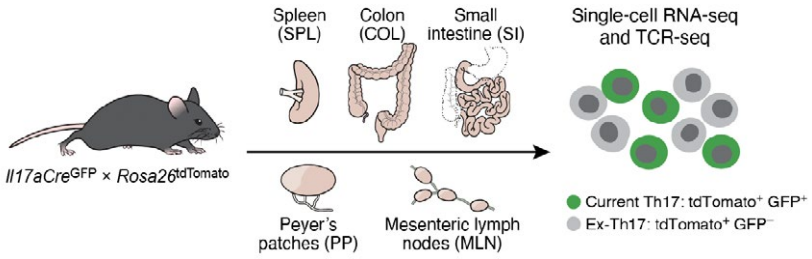

Figure 1: *Experimental set-up. We performed combined single-cell RNA-seq and TCR-seq of tissue Th17 cells. Th17 cells were isolated from Il17aCreGFP X Rosa26tdTomato mice in which all cells that ever-expressed IL-17A are marked with tdTomato expression and cells that currently express IL-17A are marked with GFP expression.*

Discovery of a homeostatic and pathogenic Th17 population during autoimmunity

The induction of EAE resulted in the generation of two Th17 cell populations, a major pathogenic Th17 cell population, marked by CXCR6, that migrated specifically to the CNS, and highly expressed the pro-inflammatory cytokines GM-CSF and IFNγ (**Figure 2**). However, in addition, a smaller population of homeostatic, stem-like TCF1$^+$ Th17 cells, marked by SLAMF6, was induced, and migrated to the intestine and associated lymphoid tissue. Combined scRNA- and TCR-seq revealed that the stem-like SLAMF6$^+$ population and the pathogenic CXCR6+ Th17 population are directly related to each other, in that the SLAMF6$^+$ population gives rise to the CXCR6$^+$ population. Thereby, the

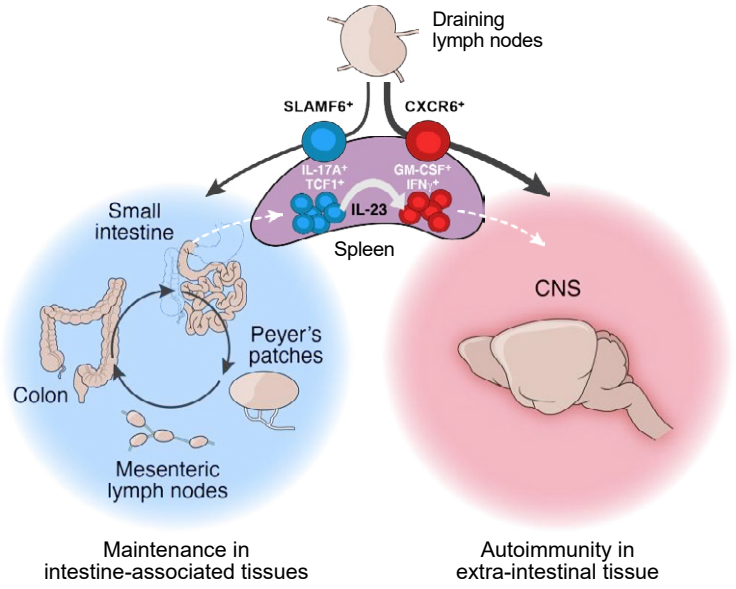

Figure 2: *A model of the conversion of the homeostatic TCF1+ IL-17A+ SLAMF6+ Th17 population to the pathogenic GM-CSF+ IFN+ CXCR6+ Th17 population during the development of neuroinflammation.*

stem-like SLAMF6+ population forms a reservoir in the gut maintained by microbiota that replenish pathogenic Th17 cells and directly contribute to inflammation in the brain. These results identified for the first time a mechanism linking homeostatic, gut T cells with pathogenic T cells by direct conversion of the stem-like intestinal cells to pathogenic cells. I am hopeful that these novel mechanistic insights into the gut-brain axis and the relationship of different pathogenic CD4+ T cell populations to one another can in the future be applied to other autoimmune and inflammatory diseases and could thereby inform therapeutic targeting of human autoimmune diseases. Importantly, the stem-like, intestinal SLAMF6+ Th17 cells could be the main population replenishing pathogenic cells during autoimmune diseases thereby being responsible for chronicity and relapses observed across many human autoimmune conditions (11). Therefore, targeting the SLAMF6+ Th17 population for the treatment of autoimmune diseases could, in contrast to current treatment options, specifically prevent disease chronicity and relapses.

Analysis of the chromatin landscape of Th17 cell heterogeneity

After the identification of homeostatic and pathogenic Th17 cells in vivo, I was interested in identifying regulatory mechanisms underlying Th17 cell heterogeneity to specifically identify targets for the treatment of autoimmune diseases. Together with collaborators in the laboratory of Aviv Regev at the Broad Institute, I performed combined ATAC-seq and RNA-seq of non-pathogenic and pathogenic Th17 cells both in vitro and in vivo as well as of other CD4+ T cell subsets to leverage the accessible chromatin landscape of Th17 cells to analyze the regulation of different Th17 cell phenotypes (12). Interestingly, we found non-pathogenic Th17 cells and pathogenic Th17 cells to exhibit vastly distinct chromatin landscapes, suggesting distinct regulatory mechanisms driving the different Th17 cell phenotypes. By modeling a regulatory network across Th17 heterogeneity, we predicted and validated the transcription factor BTB Domain and CNC Homolog 2 (BACH2) as a novel negative regulator of Th17 pathogenicity. We found BACH2 to restrain pathogenic programs (CXCR6+) in Th17 cells and to enhance homeostatic, stem-like programs (SLAMF6+) in Th17 cells thereby limiting the induction of tissue in-

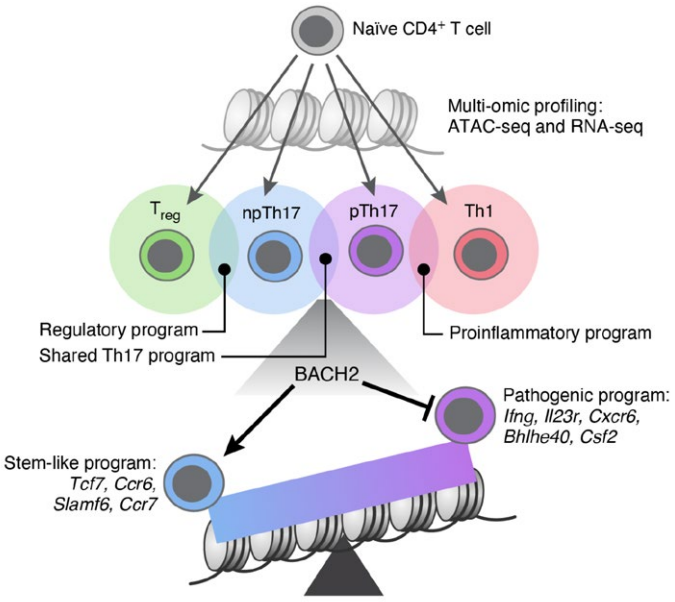

Figure 3: Summary figure depicting shared accessible chromatin features in CD4+ T cells and highlighting BACH2 as a novel regulator of Th17 cell function.

flammation and autoimmunity (**Figure 3**). Furthermore, analysis of a large-scale study of MS variant genome-wide association showed that BACH2 variants are also associated with autoimmune disease risk in humans. Together, we developed a framework to leverage chromatin accessibility profiles to yield novel insights into the regulation of $CD4^+$ T cell diversification, validated BACH2 as a promising novel target for autoimmunity, and yielded a foundation for future explorations of novel drivers of Th17 differentiation and function.

While my research contributed to our mechanistic understanding of the "gut-brain axis" and the role of Th17 cell heterogeneity for autoimmune diseases, important points remain to be addressed until we can translate our findings to treatments in the clinic. In the future, it will be important to identify the antigen specificities of the TCRs in the $SLAMF6^+$ and $CXCR6^+$ populations to identify the pathogenic antigens the Th17 cells react to and to explore if cross-reactivity to microbiota is involved in tissue-specific autoimmunity. It will also be important to identify the factors driving the conversion of the homeostatic cells to the pathogenic cells and to analyze whether similar mechanisms induce pathogenic Th17 cells in autoimmune inflammation at other tissue sites besides the brain.

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BINDER Innovation Prize
How cells compartmentalize their chromatin

Fabian Erdel

Summary
The cellular interior is compartmentalized, allowing the separation of biochemical activities in space and time. In the cell nucleus that lacks internal membranes, this is achieved via the formation of membrane-less subcompartments, which accumulate molecules involved in specific cellular activities. Some of these substructures form at distinct sites on chromatin, such as nucleoli at clusters of ribosomal DNA or 53BP1 nuclear bodies at DNA breaks, while others such as nuage granules are partitioned from chromatin. Several mechanisms have been invoked to explain the formation of such subcompartments, including different types of phase separation. Here, I briefly discuss these mechanisms along with the properties of the resulting subcompartments and the strategies to identify them in living cells. I will also discuss our recent work that challenges the notion that membrane-less subcompartments are mostly formed by liquid-liquid phase separation, but rather shows that cells use different mechanisms to form subcompartments of different flavors that correlate with their functions.

Demixing the nuclear content
The content of the cell nucleus is well-mixed via passive diffusion, which enables the rapid transport of molecules from one place to another (Phair and Misteli, 2000). Typically sized proteins can travel through the entire nucleus in only a few seconds, as shown for mono- and multimers of inert fluorescent proteins (Bancaud et al., 2009; Baum et al., 2014). Accordingly, concentration gradients within the nucleus are quickly balanced on these timescales, which means that local accumulations of diffusive molecules will rapidly disappear. Nevertheless, the nucleus is compartmentalized, i.e., it contains substructures in which distinct sets of proteins, RNA molecules and chromatin regions accumulate. This phenomenon can be referred to as "demixing", as it is the opposite of diffusive mixing that gives rise to uniform distributions of molecules. There are different scenarios to explain why some concentration gradients in the nucleus are not balanced by diffusion so that subcompartments can persist (**Fig. 1**). One is the *interaction with clustered binding sites* (ICBS) scenario, in which molecules of interest bind to segments of chromosomes, which are large and move only very slowly over larger distances (Erdel, 2020; Strickfaden et al., 2010). Consequently, the complexes of proteins and slowly moving chromatin segments cannot rapidly diffuse away but rather form a persisting subcompartment. Another scenario is *liquid-liquid phase separation* (LLPS), in which molecules of interest establish multivalent interactions with each other, which means that each of them simultaneously interacts with multiple neighboring ones. As a consequence, two phases are formed: a dense phase where the respective molecules establish a dense network of multivalent interactions (Choi et al., 2020; Sanders et al., 2020), and a dilute phase where the respective molecules are far apart from each other and do not interact with each other. The individual molecules in both the ICBS and the LLPS scenario are usually dynamic, which means that they exchange between the substructures and their surroundings and can explore the entire nucleus. Yet, the respective nuclear subcompartment as a whole persists, because each molecule that leaves is readily replaced by a molecule that newly arrives. The LLPS scenario does not necessarily involve chromatin, but the resulting condensates can be associated with chromatin. For subcompartments that contain chromatin, be they formed by ICBS or LLPS, the enclosed chromatin regions can be clustered and/or compacted via a process called *polymer-polymer phase separation* (PPPS), involving bridging interactions between chromatin segments (Erdel and Rippe, 2018). These bridges can be mediated by proteins that bind simultaneously to two chromatin segments, or by proteins that simultaneously bind to one chromatin segment and to other chromatin-binding proteins. Thus, PPPS can conceptually be driven by any chromatin-binding protein that forms dimers or higher-order multimers (Erdel, 2023). PPPS and LLPS are not mutually exclusive but rather describe distinct molecular processes, i.e., chromatin bridging (PPPS) and the establishment of a dense multivalent interaction network (LLPS), which can occur together or in isolation, and each of which has dedicated functional consequences as discussed below. Our recent work shows that both mechanisms are not always coupled in cells.

The demixing mechanism determines the properties of nuclear substructures
Subcompartments formed by the different demixing mechanisms mentioned above exhibit different properties that affect

their function. Most of these differences relate to the presence of a dense network of multivalent interactions in LLPS and the absence of such a network in ICBS (Choi et al., 2020; Erdel and Rippe, 2018; Muzzopappa et al., 2022). This interaction network influences the molecular dynamics and the physicochemical environment of the subcompartment. On the one hand, molecules that are embedded in such a network move more slowly and need more time to leave the network, which spans the entire dense phase, because interactions have to be broken. This means that such an interaction network will tend to increase the viscosity and create an interfacial barrier that reduces molecular exchange with the surroundings (Muzzopappa et al., 2022). This has consequences for the biochemical reactions in the subcompartment, whose rates depend on the viscosity and the influx and efflux of substrates and products. On the other hand, a dense interaction network along with a high concentration of macromolecules that establish these interactions changes the local physicochemical environment, and molecules that can engage in these interactions selectively partition into the dense phase. This applies to cellular macromolecules, but also to exogenous molecules that are added to the cell from outside, such as cancer therapeutics (Klein et al., 2020). Altered solution properties have also been shown to affect the conformation of cellular macromolecules in the dense phase (Wei et al., 2017), which may modulate their activity. Another important hallmark of LLPS is that the separation into a dilute and a dense phase occurs abruptly if a threshold concentration of interacting molecules or a threshold level of crowding is exceeded, while there is no such discontinuity in ICBS. In this respect, LLPS is an "all-or-none" mechanism, in which a substructure is formed or not, while in ICBS molecules gradually partition into a substructure as concentrations or crowding levels increase. Taken together, the question if a subcompartment is formed by LLPS or ICBS has several implications for understanding its molecular composition, material properties and stability, as well as for the transport and conformation of the individual molecules it contains. At the level of chromatin, the spatial reorganization that occurs during PPPS modulates genomic contact probabilities, and chromatin bridges alter the stiffness of the subcompartment. Thus, the question if subcompartments are formed via PPPS has con-

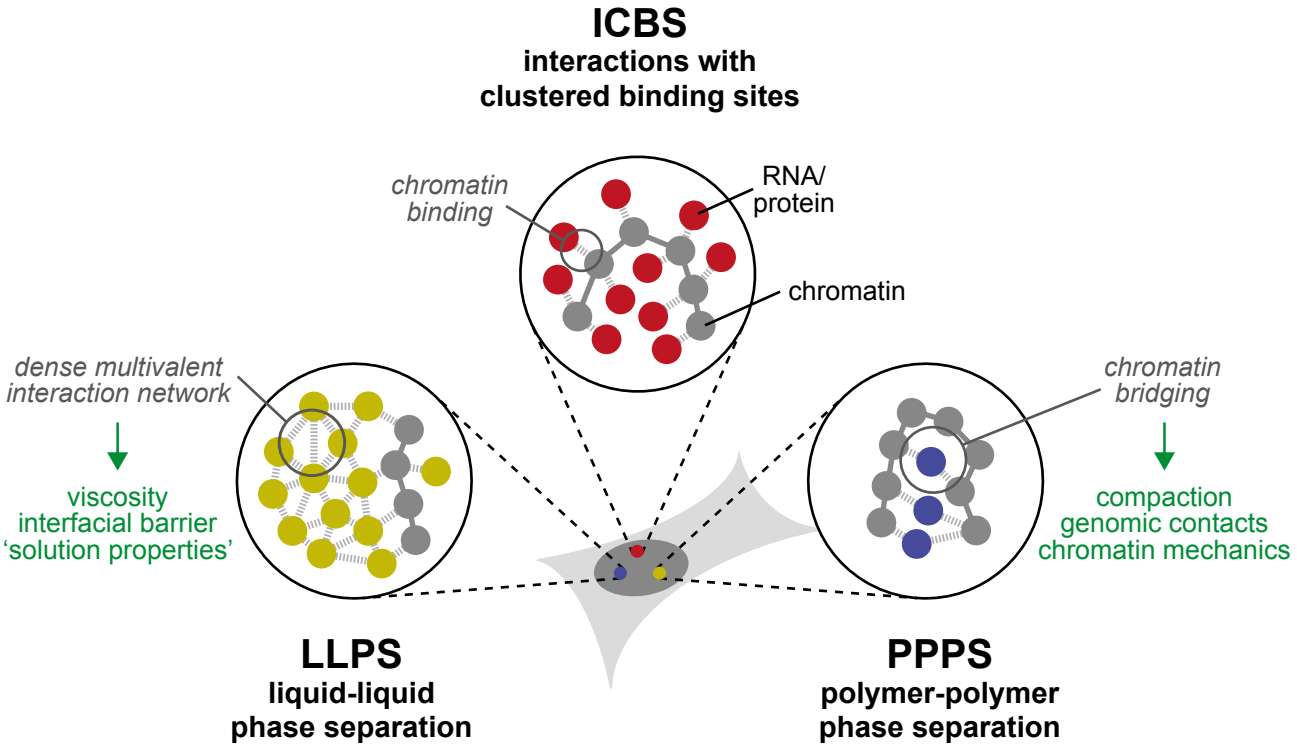

Figure 1: *Different mechanisms can explain the demixing of molecules within the nucleus into membrane-less subcompartments. On the one hand, proteins/RNA molecules can interact with clustered binding sites (ICBS) present on chromosomes, forming local accumulations that persist because chromosomes diffuse only very slowly over larger distances. On the other hand, proteins/RNA molecules can undergo liquid-liquid phase separation (LLPS), which involves the establishment of a dense multivalent interaction network that affects the viscosity and 'solution properties' of the subcompartment and creates an interfacial barrier that reduces molecular exchange with the surroundings. Chromatin within a subcompartment can undergo polymer-polymer phase separation (PPPS), which is driven by bridging interactions among chromatin segments and affects long-range genomic contacts and chromatin mechanics.*

sequences for understanding the regulation of gene expression and nuclear mechanics.

Identifying the demixing mechanisms driving nuclear compartmentalization

As the demixing mechanism plays an important role in determining the behavior of a nuclear subcompartment, it is important to identify it in the context of a living cell. This task has turned out to be notoriously complicated because it requires non-invasive live-cell assays to identify dense multivalent interaction networks, chromatin bridges, and/or their functional consequences. We have addressed this issue by developing tools to measure the local viscosity and the strength of the interfacial barrier using fluorescence fluctuation microscopy (Erdel et al., 2020; Muzzopappa et al., 2022). The latter was accomplished with a modified fluorescence recovery after photobleaching (FRAP) approach that we termed *MOCHA-FRAP* for model-free calibrated half-FRAP (**Fig. 2**). In this assay, labeled molecules in one half of the subcompartment are bleached, and the fluorescence intensity in the bleached and the non-bleached half is monitored over time. In the presence of an interfacial barrier, molecules show a preference to stay within the subcompartment instead of moving across its interface into the surrounding nucleoplasm. The stronger this barrier, the more likely a bleached molecule moves to the non-bleached half, which is part of the same subcompartment, instead of crossing the interface to change place with a molecule from outside. The preference for internal mixing is therefore seen as a pronounced intensity decrease in the non-bleached half. The MOCHA-FRAP method quantifies this intensity decrease and converts it into the strength of the interfacial barrier. We have used MOCHA-FRAP to study several nuclear substructures side-by-side as explained in the next section.

Cells use different mechanisms to compartmentalize their chromatin

Cells partition their chromatin into distinct subcompartments that serve specific functions. Silent genomic regions are packaged into heterochromatin foci that contain heterochromatin protein 1 (HP1) or into Polycomb bodies that contain several Polycomb group proteins. Active genomic regions are also partitioned into distinct nuclear substructures, including for example nucleoli, the production sites of ribosomal RNAs, and transcription factories or transcriptional condensates, which contain genes that are actively transcribed by RNA polymerase II. Other chromatin-associated activities besides transcription are also compartmentalized, for example DNA repair that occurs in dedicated foci containing 53BP1. To gain a comprehensive picture of how cells compartmentalize their chromatin, we applied the MOCHA-FRAP technique to a panel of different nuclear substructures (**Table 1**). In general, we found that cells contain chromatin subcompartments with and without interfacial barriers, which strongly indicates that cells do not rely on a single mechanism to form chromatin subcompartments. More specifically, silent chromatin compartments associated with HP1 in mouse fibroblasts lacked an interfacial barrier, while RNA-rich nucleoli in the same cells exhibited such a barrier (Erdel et al., 2020; Muzzopappa et al., 2022). Compact chromatin domains decorated with the ubiquitin ligase TRIP12 lacked an interfacial barrier (Vargas et al., 2023). Early DNA repair foci containing poly (ADP-ribose) chains exhibited a barrier, while late DNA repair clusters that were devoid of poly (ADP-ribose) chains and formed a distinct chromatin compartment, the 'D' compartment, lacked such a barrier (Arnould et al., 2023). These results show that chromatin subcompartments come in different flavors, with cells using different demixing mechanisms to specifically regulate their properties according to the required activity. These observations suggest that some biochemical activities are compatible with the default physicochemical environment of the nucleoplasm, which is mostly unchanged in subcompartments formed by ICBS/PPPS, while other activities are favored in special environments that are created via LLPS. Although more work is needed to extract general principles, our current data suggest that chromatin subcompartments enriched in charged polymers with low complexity, i.e., RNA molecules and poly (ADP-ribose) chains, tend to be formed by LLPS, while subcompartments that lack such molecules, for example undamaged heterochromatin domains, tend to be formed by ICBS/PPPS. We are currently investigating this hypothesis using single-molecule reconstitution approaches.

Outlook

Despite the recent progress in understanding phase separation mechanisms and the resulting biomolecular condensates, there are still many questions that remain unanswered. On the technical side, recent fluorescence microscopy approaches such as MOCHA-FRAP make it now possible to distinguish different types of subcompartments from each other in living cells, which represents an important step toward dissecting the biophysical mechanisms that are at play. A limitation of the current approaches is the minimum required size of the subcompartments of interest, making it difficult to investigate small substructures at or below the diffraction limit, such as transcriptional condensates. We are currently developing approaches to address this issue. Complementary strategies that resolve the solution properties of nuclear substructures via fluorescence-based sensors have recently been introduced. These rely on the sensitivity of fluorescent probes to the local physicochemical environment, e.g., pH, polarity and viscosity (Guidotti et al., 2022; Martin et al., 2015). I anticipate that future developments in this field will help to complement the assays that are based on fluorescently tagged cellular proteins. The same applies for label-free microscopy approaches, such as quantitative phase microscopy and Brillouin microscopy, which can probe key aspects of biomolecular condensates, such as their composition and mechanics, in unperturbed cells (McCall et al., 2023; Schlüßler et al., 2022). Despite these new possibilities in detecting phase separation

processes and in mapping the properties of nuclear subcompartments, some challenges will probably still remain. One is to clearly pin down the biological function of phase separation once it is detected. We currently lack tools to systematically and selectively perturb dense multivalent interaction networks without changing the interactomes of the molecules involved in these networks, which would be very helpful to dissect their function. This could be conceptually possible by reducing interaction valencies without (significantly) changing affinities. The development of new classes of condensate-modifying drugs might make this possible (Patel et al., 2022). Another key challenge is to understand how molecules selectively partition into the correct subcompartment although the interactions driving phase separation are supposedly similar across different cellular condensates. Addressing these questions will likely keep researchers in the field busy in the near future.

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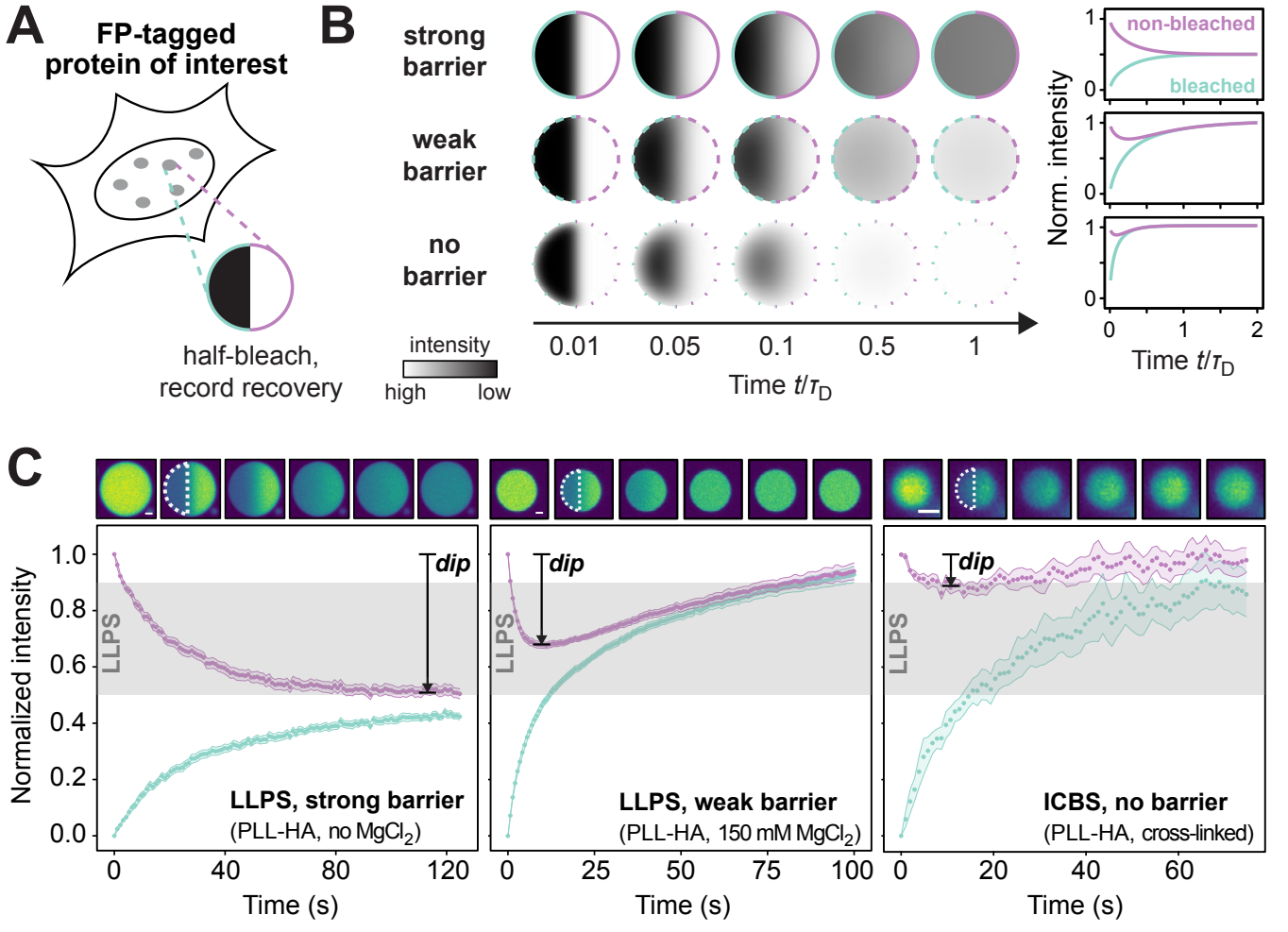

Figure 2: *Subcompartments formed by different mechanisms can be distinguished using model-free calibrated half-FRAP (MOCHA-FRAP), which detects the interfacial barriers that are created by dense multivalent interaction networks.* **(A)** *MOCHA-FRAP involves bleaching fluorescently tagged proteins in one half of the subcompartment of interest, followed by the analysis of the intensity in the bleached and the non-bleached half.* **(B)** *Theoretically calculated fluorescence recoveries for subcompartments with or without interfacial barriers. The recovery curves for both halves in the different scenarios are shown on the right. The presence of a barrier translates into an intensity decrease ("dip") in the non-bleached half. Images adapted from (Erdel et al., 2020).* **(C)** *MOCHA-FRAP experiments for reconstituted model compartments formed by LLPS or ICBS. Compartments were made from labeled poly-L-lysine (PLL) and unlabeled hyaluronic acid (HA), which can undergo LLPS in the test tube. Interfacial barriers were modulated by the addition of $MgCl_2$, which weakens the interactions between PLL and HA. ICBS was mimicked by chemically cross-linking unlabeled PLL-HA condensates to obtain a large cluster of binding sites, and by subsequently adding labeled PLL that transiently interacts with them. Images adapted from (Muzzopappa et al., 2022).*

lier career at the DKFZ in Heidelberg and at Columbia University in the City of New York. I particularly thank my previous supervisors Karsten Rippe and Eric Greene who have trained, inspired and supported me. I am grateful for the funding by the European Research Council (ERC-2018-StG 804023), the French Foundation for Medical Research (FRM), and the French National Centre for Scientific Research (CNRS), which has made my research possible. I also thank my family and friends for their enduring support.

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Protein	Subcompartment	Barrier (kT)	Mechanism	Reference
Ddx4	Nuage granule	0.07	LLPS	(Muzzopappa et al., 2022)
Npm1	Nucleolus	0.01		(Muzzopappa et al., 2022)
53BP1	Early DNA repair site	0.03		(Arnould et al., 2023)
	D compartment	0	ICBS/ PPPS	
Cbx5/ HP1α	Heterochromatin	0		(Muzzopappa et al., 2022)
TRIP12	Condensed chromatin	0		(Vargas et al., 2023)

Table 1: Demixing mechanisms and interfacial barriers for various nuclear subcompartments based on MOCHA-FRAP. *We used MOCHA-FRAP to study several nuclear substructures in mammalian cells. The results show that cells contain subcompartments with or without interfacial barriers, which reflects the presence or absence of a dense multivalent interaction network, a hallmark of LLPS.*

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BINDER Innovation Prize	2023
ERC Starting Grant	2018
ATIP Avenir Fellowship	2018
EMBO Long-Term Fellowship	2014
Selection by Scientific American as promising young scientist under 30	2013
Nikon Young Scientist Award	2013
Annemarie Poustka PhD Fellowship	2009
Otto Haxel Award	2009

Werner Risau Prize
Incongruence between transcriptional and vascular pathophysiological cell states

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Summary
The cellular The Notch pathway is a major regulator of transcriptional specification and vascular biology. Previous studies have suggested that targeting the ligand Dll4 or the Notch-receptors results in similar angiogenesis outcomes and vascular neoplasms. Here, we analyze single and compound genetic mutants for all Notch signaling members and find very significant differences in the way ligands and receptors regulate liver vascular homeostasis. Loss of Notch receptors or Rbpj leads to minor vascular pathology featuring hypermitogenic cell-cycle arrest and senescence. In contrast, loss of Dll4 triggers a strong Myc-driven transcriptional switch towards cell proliferation and sprouting and major pathology. Targeting of Myc completely suppressed these angiogenic cell states induced by Dll4 loss-of-function. However, this did not avoid the vascular abnormalization and organ pathology. Inhibition of MAPK/ERK, Rac1, Pi3K/mTor or NO signalling also did not prevent it. Only anti-VEGF prevented the vascular pathophysiology induced by Dll4 loss, but without fully suppressing its transcriptional and metabolic programs. This study shows incongruence between single-cell transcriptional states, vascular phenotypes and related pathophysiology. Our findings also establish that it is the vascular structure abnormalization, rather than neoplasms, that causes the reported anti-Dll4 toxicity.

Background
Notch is a cell-to-cell ligand-receptor signaling pathway that has a major influence on cell transcription and biology[1] playing important roles in several diseases[2]. General Notch signaling or γ-secretase inhibitors have been used in the clinics, with undesired side effects, including disruption of the normal intestinal stem-cell differentiation[2,3]. Specific blocking antibodies are now available that target the various ligands and receptors of the Notch pathway[4-8]. Given the specificity of Dll4 expression in endothelial cells (ECs), targeting this ligand was initially thought to be an effective and safe strategy for specifically modulating Notch signalling and angiogenesis in disease, such as during tumor growth[6,7]. However, anti-Dll4 was later shown to induce a loss of endothelial quiescence and vascular neoplasms, which were proposed to be the main cause of pathology in several organs[5,8,9]. This toxicity diminished the clinical appeal of Dll4/Notch blockers in cancer or cardiovascular disease settings.

Here, we characterized the impact on adult mice vascular homeostasis of single or compound targeting of all Notch signaling members. High-resolution scRNA-seq and 3D confocal microscopy of adult liver vessels revealed very significant differences in the way each Notch member regulates vascular signaling, structure and single-cell states. γ-secretase inhibitors or removal of Notch receptors did not cause significant vascular or organ disease. Abnormal proliferating and sprouting single-cell states were generated only after Dll4 targeting. Surprisingly, suppression of these angiogenic cell states by additional genetic or pharmacological targeting was insufficient to prevent vascular and organ disease. Conceptually, our data shows that the major transcriptional changes and angiogenic cell states elicited by targeting Dll4 correlate with, but do not cause, the observed vascular pathophysiology. Instead, we propose that it is the unrelated vascular structure abnormalization and malfunction that leads to organ pathology and the reported toxicity of anti-Dll4[5,8].

Main Results
Notch pathway expression and signalling in adult organs ECs
To elucidate the role of Notch signaling in global vascular homeostasis, we first assessed its activity in different organ vascular beds by immunodetection of the activated form of the Notch1 intracellular domain (N1ICD^{Val1744}). This epitope was detected in ~50% of all organ ECs (Fig. 1a, 1b). Bulk RNA-seq analysis revealed that *Dll4* and Notch1 are the most expressed ligand–receptor pair in quiescent vessels of most organs (Fig. 1c, 1d). Adult mice with induced deletion of Dll4 in ECs (Dll4^{iDEC} - Dll4$^{flox/flox}$ Cdh5-CreERT2) led to a significant reduction in N1ICD^{Val1744} and Hey1 signals in most organs quiescent ECs (Fig. 1e-i). This indicates that Dll4 is the main functional ligand responsible for triggering Notch activity in most quiescent vessels. Only in lungs we observed compensatory upregulation of *Dll1* (Fig. 1i). *Dll4* deletion elicited remarkably different gene expression signatures among different organ vascular beds, with the adult liver endothelium presenting the most pronounced changes in gene expression (Fig. 1j, k). Despite significant transcriptional changes in most organs ECs, only the endothelium of the heart, muscle, and liver showed an increase in the frequency of cycling or activated Ki67+ cells upon *Dll4* deletion (Fig. 1l-n), and these were the only organs with clear alterations in the 3D vascular architecture after the loss of Dll4–Notch signaling (Fig. 1o).

Targeting Dll4 induces heterozonal responses in liver vessels

The previous RNA-seq and histological data revealed the adult liver endothelium as the most reactive vascular bed to the targeting of Dll4–Notch signaling. Rats and chimpanzees treated with anti-Dll4 antibodies also developed significant liver vascular neoplasms and disease[5,8], and therefore we focused our analysis on this organ. To gain deeper insight, we performed a high-resolution spatiotemporal phenotypic analysis after targeting Dll4 for 2 days to 3 weeks. The increase in vascular density after targeting Dll4 was relatively slow and progressive, only becoming noticeable one week after genetic deletion (Fig. 2a-c). Endothelial proliferation peaked at day 4, and was sustained after, leading to a progressive increase in vascular density and the total number of ECs (Fig. 2b-d). Proliferation of neighboring hepatocytes was also increased, peaking after the peak in endothelial proliferation (Fig. 2e), suggesting that Dll4^{KO} ECs secrete angiocrine factors inducing hepatocyte proliferation, as shown before during liver regeneration10.

The effect of Dll4 targeting was, however, notably heterogeneous and zonal. Only vessels around the central veins (CVs) and with a known venous identity11 had a higher number of ECs (Fig. 2f, g), larger nuclei (Fig. 2h), and expression of cell-cycle (Fig. 2i, 2j) and apoptosis (Fig. 2k) markers. Therefore, the previously reported anti-Dll4–driven liver histopathology and increase in cell proliferation8 is now found to be mainly associated to the central-vein sinusoids. Paradoxically, the portal vein (PV) sinusoids, which have arterial identity and the highest Dll4 expression and Notch activity (Fig. 2l-n), showed a minor increase in EC proliferation (Fig. 2i) despite a significant loss in the expression of arterial genes (Fig.2o). Besides the cell-cycle marker Ki67, we also analyzed more specific S-phase (EdU) and cell-cycle arrest/senescence (p21) markers. This analysis revealed expression of p21 in 30% of Dll4^{iDEC} ECs in the venous vessels around the central veins (Fig. 2p). Among Ki67+ ECs, 40% were positive for EdU and 25% for p21 (Fig.2q). This shows that there is a mix of productive cell division (EdU+) and arrest (p21+) after Dll4 loss in liver ECs. Pulse–chase single-cell ifgMosaic tracking revealed that relatively few of the Ki67+ ECs had the ability to divide and clonally expand after Dll4 targeting, with some cells dividing 6 to 50 times more than their neighbours (Fig. 2r). All of these progenitor cells were located in the sinusoids around central veins (Fig. 2r iii).

Loss of Notch1 or Rbpj in LSECs induces hypermitogenic arrest

Notch ligands and receptors can be targeted with a range of pharmacological compounds and antibodies[4–7], and so far only Dll4-targeting antibodies have been reported to cause major vascular disease[5,8]. In contrast, genetic deletion of *Notch1* or *Rbpj* in mice has been suggested to cause vascular phenotypes very similar to the genetic deletion of *Dll4*, during angiogenesis and in adult vessels[9,12–15]. We therefore investigated if deleting Notch1 or *Rbpj*, the master regulator of all Notch receptor signaling, induced vascular pathology similar to that induced by the loss of Dll4 (Fig. 3a). Surprisingly, *Notch1* and *Rbpj* deletion for 2 or 4 weeks did not significantly increase EC proliferation and related vascular pathophysiology (Fig. 3b-e), despite these mutant cells having even higher phospho-ERK (P-ERK) activity than ECs lacking Dll4 (Fig. 3f-h). We next compared the transcriptome of Dll4^{iDEC} and Rbpj^{iDEC} vessels. ECs from both mutant lines showed a similar upregulation of genes related to cell-cycle activation and metabolism (Fig. 3i) and had enlarged nuclei (Fig. 3j). However, compared with Dll4^{iDEC} livers, Rbpj^{iDEC} livers had significantly less vascular expansion (Fig. 3k) and stronger upregulation of p21 (Fig. 3l), a cell-cycle inhibitor frequently upregulated in senescent or hypermitogenically arrested cells[16]. We also identified a significant increase in the number of binucleated p21+ ECs, suggestive of replicative stress and G2 arrest of the mutant cells (Fig. 3m). RNA-seq analysis revealed signatures of genetic pathways linked to G2M checkpoints, chromosome segregation, and general replicative stress and senescence in Rbpj^{iDEC} ECs (Fig. 3n). To determine the functional impact of p21 upregulation, we analyzed compound Rbpj^{iDEC} p21^{KO} mice (Fig. 3o). p21 loss did not affect the minor vascular sinusoid dilation seen in Rbpj^{iDEC} livers, but did increase the frequency of cycling (Ki67+) and apoptotic (Cleaved caspase3+) cells (Fig. 3p-r), in line with the role of p21 as a cell-cycle and apoptosis inhibitor17, particularly in hypermitogenically activated RbpjKO cells. This dual and paradoxical effect of p21 loss on both cell proliferation and apoptosis may explain the relatively mild increase in EC numbers in Rbpj^{iDEC} p21^{KO} livers compared with the fully arrested Rbpj^{iDEC} liver vessels. These results suggest that loss of Dll4 induces a reduction in Notch signaling that results in a mixed population of proliferative and arrested ECs, whereas the complete loss of Notch signaling induces mostly hypermitogenic arrest, without productive cell division.

Targeting Dll4 and Notch induces incongruent cell states

We next performed single-cell RNA-seq to identify possible differences in vascular single-cell states induced by targeting either Dll4, Notch1 or Rbpj. This analysis was performed on cells expressing the Cdh5-CreERT2 and *iSuRe-Cre* alleles[18] to guarantee endothelium-specific recombination, labeling, and full genetic deletion of all the floxed genes used in this study (Fig. 4a-b). Altogether, the scRNA-seq data analysis showed the existence of 10 clearly defined cell clusters (Fig. 4c-e). The deletion of *Rbpj*, *Notch1*, and Dll4 resulted in a significant decrease in Notch signaling and *Hes1* expression (Fig. 4b) and in the loss of the arterial sinusoidal capillaries transcriptional C1a cluster. However, only the loss of Dll4 was able to induce a very pronounced loss of liver sinusoidal genes and capillarization[19,20] and a tip-cell transcriptional program (C4). This program was characterized by the downregulation of Gata4[19], Maf[21] and the

venous *Wnt2* gene expression (Fig. 4f-h) and very high expression of the tip-cell markers *Kcne3*, *Esm1*, *Angpt2* and *Apln*, as well as *Myc* and its canonical target *Odc1* (Fig. 4i). Paradoxically, Notch1^{iDEC} and Rbpj^{iDEC} liver ECs, in which the decrease in Notch signaling was more pronounced (Hes1 expression in Fig. 4b), showed a more moderate metabolic activation, and most of these mutants ECs clustered either in the venous C1v or in the activated C3 cluster and did not reach the extreme C4 tip-cell state (Fig. 4c,d).

Histology confirmed that indeed only the *Dll4^{iDEC}* mutants had a significant population of Esm1+ tip cells and these were mostly present in the venous sinusoidal capillaries interconnecting the liver central veins (Fig. 4j, k), where EC proliferation and density are the highest (Fig. 2f-i). The upregulation of the global cell-cycle marker Stmn1 in *Dll4^{iDEC}* livers (Fig. 4l) correlated with the 6-fold higher frequency of Ki67-protein+ cells in these mutants than in the Notch1 and Rbpj mutants (Fig. 3d). Most Esm1+ tip cells were not Ki67+, in accordance with their higher sprouting activity and arrested nature, but had proliferating Ki67+ cells as close neighbors (Fig. 4j, m). *Notch1^{iDEC}* and *Rbpj^{iDEC}* ECs showed significant upregulation of the replication-stress/senescence markers p21 (cdkn1a), p53 (trp53), and p16 (cdkn2a) (Fig. 4n). These cells undergo hypermitogenic S-G2-M arrest (Fig. 3m, 3n), without becoming Kcne3+/Esm1+ sprouting tip cells (Fig. 4c, d), which is in contrast to the current understanding of sprouting angiogenesis[22,23].

Deletion of *Notch1/2/4* in ECs, similarly to Rbpj loss, results in even lower Hes1 expression and higher p21 expression (arrest); however, this does not result in the induction of tip cells (Esm1+/Kcne3+) or proliferating Stmn1+ cells (Fig. 4o-q).

We also tested a general γ-secretase inhibitor (DBZ), that is known to block Notch signaling and elicit strong effects on tumor and retina angiogenesis[6], similarly to anti-Dll4. However, this compound had a very weak effect on quiescent vessels, similar to the changes seen in Dll4 heterozygous livers (Fig. 4r-t). This suggests that in order to fully activate quiescent ECs and induce significant numbers of tip cells and vascular abnormalization, pronounced and continuous loss of Dll4 signaling must be sustained for about a week, which can be achieved with genetic deletion or blocking antibodies5 but not with small molecule inhibitors targeting Notch.

Deletion of all other Notch ligands do not elicit pathology

Besides Dll4, other Notch ligands are also expressed in liver ECs (Fig. 5a). The Notch signaling target Hes1 is more expressed in *Dll4^{iDEC}* than in *Notch1^{iDEC}*, *Rbpj^{iDEC}*, or *Notch1/2/4^{iDEC}* mutants (Fig. 4b, 4q), suggesting that the other weakly expressed Notch ligands (Dll1, Jagged1, and Jagged2) may partially compensate the loss of Dll4 and induce residual Notch signaling essential for the induction of the tip-cell state. Notably, *Jagged1* mRNA was barely detectable in bulk or scRNA-seq data of quiescent liver ECs (Fig. 1d and Fig.5a), but its protein was clearly expressed in liver vessels (Fig. 5b). Deletion of all three ligands (*Jag1*, *Jag2*, and *Dll1*) did not alter vascular morphology, induce pathology, or increase the frequency of Ki67+ cells, confirming that Dll4 is the main Notch ligand in quiescent vessels (Fig. 5c-g). Liver blood profiling revealed an increase in the percentage of neutrophils, but this was also seen in circulating blood, suggesting a systemic rather than organ-specific role of these ligands (Fig. 5h, i). In agreement with this, scRNA-seq data analysis confirmed that most mutant ECs remained quiescent and did not become activated or form tip cells (Fig. 5j-l). Moreover, deletion of *Jag1/2/Dll1* in ECs did not compromise the portal sinusoids arterial identity (Fig. 5k, m), instead revealing a significant and counterintuitive increase in the Notch signaling target *Hes1* and an increase in the arterial gene *CD34*, together with a very pronounced decrease in the expression of the venous-enriched *Wnt2* gene (Fig. 5n). This counterintuitive increase in Notch signaling was also observed before after the loss of Jagged1 during angiogenesis[24].

Myc loss prevents *Dll4^{iDEC}* transcriptional states but not pathology

We next aimed to determine the molecular mechanisms responsible for the unique EC activation, tip-cell signature, and vascular pathology induced by targeting Dll4. As mentioned above, *Myc* and its target *Odc1* were among the most strongly upregulated genes in Dll4 mutant ECs, compared with Notch1 and Rbpj mutants. Myc is known to activate important ribosome biogenesis and protein translation pathways, favoring cell growth[25]. Dll4^{iDEC} livers showed upregulation of a large range of canonical E2F, Myc, mTORC1 and ribosomal (*Rpl*) genes, particularly in the activated, proliferating, and endothelial tip-cell clusters (Fig. 6a). This hypermetabolic transcriptional status was confirmed by mass spectrometry (MS) analysis of protein lysates obtained from freshly isolated liver ECs (Fig. 6b-f), providing a high-depth proteomic analysis of the endothelial tip-cell state induced by targeting Dll4. We also independently confirmed *Myc* mRNA and protein upregulation in Dll4KO vessels (Fig. 6g-h).

We next investigated the implication of Myc in the *Dll4^{iDEC}* transcriptional program and subsequent vascular-related pathology. Myc loss (in *Dll4/Myc^{iDEC}* animals) almost entirely blocked the EC activation induced by *Dll4* loss, and very few ECs were in the activated (C3) and tip-cell (C4) clusters (Fig.6i-l). Consistent with the scRNA-seq data, frequencies of proliferating (Ki67+) and tip (Esm1+) cells in *Dll4/Myc^{iDEC}* mutants were similar to those in wildtype animals (Fig. 6m). Myc activity is thus essential for the strong metabolic and biosynthetic phenotype of *Dll4^{KO}* liver ECs and the appearance of the abnormal cell states. Surprisingly, despite this strong transcriptional and cell states reversion to a quiescent state, *Dll4/Myc^{iDEC}* mutant vessels were still highly ab-

normal and dilated (Fig. 6n). The vascular abnormalities in *Dll4/Myc*^{iDEC} mutant livers were not in accordance with their more quiescent scRNA-seq profile (Fig.6j-l), nor with the significantly lower frequencies of Ki67+ and Esm1+ cells (Fig. 6m). Interestingly, *Dll4/Myc*^{iDEC} livers retained hallmarks of tissue hypoxia and inflammation (Fig. 6l). Altogether, these data indicate that the vascular structure abnormalization observed in Dll4 mutant livers is not driven by the detectable changes in endothelial transcriptional programs or the proliferative and tip endothelial cell states.

Anti-VEGF prevents the Dll4iDEC pathology with a lower impact on transcription

Among the few GSEA Hallmark pathways whose upregulation in *Dll4* mutants was not altered in *Dll4/Myc*^{iDEC} vessels was the hypoxia pathway and inflammatory response (Fig. 6l). Hypoxia is known to induce expression of VEGFA, which can induce vascular expansion without the need for proliferation[26]. Therefore, we explored if anti-VEGF would be able to prevent the appearance of the activated vascular cell states, vascular enlargement and liver pathology induced by *Dll4* deletion. Unlike Myc loss, anti-VEGF treatment reduced both the vascular expansion and the liver pathology induced by Dll4 deletion (Fig. 7a-d). scRNA-seq analysis confirmed the almost complete loss of the tip-cell (C4) and proliferating (C5) single-cell states, as well as a significant reduction in the activated cell states (C3), with a general return to the quiescent cell states (Fig. 7e-i). scRNA-seq and histology data also revealed a depletion of VEGFR2/Kdr+ sinusoidal capillaries by anti-VEGFA (Fig. 7b-e, i). Anti-VEGFA rescued the expression of the blood flow and shear stress responsive genes Klf2 and Klf4 (Fig. 7j), suggesting a normalization of vessels and blood flow.

These results show that anti-VEGF prevents not only the appearance of the abnormal single-cell states induced by Dll4 targeting, as Myc loss also does, but in addition it also prevents the vascular expansion and blood flow abnormalities associated organ pathology. However, blocking VEGF had a much lesser impact than Myc loss on the *Dll4*^{KO}-transcriptional signature (Fig. 7k). Anti-VEGFA treatment of *Dll4*^{iDEC} livers attenuated, but did not completely downregulate, many of the genes associated with metabolic and biosynthetic activities (Fig. 7l). This suggests that even though *Dll4*^{iDEC} + anti-VEGF-treated ECs are transcriptionally and metabolically more active than *Dll4/Myc*^{iDEC} ECs, paradoxically only the latter form abnormal and enlarged vessels that result in organ pathology.

Inhibition of major signalling pathways did not prevent Dll4iDEC pathology

VEGFA induces many important and often difficult to distinguish endothelial functions, such as proliferation, sprouting, cell size, survival, and permeability[27-29]. VEGF is thought to execute its effects on sprouting and angiogenesis mainly through ERK signaling[30,31]. However, administration of a highly effective ERK/MEK signaling inhibitor (SL327) had a much more modest effect than anti-VEGF, and only partially reduced the number of activated and tip ECs (Fig. 7m-s). The VEGF-dependent vascular enlargement or expansion could be alternatively mediated by increased Rac1[32], Pi3K/mTor[33,34] or nitric oxide (NO)[35,36] signalling. However, the inhibition of these pathways also did not prevent the vascular pathophysiology induced by targeting Dll4 (Fig. 8). Rapamycin did effectively prevent the increase in the number of ECs, but not vascular dilation and pathology. Thus, the vascular pathophysiology effects of anti-VEGF, and anti-Dll4, are broader and independent of these signalling pathways activity.

Overall, these results show that the genetic and pharmacological modulation of single-cell states related with endothelial dedifferentiation, activation, proliferation and sprouting often do not correlate with adult vascular phenotypes, function and ultimately organ pathology.

Discussion

Notch is one of the most important pathways for vascular development by enabling the necessary differentiation of ECs during angiogenesis[28,37,38]. Here, we extend on previous observations that Notch also plays an important role in the homeostasis of several organ vascular beds[8,9,39]. Dll4 is active in all organ vascular beds, and its loss impacts the transcriptome of most quiescent ECs; however, Dll4 targeting only effectively activates vascular growth in the heart, muscle, and liver. Even though the existence of 4 Notch receptors and 5 ligands allows for the possibility of multiple quantitative and qualitative signaling combinations and redundancy, our results confirm that Dll4 and Notch1 are clearly the most important Notch ligand-receptor pair for maintaining the global homeostasis of ECs.

Previous work suggested that Dll4 and Notch1/Rbpj have similar functions in vascular development and homeostasis[6-8,15,24,40,41], with only Jagged ligands shown to have opposite functions in Notch signaling and angiogenesis24. In this study, we show that Dll4 can have distinct functions from its receptors in vascular biology. It was possible to identify this difference only thanks to the use of scRNA-seq and high-resolution confocal analysis of liver vessels morphology; bulk RNA-seq analysis did not reveal significant differences between the transcriptomes of Dll4 and Rbpj mutants. The loss of Dll4, unlike the loss of Notch receptors or Rbpj, elicits a unique cascade of changes that culminates in the loss of sinusoidal marker genes and upregulation of Myc, similar to the loss of Gata4[19]. *Dll4*^{iDEC} liver vessels lose all quiescent arterial and venous cell states. The arterial cells become highly activated, and the venous cells show either tip-cell or proliferating cell signatures. Paradoxically, although Dll4 loss induces a weaker loss of Notch signaling than is induced by the loss of Notch receptors or Rbpj, it elicits a much stronger metabolic activation and expansion of the liver endothelium. This

may be in part related to the bell-shaped response of ECs to mitogenic stimuli, as we previously showed during retina angiogenesis[16]. Our data indicate that full loss of Notch, or Rbpj, induces stronger ERK signaling and hypermitogenic arrest associated with hallmarks of cellular senescence, whereas *Dll4*^{iDEC} vessels retain a residual level of Notch signaling, that instead effectively induces strong Myc-driven ribosome biogenesis and a metabolic switch towards active protein synthesis and cell growth that drives both EC proliferation and the generation of tip cells. The pro-proliferative effect of targeting Dll4 in quiescent vessels is in contrast to the hypermitogenic cell-cycle arrest that occurs after targeting Dll4 during embryonic and retina angiogenesis[16,42], presumably a reflection of the significantly lower levels of growth factors, including VEGF, in adult organs.

High resolution confocal microscopy revealed the heterozonal effect of Dll4 targeting. The induction of EC proliferation and tip cells was restricted to the most hypoxic liver venous sinusoids, precisely the ones with lower expression of Dll4 and Notch. Previous research showed that liver venous sinusoids have higher baseline activity of several tyrosine kinase signaling pathways[43], which may explain the observed zonal effect of Dll4 targeting.

The temporal analysis of the effects of Dll4 targeting on the adult liver vasculature also revealed that it takes at least a week for the full transcriptional reprogramming of quiescent ECs and the vascular expansion and organ pathology to become noticeable. During angiogenesis, this transcriptional and vascular morphology switch is already evident after 24 hours of anti-Dll4 treatment[16]. This slow transcriptional reprogramming of quiescent ECs by Dll4 targeting may be related with the much lower levels of growth factors and nutrients availability in adult organs. The slow nature of this reprogramming may also explain the lack of effect of the small molecule inhibitor DBZ on quiescent ECs. Unlike anti-Dll4 or genetic deletion, which result in continuous loss of signaling, the less stable small-molecule inhibitor DBZ elicited no significant change in the quiescent vascular cell transcriptional states and phenotypes, whereas it is very effective during retina angiogenesis[15,16]. These findings have implications for selecting the most effective and safest way to target Notch in the clinics, with blocking antibodies targeting Dll4 versus antibodies targeting Notch receptors, or the use of small-molecule inhibitors. Our data indicate that Notch-receptor targeting antibodies or small-molecule γ-secretase inhibitors do not induce significant liver vascular pathology, and should be as effective as anti-Dll4 at dysregulating tumour or ischemia-related angiogenesis, which can be beneficial in some therapeutic settings. It has also been shown that is possible to modulate the stability and pharmacokinetics of anti-Dll4 to decrease its toxicity and still maintain its therapeutic and angiogenesis efficacy[5].

Our analysis also confirms the importance of Myc for the biology of ECs in the absence of Dll4. We previously reported that *Myc* loss rescues the ability of *Rbpj*^{KO} or *Dll4*^{KO} ECs to form arteries[42]. Here, we show that Myc loss abrogates the generation of activated, proliferative, and sprouting tip cells after Dll4 targeting, but surprisingly, this return to genetic and phenotypic quiescence is insufficient to prevent Dll4-targeting–induced vascular expansion, dysfunction, and consequent organ pathology. In contrast, anti-VEGF treatment did not completely abrogate the Dll4-targeting genetic program, but was able to prevent the associated vascular and organ pathology. This effect of anti-VEGF was however not reproduced by inhibition of the MAPK/ERK, Rac1, Pi3K/mTor or NO signaling. This suggests a broader role for anti-VEGF in preventing the pathologic vascular enlargement and remodelling after anti-Dll4, that could be also related to its effect on liver EC survival. Our data suggest that the action of VEGF on the vascular expansion and survival is independent of its direct effect on these signalling pathways[30,32-36], and also independent of cell proliferation and sprouting, as also previously proposed[26,44]. The sum of these findings also suggests that the recently developed bispecific antibody targeting both Dll4 and VEGF simultaneously (Navicixizumab, OncXerna) may be less toxic than the use of anti-Dll4 alone[45].

Altogether, the data obtained with several compound mutant and pharmacological approaches show that most of the transcriptional changes and angiogenic cell states elicited by targeting Dll4 correlate with, but do not cause, vascular pathophysiology (Fig. 9). Therefore, vascular neoplasms are not the cause of the previously reported anti-Dll4 toxicity[8]. Instead, we propose that is the unrelated venous sinusoids enlargement and architecture abnormalization that leads to vascular malfunction, blood accumulation, inflammation, hypoxia and altogether this results in subsequent organ pathology.

These data also raise questions about the general use of single-cell transcriptional or genetic states to describe and predict functional or dysfunctional vascular phenotypes and ultimately organ pathophysiology. A single-cell transcriptional state is only a small part of a cell´s phenotype and function.

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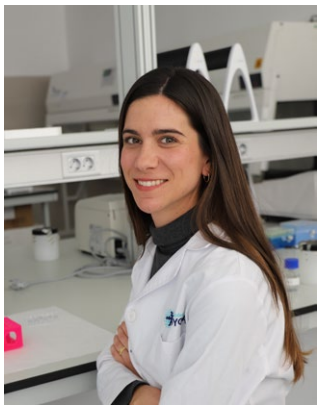

Curriculum Vitae

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Education

2021 **Ph.D.** in Molecular Biosciences (Cum Laude) Universidad Autónoma de Madrid, Madrid, Spain.
2015 **Master's** in Molecular Biomedicine, Universidad Autónoma de Madrid, Madrid, Spain.
2014 **Bacherlor's** Degree in Biotechnology, Universidad Pablo de Olavide, Sevilla, Spain.

Professional Experience

2022 **Assistant Professor and Biotechnology Degree Coordinator** at Universidad Loyola Andalucia, Sevilla, Spain.
2021 **Postdoctoral researcher**, Prof. Rui Benedito, Spanish National Centre for Cardiovascular Research (CNIC), Madrid, Spain.
Assistant Professor at Universidad Francisco de Vitoria, Madrid, Spain.
2018 **Ph.D. visiting student**, Prof. Hannah Carter, Department of Medicine, Division of medical genetics, University of California San Diego (UCSD), USA.
2015 **Ph.D. student**, Prof. Rui Benedito, Spanish National Centre for Cardiovascular Research (CNIC), Madrid, Spain.

Fellowships and Awards

2023 **Werner Risau Prize**, German Society of Cell Biology, DGZ, Germany.
2022 **Juan de la Cierva Postdoctoral fellowship**, Ministry of Science and Innovation, Spain.
2022 **Best poster prize**, Notch signaling in development, regeneration and disease, Gordon Research Conference, Maine, USA.
2019 **Residencia de Estudiantes fellowship**, Ministry of Science, Innovation and University, Madrid, Spain.
2018 **Boehringer Ingelheim Fonds Travel Grants**.
2015 **Ph.D. fellowship Obra Social la Caixa**.
2014 **Master's fellowship** from Spanish National Centre for Cardiovascular Research.
2014 **Introduction to Research Scholarship**, Universidad Pablo de Olavide, Sevilla, Spain.
2013 **DAAD Research Internship in Science and Engineering**, German Academic Exchange Service, Germany.
2010 **Becas Europa Fellowship**, Universidad Francisco de Vitoria, Madrid, Spain.
2010 **Educational Excellence Awards**, Jerez de la Frontera, Spain.

Research interest and activity

I am passionate about using new technology to better comprehend the role of blood vessels in organ homeostasis and disease. While working in Rui Benedito's laboratory at the Spanish National Centre for Cardiovascular Research, I was involved in the development of novel genetic tools to perform lineage tracing and to enhance the efficiency and reliability of conditional mutagenesis and gene function analysis in mouse models (Pontes-Quero, et al., Cell. 2017; Fernández-Chacón et al., Nat Commun. 2019). These tools have allowed me to investigate how different Notch ligands and receptors regulate the molecular and cellular mechanisms that maintain adult vascular homeostasis, as detailed in my recent publication in Nature Cardiovascular Research (Fernández-Chacón et al., 2023). Currently, I am exploring the endothelial contribution in the human aortic valve calcification.

Publications

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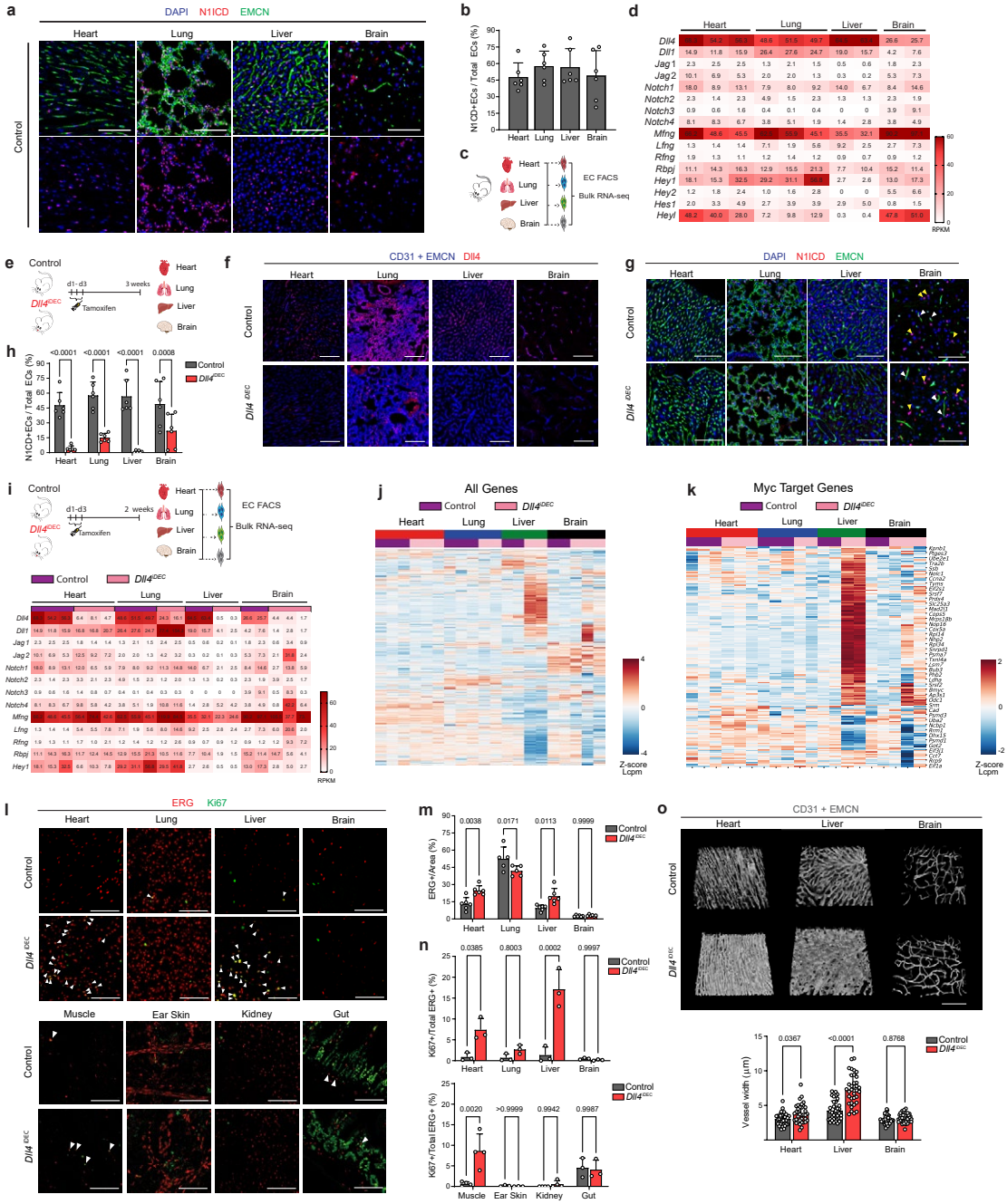

Figure 1: Dll4 deletion leads to EC activation and proliferation only in some vascular beds. a,b, *Notch1 signalling activity (cleaved Val1744 N1ICD) in quiescent endothelium (DAPI+Endomucin+, abbreviated as EMCN).* **c,** *Schematic representation to illustrate the bulk RNA-seq experiment performed with adult ECs isolated by FACS.* **d,** *Heatmap with RNA-seq RPKM (Reads per kilo base per million mapped reads).* **e,** *Experimental layout for the inducible deletion of Dll4 in Cdh5+ ECs (Dll4^{iDEC}) with Cdh5(PAC)-CreERT2.* **f,** *Expression of Dll4 protein in CD31+EMCN+ vessels.* **g,h,** *Dll4 deletion significantly reduces Notch signalling activity (cleaved Val1744 N1ICD) in all quiescent vascular beds. In brain micrographs, white arrowheads indicate ECs and yellow arrowheads non-ECs. Note that whereas N1ICD is maintained in non-ECs, most N1ICD signal disappears from the ECs in Dll4^{iDEC} brains.* **i,** *Schematic representation to illustrate the bulk RNA-seq experiment performed with adult ECs. Below a heatmap showing the relative expression of all Notch pathway components and canonical target genes in control and Dll4^{iDEC} mutant ECs.* **j,** *Unsupervised hierarchical clustering showing stronger gene expression changes in Dll4^{iDEC} liver ECs compared to the other organs. Z-score lcpm: Z-score of the logarithmic counts per million.* **k,** *Unsupervised hierarchical clustering showing strong upregulation of Myc target genes in Dll4^{iDEC} liver ECs compared to the other organs.* **l–n,** *Dll4 deletion results in increased EC proliferation (Ki67+ERG+ cells) in some organs but not others.* **o,** *3D reconstruction images from thick vibratome sections show vessel (CD31+EMCN+) enlargement in Dll4^{iDEC} heart and liver but not in brain. Data are presented as mean values +/- SD. For statistics see Source Data File 1. Scale bars, 100 µm.*

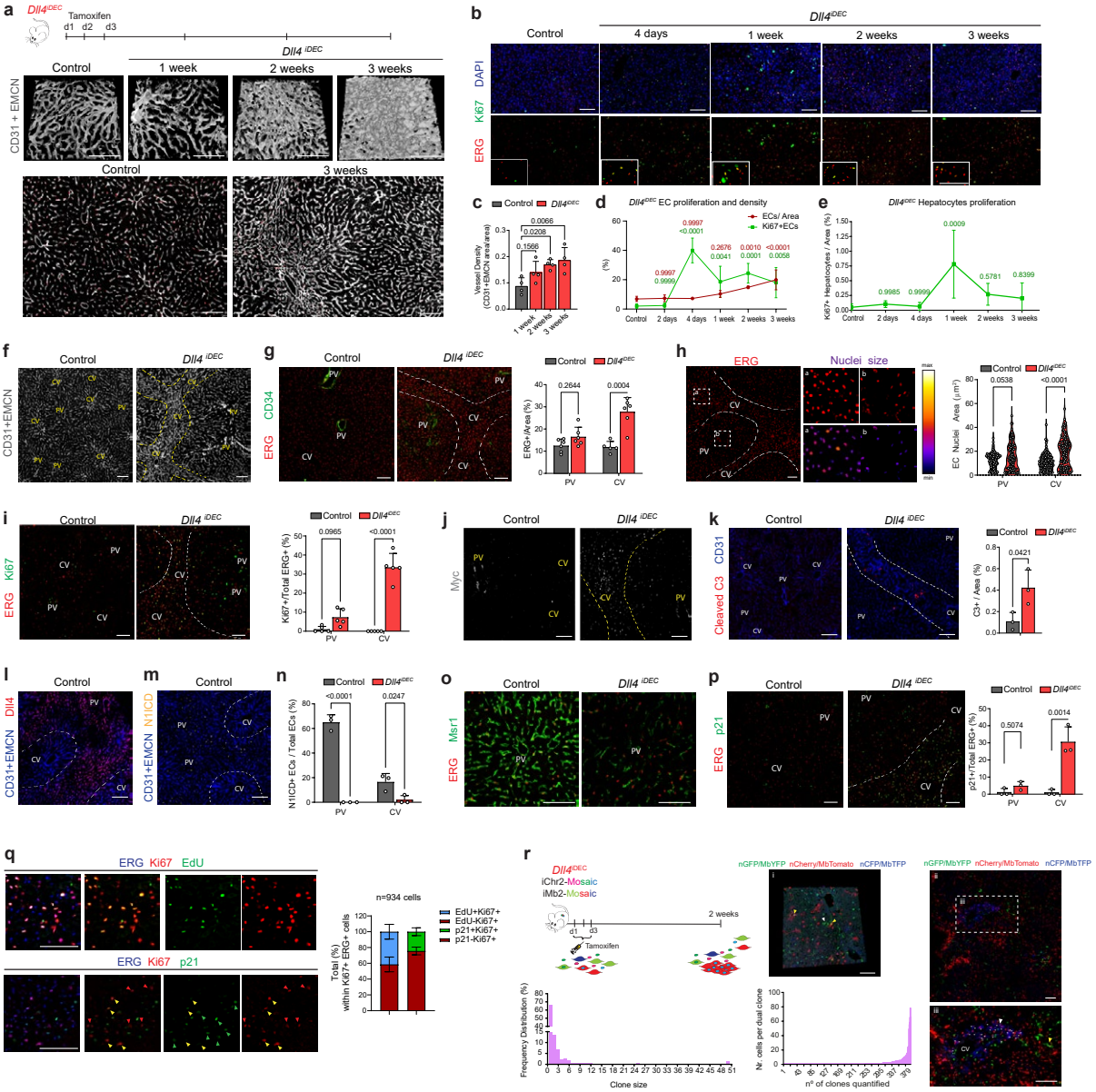

Figure 2: Targeting Dll4 induces heterozonal responses in liver vessels. a, *Experimental layout for the inducible deletion of Dll4 in Cdh5+ ECs (Dll4^{iDEC}) with Cdh5(PAC)-CreERT2. 3D projection of confocal images from thick vibratome sections.* **b–e,** *Analysis of EC (ERG+ cells) and hepatocyte (ERG-DAPI+) proliferation (Ki67+) and cell number.* **f,** *Representative confocal micrographs showing that the abnormal vascular pattern observed in Dll4^{iDEC} livers is located in the central veins (CV)-connecting sinusoids, but not in ECs surrounding portal veins (PV). Yellow dashed lines highlight the CV affected area.* **g,** *EC density in Dll4^{iDEC} liver is higher in sinusoids connecting the CVs rather than around PVs (CD34+). White dashed lines highlight the denser area.* **h,** *Dll4^{iDEC} liver section showing the increase in nuclei size mainly in CV-connecting sinusoids. White dashed lines highlight the area with higher EC density and with larger EC nuclei. Higher magnification pictures of insets (a) and (b) together with pseudocolouring of nuclear sizes (lower panels) show differences in nuclei size between CV and PV areas, respectively. Violin plots reflecting changes in cell nuclei sizes.* **i,** *Increased EC proliferation (Ki67+ERG+) in Dll4^{iDEC} liver particularly in the sinusoids connecting the CVs.* **j,** *Myc protein is upregulated mainly in ECs (ERG+ cells) around the CVs after Dll4 deletion.* **k,** *Increased apoptosis (cleaved Caspase 3) in CV areas upon Dll4 deletion.* **l–m,** *Dll4 and activated N1ICD (V1744) protein are mostly present in arterial PV areas, while being mostly undetectable in venous CV areas.* **n,** *Dll4 deletion leads to loss of N1ICD (Val1744) activation in liver ECs.* **o,** *Msr1 immunostaining showing loss of arterial identity in Dll4^{iDEC} vessels.* **p,** *p21 expression in Dll4^{iDEC} liver ECs (ERG+) is also higher in the sinusoids around the CVs.* **q,** *Dll4^{iDEC} Ki67+ liver ECs are actively dividing in S-phase (EdU+Ki67+ERG+, yellow arrowheads upper panel) and a small fraction of proliferating ECs (Ki67+ERG+) also expresses p21 protein (p21+Ki67+ERG, yellow arrowheads lower panel).* **r,** *Dual ifgMosaic single cell clonal tracking after Dll4 deletion. Images showing representative dual-labelled EC clones (yellow and white arrowheads in (i) and asterisks in (iii)). Data are presented as mean values +/- SD. For statistics see Source Data File 1. Scale bars, 100 µm.*

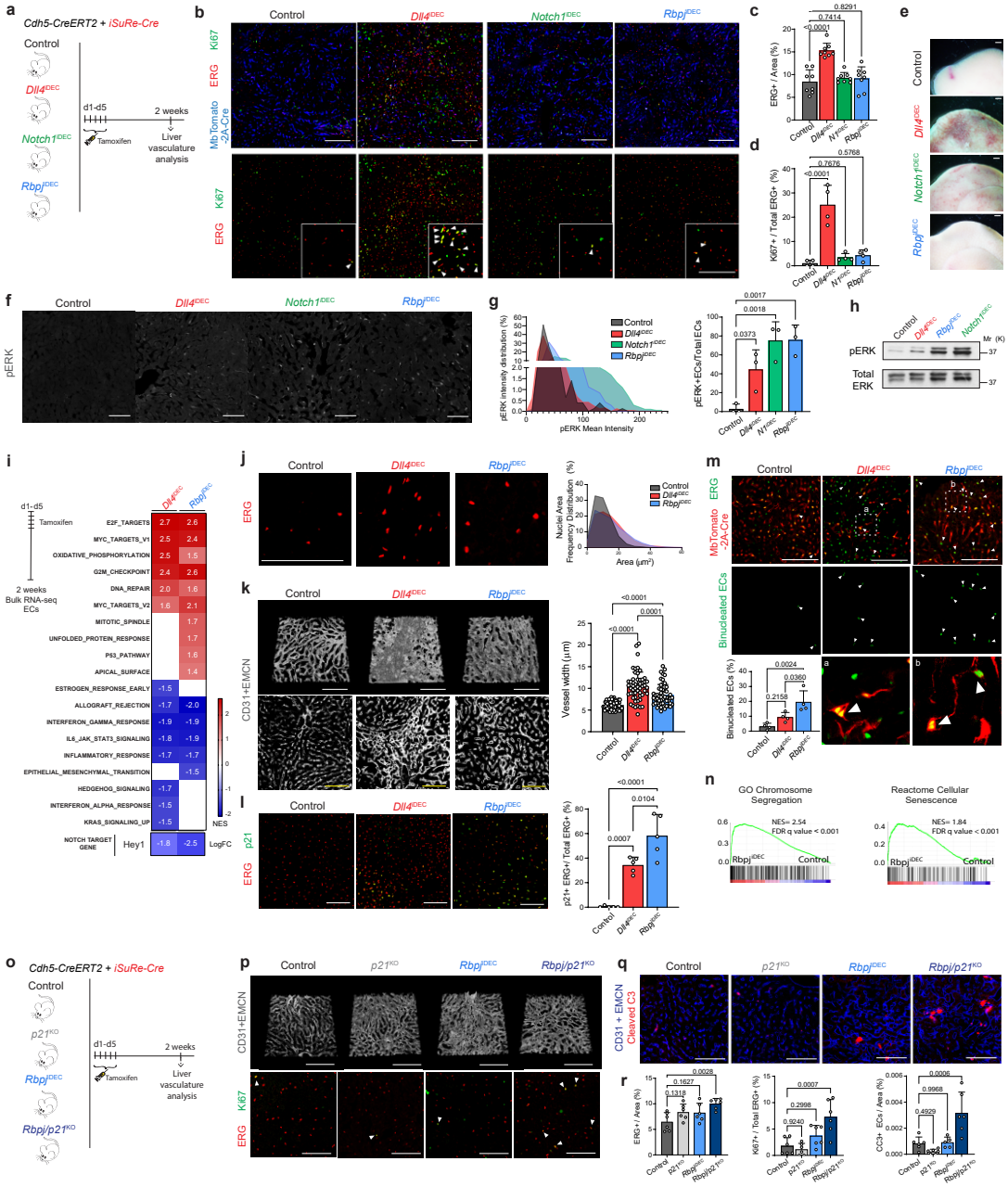

Figure 3: Deletion of Rbpj or Notch1 in liver quiescent blood vessels does not phenocopy Dll4 deletion. a, *Experimental layout for the inducible deletion of Rbpj (Rbpj^{iDEC}), Notch1 (Notch1^{iDEC}) and Dll4 (Dll4^{iDEC}) in Cdh5+ ECs. All mice contained the Cdh5(PAC)-CreERT2 and iSuRe-Cre (expressing MbTomato-2A-Cre) alleles to ensure genetic deletion of the floxed alleles.* **b–d,** *Increased EC density (ERG+ per field) and proliferation (Ki67+ERG+/ERG+) was observed only in Dll4^{iDEC} liver ECs.* **e,** *Gross liver pathology is observed exclusively in Dll4^{iDEC} livers.* **f–h,** *p-ERK immunostaining and whole liver western blot showing that the frequency of p-ERK expressing ECs and intensity levels increase in the mutants, particularly the Notch1 and Rbpj mutants.* **i,** *Heatmap with the normalized enrichment score (NES) from significant Hallmark analysis (FDR qval< 0.05) by GSEA from bulk RNA-seq data.* **j,** *Mutant liver ECs have a larger nuclei size compared with control liver ECs.* **k,** *Vascular (CD31+) dilation/expansion is more pronounced in Dll4^{iDEC} mutants. l, p21 expression in ECs (p21+ERG+) is more increased in Rbpj^{iDEC} mutants.* **m,** *Binucleated cells (white arrowheads) identified in Dll4^{iDEC} and Rbpj^{iDEC} mutants. High magnification of insets (a) and (b) are shown at the bottom.* **n,** *GSEA analysis show a positive and significant enrichment in Chromosome Segregation and Cellular Senescence-related genes in Rbpj^{iDEC} mutant liver ECs as shown by the Normalized Enrichment Score (NES).* **o,** *Experimental layout for the inducible deletion of Rbpj in a p21KO background.* **p,** *3D projection of thick vibratome sections showing the endothelial surface marker CD31 and EMCN, and proliferation (Ki67) analysis in ECs (ERG+).* **q,** *Analysis of the apoptosis marker cleaved caspase 3.* **r,** *The absence of p21 in a Rbpj^{iDEC} background results in a modest increase in EC density (ERG+), but both EC proliferation (Ki67+ERG+) and apoptosis (cleaved Caspase 3) are significantly increased. Data are presented as mean values +/- SD. For statistics see Source Data File 1. Scale bars, 100 µm, except e, 1mm.*

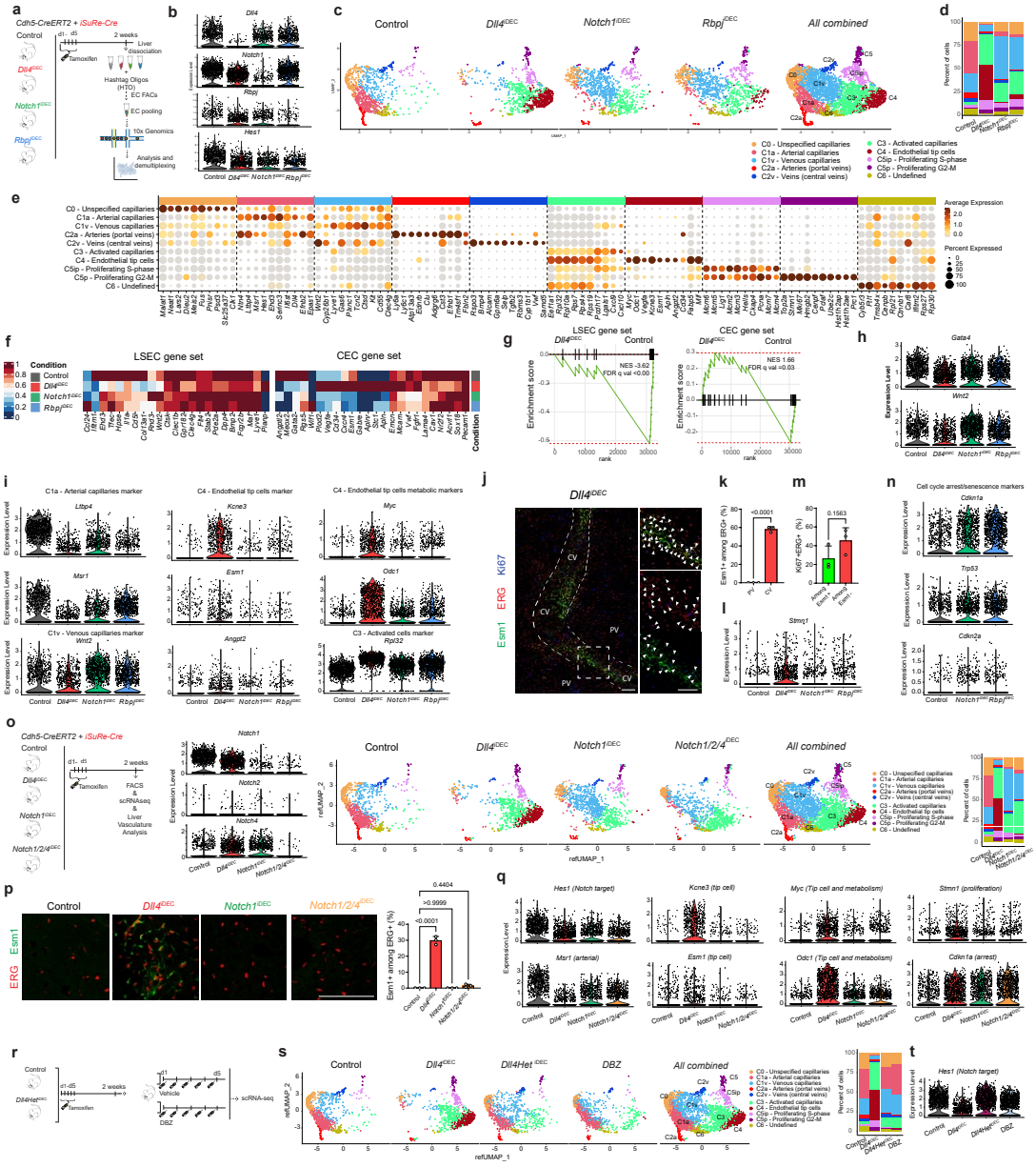

Figure 4: scRNA-seq analysis reveals significant differences between targeting Dll4 and Notch signalling. a, *Experimental layout for the inducible deletion of the indicated genes in Cdh5-CreERT2+ ECs and collection of the iSuRe-Cre+ (Tomato-2A-Cre+) cells to ensure genetic deletion.* **b,** *Violin plots showing Dll4, Notch1 and Rbpj mRNA expression in single cells and the subsequent downregulation of the Notch target gene Hes1 in all mutants.* **c,d,** *UMAPs and barplot showing the 10 identified clusters, and barplot showing the percentage of cells in each cluster in all samples.* **e,** *Dot plot showing the frequency (size) and intensity (color) of expression for the top cluster marker genes.* **f,** *Heatmap showing the indicated liver sinusoidal endothelial cell (LSEC) and continuous/capillary endothelial cell (CEC) gene expression signatures.* **g,** *Enrichment score analysis of LSECs and CEC signatures in Dll4^{iDEC} ECs.* **h,** *Violin plots showing decreased Gata4 and Wnt2 expression only in Dll4^{iDEC} mutants. i, Violin plots for some cluster marker genes.* **j,k,** *In Dll4^{iDEC} mutants, tip cells (Esm1+ERG+) are localized in the sinusoids around central veins (CVs), but not in PVs sinusoids.* **l,** *The global cell-cycle marker Stmn1 is highly upregulated exclusively in Dll4^{iDEC} liver ECs.* **m,** *Most of the Esm1+ECs are not Ki67+, but have Esm1- Ki67+ECs+ as neighbours in the CV sinusoids.* **n,** *Violin plots for the indicated genes and conditions. o, Experimental layout for the inducible deletion of the indicated genes, their violin plots, UMAPs and barplots.* **p,** *Expression of the tip cell marker Esm1 in ERG+ ECs located in CVs sinusoids.* **q,** *Violin plots showing that deletion of Notch1/2/4 results in less Notch signalling (Hes1), and less arterial marker expression (Msr1), but no induction of the tip cell programme (Kcne3/Esm1/Myc/Odc1) or the proliferation marker Stmn1. The cell-cycle arrest marker (Cdkn1a) is increased.* **r,** *Experimental layout for the inducible heterozygous deletion of Dll4 (Dll4Het^{iDEC}) for 2 weeks or DBZ treatment for 4 days in Cdh5+ ECs used for scRNA-seq.* **s,** *UMAPs and barplots obtained.* **t,** *Violin plots showing expression of the canonical Notch signalling target Hes1. Data are presented as mean values +/- SD. For statistics see Source Data File 1. Scale bars, 100 µm.*

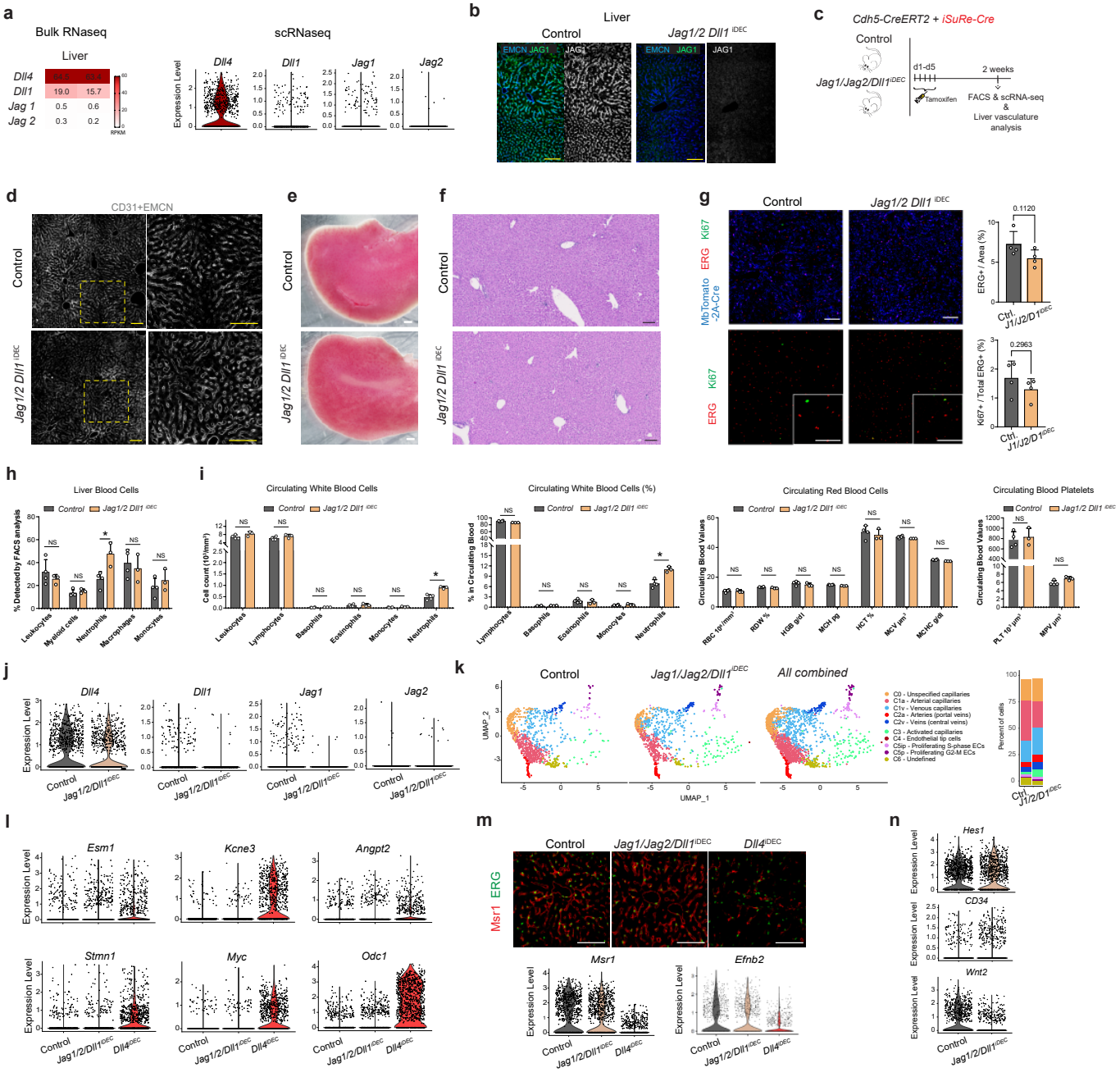

Figure 5: Deletion of Jag1/Jag2/Dll1 leads to the non-pathologic up-regulation of Notch signalling. a, *Heatmap of bulk RNA-seq reads and violin plot of single cell data showing expression of all Notch ligands in liver ECs.* **b,** *Despite its low mRNA expression, Jag1 protein is clearly detected in the adult liver quiescent endothelium (EMCN+) and absent in Jag1/Jag2/Dll1^{iDEC} mutants.* **c,** *Experimental layout for the inducible deletion of Jag1, Jag2 and Dll1 in Cdh5+ ECs. d, CD31 and EMCN+ immunostaining shows no vascular architecture changes.* **e,f,** *Macroscopic pictures and H&E staining show absence of liver pathology.* **g,** *Deletion of the three ligands does not lead to an increase in endothelial proliferation (Ki67+/ERG+ECs) or increase in EC number (ERG+ cells per field).* **h,** *Analysis by FACS of the percentage of different blood cells in livers.* **i,** *Hematological analysis of circulating (systemic) blood cells.* **j,** *Violin plots showing expression of the 4 ligands in scRNA-seq data.* **k,** *UMAPs and barplot showing the 10 identified clusters and the percentage of cells in each cluster in the two samples.* **l,** *Jag1/Jag2/Dll1 mutant ECs do not upregulate the tip-cell (Esm1/Kcne3/Angpt2), nor metabolic (Myc/Odc1), nor proliferation (Stmn1) transcriptional programme observed in Dll4 mutants.* **m,** *Immunostaining and scRNA-seq data showing that Jag1/Jag2/Dll1 mutant ECs do not downregulate the expression of the arterial markers Msr1 and Efnb2.* **n,** *Violin plot showing an increase in the Notch target gene Hes1 and the arterial gene CD34, together with a decrease in the expression of the venous Wnt2 gene in Jag1/Jag2/Dll1 mutant ECs. Data are presented as mean values +/- SD. For statistics see Source Data File 1. Scale bars, 100 µm, except e, 1mm.*

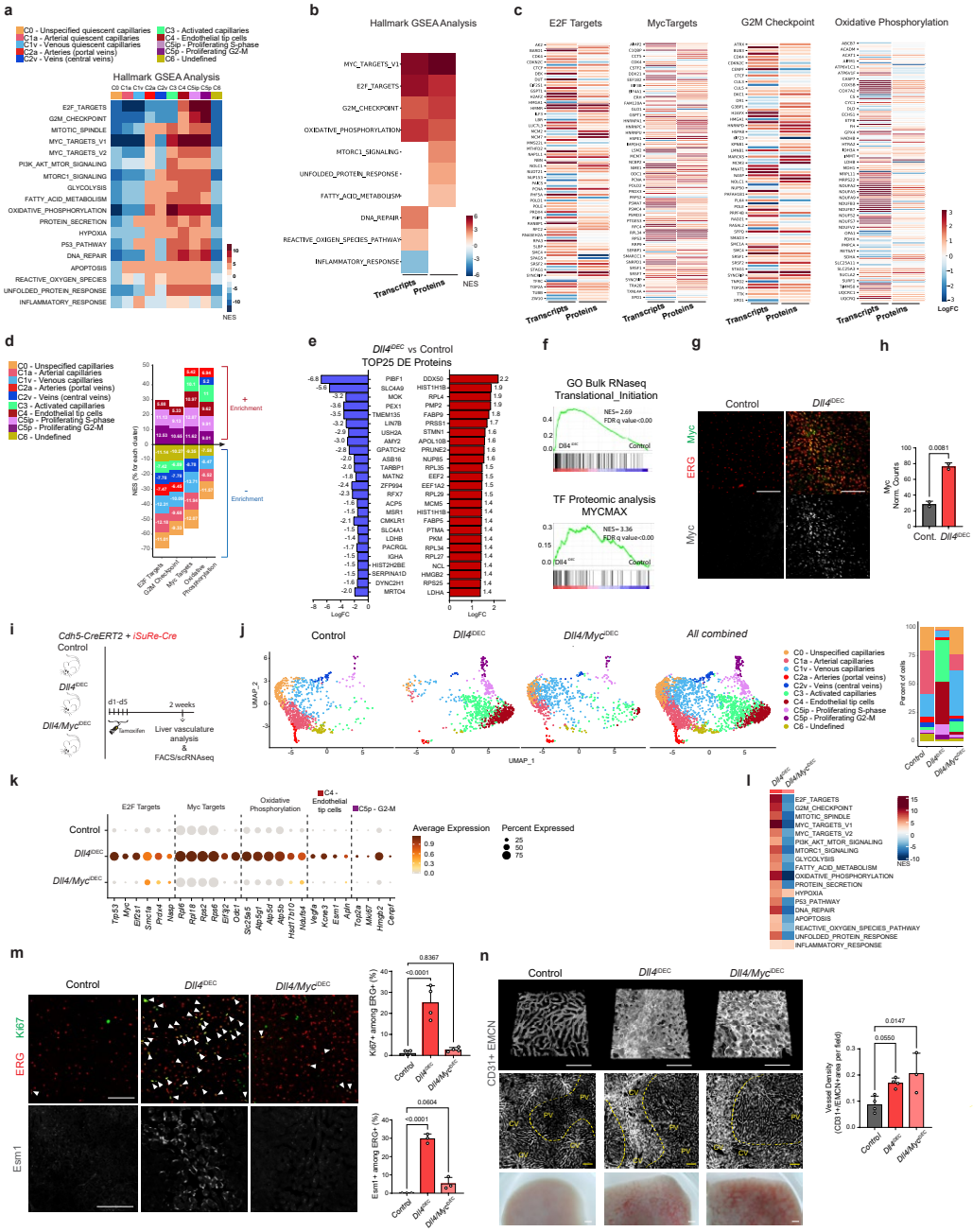

Figure 6: Myc loss prevents the Dll4KO endothelial activation and single cell states but not vascular pathology. a, *GSEA Hallmark analysis for each single cell cluster.* **b,** *GSEA Hallmark analysis performed with the Dll4^{iDEC} bulk proteome and transcriptome.* **c,** *Heatmaps showing log-FC of genes and proteins belonging to different sets.* **d,** *Barplot showing the Normalized Enrichment Score (NES) in each single cell cluster for the indicated gene sets.* **e,** *Barplot with the top differentially expressed (DE) proteins in Dll4^{iDEC} livers.* **f,** *Enrichment analysis show a significant positive enrichment in translational initiation-related genes and proteins encoded by genes that are regulated by the MycMax transcription factors.* **g,** *Micrographs showing immunostainings for the Myc protein, which is upregulated in liver ECs (ERG+ cells) after Dll4 deletion.* **h,** *Myc mRNA expression (normalized counts from bulk RNA-seq).* **i,** *Experimental layout for the inducible deletion of Dll4 and Myc in Cdh5+ and iSuRe-Cre+ ECs and scRNA-seq analysis.* **j,** *UMAPs and barplot showing the 10 identified clusters and the percentage of cells for each cluster in the different samples.* **k,** *Dot plot of the top upregulated genes in Dll4^{iDEC} liver ECs belonging to the indicated gene marker groups.* **l,** *GSEA Hallmark analysis showing the decreased expression of most gene sets in Dll4/Myc^{iDEC}.* **m,** *Double deletion of Dll4 and Myc in ECs results in a significant reversion of proliferation (Ki67+ERG+ cells) and Esm1+ expression (Esm1+ERG+) to control levels.* **n,** *3D Confocal micrographs from thick vibratome sections (top) or thin sections (bottom), and liver macroscopic pictures showing vessel enlargement and liver pathology in Dll4/Myc^{iDEC} mutants similarly to Dll4^{iDEC} mutants. Data are presented as mean values +/- SD. For statistics see Source Data File 1. Scale bar, 100 µm, except 6n lower panel, 1mm.*

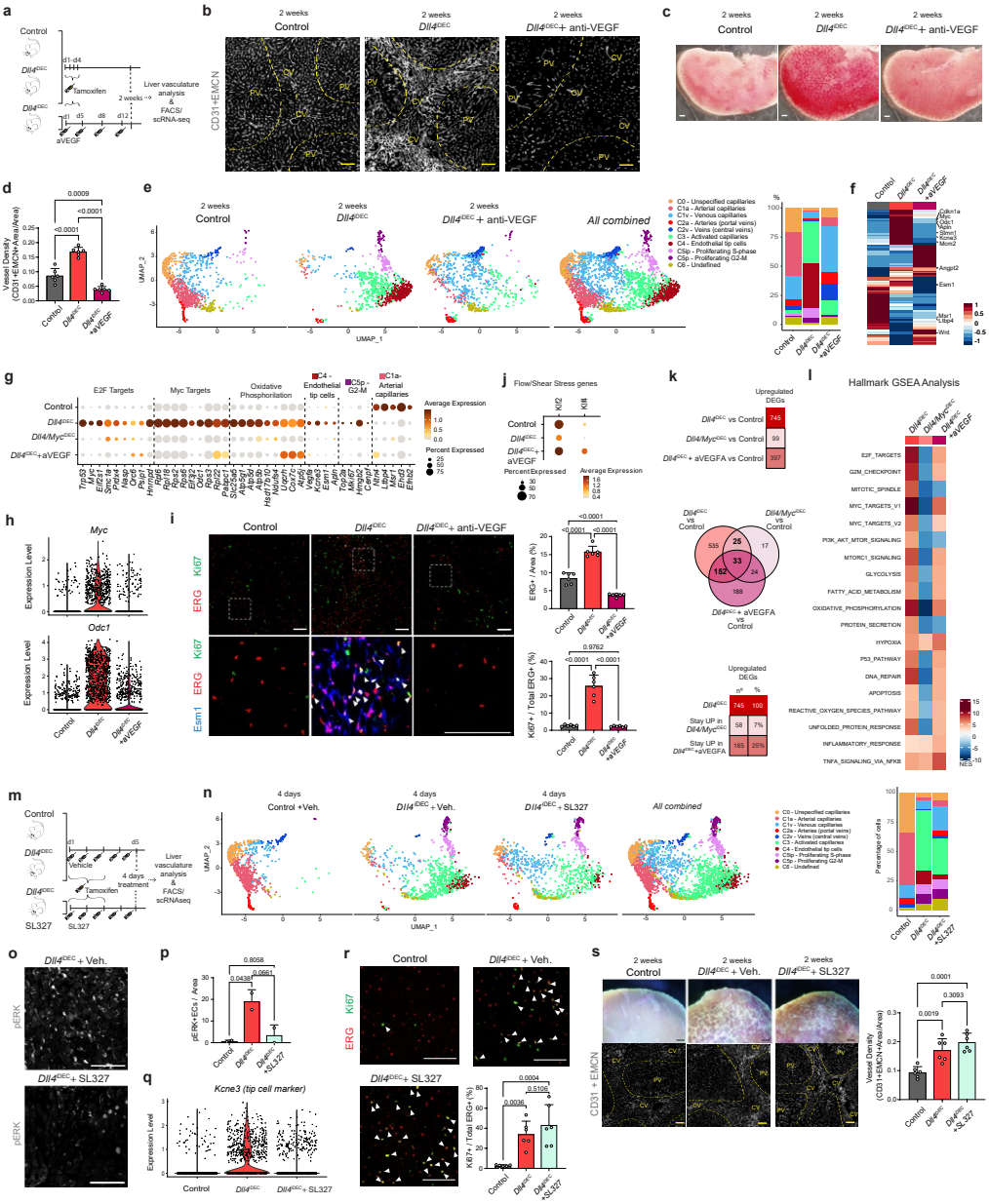

Figure 7: Vascular abnormalities and liver pathology are prevented by anti-VEGFA administration in Dll4^{iDEC} mutants by ERK-independent mechanisms. **a,** *Experimental layout for the inducible deletion of Dll4 in Cdh5+ ECs and anti-VEGFA administration.* **b,** *Confocal micrographs showing reduced CD31 or EMCN vascular immunostaining after anti-VEGFA.* **c,** *Stereomicroscope liver pictures.* **d,** *Vessel density is reduced in Dll4^{iDEC} mutants after anti-VEGFA.* **e,** *UMAPs and barplot showing the identified clusters and the percentage of cells for each cluster in indicated samples.* **f,** *Unsupervised hierarchical clustering showing gene expression changes.* **g,** *Dot plot of the top upregulated genes for each indicated gene set.* **h,** *Violin plots of scRNA-seq data showing that anti-VEGF prevents the strong upregulation of Myc and its target Odc1.* **i,** *The total number of ERG+ ECs, proliferation (Ki67+ERG+) and Esm1 expression (Esm1+ERG+) returns to control conditions after anti-VEGF administration.* **j,** *Dot plot showing expression of flow/shear stress genes.* **k,** *Number of upregulated genes for each contrast and Venn diagrams showing that when compared with Myc loss, anti-VEGF has a lower impact on the Dll4^{iDEC} upregulated genetic programme.* **l,** *GSEA Hallmark analysis confirms the more moderate effect of anti-VEGF on the Dll4^{iDEC} genetic programme when compared with Myc loss.* **m,** *Experimental layout for the inducible deletion of Dll4 and SL327 administration.* **n,** *UMAPs and barplot showing the identified clusters and the percentage of cells for each cluster in indicated samples.* **o,p,** *The administration of an ERK/MEK signalling inhibitor (SL327) results in reduced ERK phosphorylation.* **q,** *Violin plot showing that SL327 treatment inhibits the generation of tip cells (Kcne3+).* **r,** *The administration of SL327 does not change the frequency of proliferating Ki67+ ECs (Ki67+ERG+).* **s,** *Abnormal vasculature (CD31+EMCN+) associated to liver pathology still occurs after SL327. Data are presented as mean values +/- SD. For statistics see Source Data File 1. Scale bars, 100 µm, except in* **c** *and* **s** *upper panel, 1mm.*

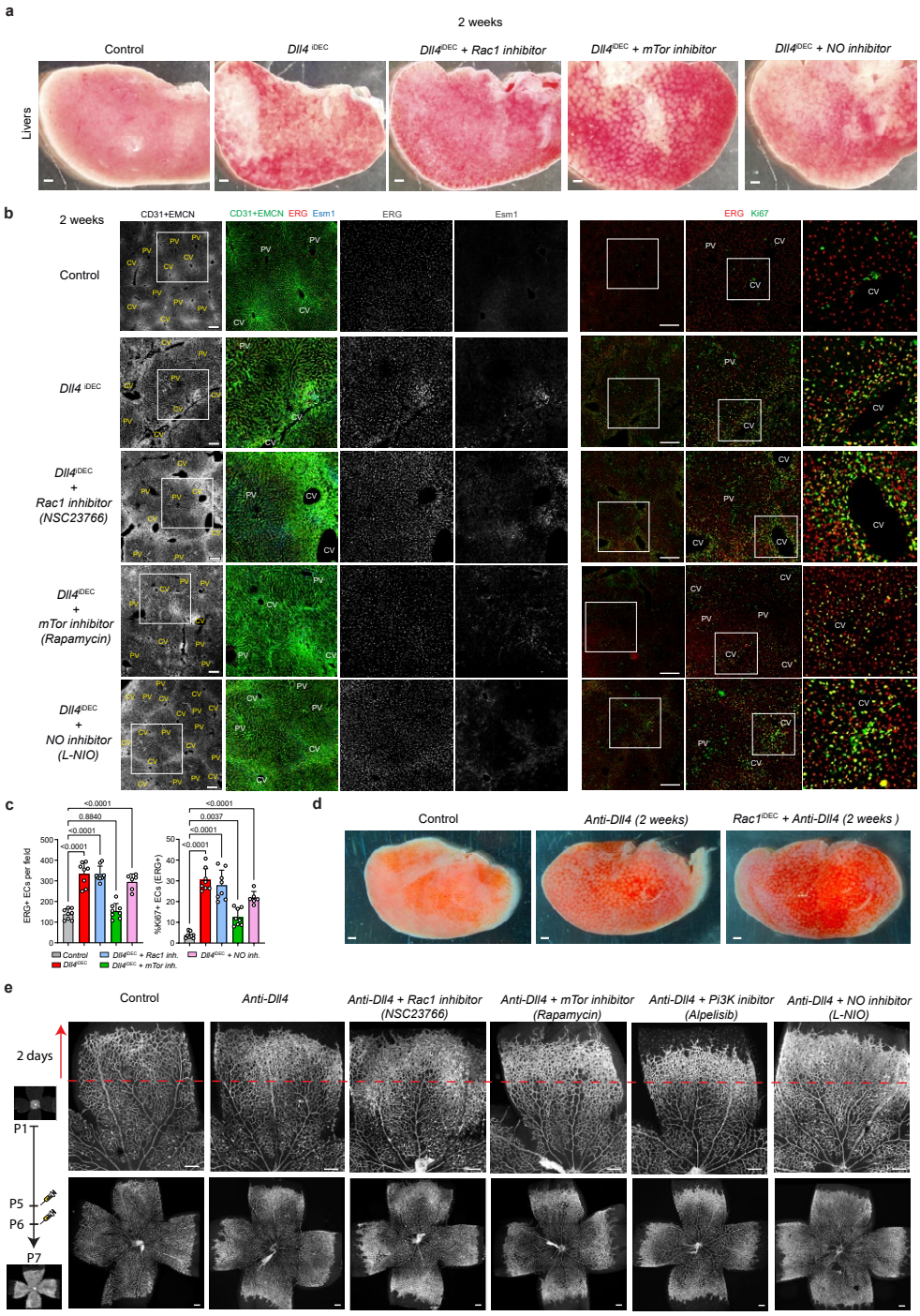

Figure 8: Inhibition of Rac1, mTor and NO signalling does not prevent the vascular pathophysiology induced by Dll4 targeting. a, *Stereomicroscope images showing adult liver vascular defects and blood accumulation after Dll4 deletion and treatment with different inhibitors for 2 weeks.* **b,** *Confocal micrographs showing that the expansion and abnormalization of the liver sinusoids (CD31+EMCN+), particularly around central veins (CVs), observed after Dll4 deletion, is not prevented by the administration of the indicated compounds. On the right, images show EC (ERG+ nuclei) proliferation (Ki67+).* **c,** *Charts showing quantification of EC density/numbers and proliferation. Note that mTor inhibitor treated liver ECs do not proliferate significantly (same ERG+ content), despite a fraction being Ki67+.* **d,** *Deletion of Rac1 with Cdh5-CreERT2 in adult livers endothelium (Cdh5+) does not prevent the vascular pathology induced by blocking Dll4 with REGN1035.* **e,** *The use of the indicated inhibitors in postnatal mouse retina angiogenesis assays for 48h, confirms that they do not prevent the increase in vascular expansion/density (IsolectinB4 labelling) induced by Anti-Dll4. Note that 2 days of angiogenesis growth correspond to the vasculature formed above the red dashed line. Data are presented as mean values +/- SD. For statistics see Source Data File 1. Scale bars, 200 µm, except in a and d, 1mm.*

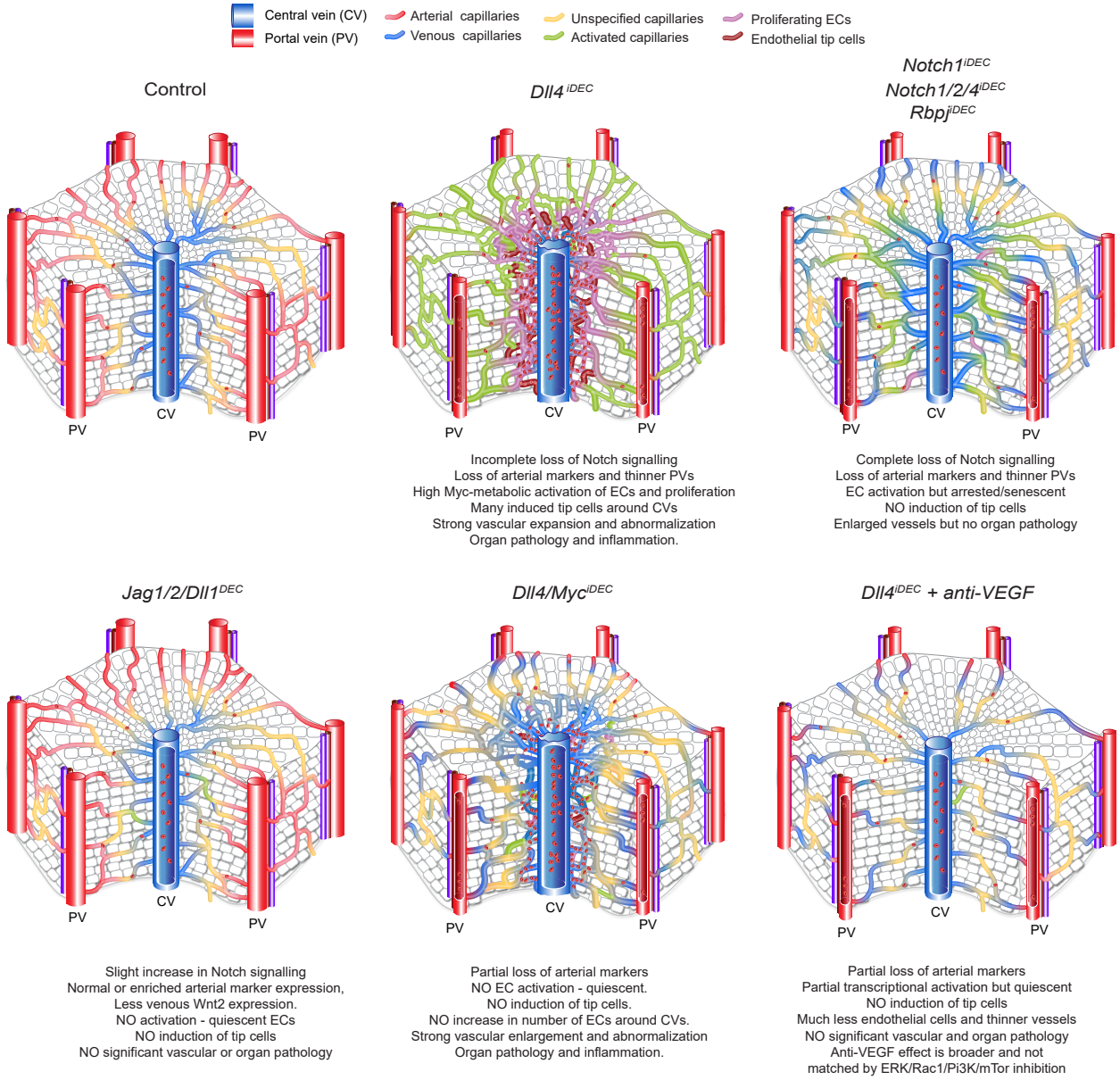

Figure 9: Incongruence between cell states, vascular morphology and pathophysiology. *Illustration showing the main endothelial cell states, phenotypes and characteristics of control and mutant livers. Targeting of the ligand Dll4 triggers incomplete loss of Notch signalling which results in the loss of arterial markers, reduced PV caliber and a strong Myc-driven metabolic activation associated with a well-defined cluster of proliferating and tip cells located in the sinusoids around the central veins. Dll4 mutant ECs have very high ribosome biogenesis, protein synthesis and oxidative phosphorylation favouring cell growth and metabolism. This genetic activation correlates with a significant increase in the number of proliferating and sprouting ECs, vascular expansion, abnormalization and subsequent organ pathology associated to the abnormal blood flow in CV sinusoids. The loss of Notch receptors or Rbpj leads to complete loss of Notch signalling and also the loss of the arterial transcriptional program and reduced PV caliber, but in this case most liver sinusoidal ECs undergo an hypermitogenic MAPK-driven cell-cycle arrest and show cellular senescence features. In contrast to Dll4 mutant ECs, Notch or Rbpj mutant ECs do not effectively proliferate or sprout and there is no significant vascular and organ pathology in mutant livers. Loss of all other Notch ligands leads to a mild increase in Notch signalling, without any associated vascular pathophysiology. Loss of Myc prevents most of the Dll4 mutant transcriptional program activation and cellular states. However, even in the absence of proliferating, sprouting and activated cells, Dll4/Myc^{iDEC} mutant livers still have abnormal and expanded CV sinusoids and significant organ pathology. Targeting VEGF only partially reduces the Dll4 mutant genetic programs, but it is enough to prevent most of the activated and tip cell states, being the cells in a quiescent state. It also induces the very significant loss of ECs, which overall prevents the vascular enlargement and associated organ pathology. The effect of anti-VEGF is broader and is not matched by the use of inhibitors targeting the ERK, Rac1 and Pi3k/mTor signalling. This work shows that single cell transcriptional states often do not correlate with observed vascular phenotypes and organ pathology.*

Meeting Report DGZ workgroup „Membrane Organization & Contact Sites":

26th Meeting on Signal Transduction 2023 by the Signal Transduction Society (STS)

The STS Meeting in 2023 was particularly memorable, as the Signal Transduction Society celebrated its 25th anniversary. The 26th Meeting on Signal Transduction took place at the Leonardo Hotel Weimar from November 6th to 8th, 2023 and featured eight workshops addressing various aspects in signal transduction, each introduced by invited renowned keynote speakers. The DGZ workgroup "Membrane Organization and Contact Sites", led by Julia Groß and Maria Bohnert, was involved in the organization of the workshop Infection and Inflammation with Carmen Buchrieser, Institute Pasteur, Paris, France, as a keynote speaker. Besides the DGZ, the signaling study groups of the German Societies for Immunology (DGfI), for Biochemistry and Molecular Biology (GBM), and for Pharmacology (DGP) were jointly involved in the organization of the scientific program. Furthermore, the SFB1423 Structural Dynamics of GPCR Activation and Signaling (Leipzig) and the Excellence Cluster for Integrative Biological Signalling Studies (CIBSS, Freiburg) significantly enriched the scientific program.

The first workshop on Monday midday commenced with Hinrich Abken from Regensburg who highlighted new developments in the T cell-based adoptive immunotherapy with chimeric antigen receptor (CAR) modified T cells and T cells redirected for unrestricted cytokine release and killing (TRUCKS). The second keynote speaker in this Immune Cell Signaling workshop was Dietmar Zehn (Munich) who thematized the diverse roles of exhausted T cells in acute and chronic infection. The following four selected short talks provided interesting insights into immune cell signaling and function. The afternoon was dedicated to the General Assembly of the STS and the first poster session, featuring presentations of highly interesting scientific work.
Following dinner, the STS celebrated its 25th Anniversary with a special workshop in which colleagues closely associated with the STS, either as founding member, such as Harald Krug (Munich), the STS Medalist 2019, namely Alfred Wittinghofer (Dortmund), or as STS Science Awardees, like Melanie Brinkmann (Braunschweig) and Geert Bultnyck (Leuven, BE). They all shared their science and their affiliation with the STS in highly entertaining talks. At the following STS reception on the gallery in front of the Belvedere exhibition hall, all participants enjoyed friendly conversations, enjoyable moments and, naturally, drinks, celebrating the STS.

The second day started with a workshop on Hot Topics in Signal Transduction. Here, Maria Christophorou (Cambridge, UK) provided fascinating insides into the process of chromatin citrullination and its possible function in somatic cell reprogramming. Katharina Höfer (Marburg) introduced the ADP-ribosyltransferase ModB and RNAylation as a new mechanism during bacteriophage infection. The Excellence Cluster for Integrative Biological Signalling Studies (CIBSS, Freiburg) was represented by Björn Lillemeier (Freiburg) and Peter Walentek (Freiburg). In the two keynote talks of this workshop entitled From Basic Research to Innovation the phosphorylation kinetics of SHP2 (B. Lillemeier) and Notch signaling events (P.Walentek) were discussed. New highlights in G Protein-mediated signaling were presented by Evgeni Ponimaskin, Hannover, who focused in his keynote lecture on the role of serotonin receptors in dementia treatment. Claudia Stäubert (Leipzig, SFB 1423) highlighted new aspects in succinate receptor 1 signaling, while Nicole Scholz (Leipzig, SFB 1423) concentrated her presentation on the adhesion GPCR (aGPCR) family and a novel transgenic sensor system designed to track GPCR dissociation. Each workshop concluded with three excellent short talks selected from the submitted abstracts.

The afternoon of the second day traditionally is reserved for a second poster session and the STS Honorary Medal award lecture, which can be considered as the highlight of the STS Meeting. In 2023, the STS awarded its Honorary Medal to Prof. Dr. Julian Downward (Francis Crick Institute London, UK). Julian Downward resolved the intricate mechanisms of the Ras pathway and the underlying molecular switches that drive normal as well as oncogenic signaling events in cancer development. Almut Schulze (Heidelberg), who had worked for a long time with J. Downward, delivered a wonderful, personal laudation, emphasizing Julian's achievements, followed by the ceremonial presentation of the medal by the STS council. Afterwards, J. Downward presented his 'Honorary Medal Lecture' in which he gave a highly informative overview of his lifetime Ras research, followed by a discussion of his influential work and its future implications.

On the last day of the STS Meeting, Dirk Brenner (Luxembourg) stepped in as a keynote speaker for the workshop Differentiation, Stress and Death thereby presenting his new study on the effect of ROS on Th17 differentiation. The workshop Infection and Inflammation was started by Carmen Buchrieser (Paris, France). In her talk, she introduced the concept of miRNA-like regulation of host cell signalling pathways by Legionella pneumophila and thus presented a new feature of L. pneumophila host-pathogen communication to the audience.

In addition to the opportunity to present the own research as a selected short talk, 38 posters added high quality research to the scientific program of the meeting. All posters were first presented as a one minute presentation in the legendary My poster in a nutshell session and afterwards shown and discussed during one of the two poster sessions The best posters were awarded with prizes of up to 250 Euro, awarded to Nadja Blasey (Bochum), Victoria Brickau (Witten/Herdecke), Celine Buchmann (Zweibrücken), and Adrian V. Fricke (Marburg). Moreover, eight young STS members were supported by STS travel grants, one of which was sponsored by Biomol.

The upcoming 27th STS Meeting is scheduled for November 4th to 6th, 2024, at the Leonardo Hotel in Weimar. For further information, please visit the STS web page on https://www.sigtrans.de, the STS Facebook account, or check on X formerly Twitter (@signalsociety).

We hope to see you in Weimar in November 2024 when we will again dive into the world of signal transduction.

Best wishes,
Klaudia Giehl (on behalf of the STS council)

Prof. Dr. Klaudia Giehl (STS President)
Justus-Liebig-Universität Gießen
Signaltransduktion zellulärer Motilität
Medizinisches Forschungszentrum Seltersberg

The STS Honorary Medal is given to Julian Downward (3. from left). Almut Schulze, Katharina Hieke-Kubatzky (STS council), and Klaudia Giehl (STS council) (from left to right).

Handover of the STS Travel Grants by the STS council member Detlef Neumann (1. from left) and Klaudia Giehl (2. from right).

Protokoll der Mitgliederversammlung 2023 der Deutschen Gesellschaft für Zellbiologie e.V.

Versammlungsleiter und Protokollführer: Prof. Dr. Roland Wedlich Söldner, Präsident

Geschäftsführerin: Prof. Dr. Gislene Pereira

Die Mitgliederversammlung fand am 12.12.2023, 12.30 Uhr – 14.00 Uhr, online über Zoom statt.

Alle Mitglieder wurden rechtzeitig durch Ankündigung in unserem Online-Mitgliederjournal „Cell News" sowie mehrmals über E-Mail eingeladen und über die Tagesordnung und Zugangsdaten zur Versammlung informiert.

Tagesordnung:

1. Bestätigung des Protokolls der letzten Sitzung
2. Jahresbericht des Präsidenten mit anschließender Diskussion
3. Geschäfts- und Kassenbericht über das abgelaufene Kalenderjahr
4. Bericht der Rechnungsprüfer:in
5. Entlastung des Vorstandes
6. Mitgliederversammlungen online über zoom
7. Sonstiges

1. Bestätigung des Protokolls der letzten Sitzung

Das Protokoll der letzten Mitgliederversammlung 2022 (online über Zoom am 15.12.2022) war in unserem Online-Mitgliederjournal „Cell News", Ausgabe 1/2023 veröffentlicht worden und wird bestätigt.

2. Jahresbericht des Präsidenten

Roland Wedlich-Söldner berichtet über die Aktivitäten der DGZ in 2022 und 2023. Zu den Mitgliederzahlen informiert er, dass wir im Jahr 2022 13 neue Mitglieder gewinnen konnten und 39 Austritte verzeichnen mussten. Zum Zeitpunkt der Mitgliederversammlung hatten wir für das laufende Jahr 2023 33 Neuzugänge und 53 Austritte. Die aktuelle Mitgliederzahl beträgt 703.

Die DGZ-Preise 2023 waren wieder in einem Webinar am 29.11.2023 verliehen worden. Preisträger*innen waren Dr. Alexandra Schnell (Nikon Young Scientist Award), Dr. Macarena Fernández Chacón (Werner Risau Preis), Dr. Florian Wilfling (Walther Flemming Award), Dr. Fabian Erdel (BINDER Innovationspreis) und Prof. Dr. Jochen Guck (Carl Zeiss Lecture). Die Preisträger:innen hielten Online-Vorträge, die von den Mitgliedern im gemeinsamen Zoom-Webinar verfolgt werden konnten. Die Mitglieder wurden informiert, dass der Werner Risau Preis 2023 anlässlich des 25. Todestages von Werner Risau zum letzten Mal verliehen wurde. Die restlichen Mittel werden in den DGZ-Etat überführt.

Als Publikationsorgan der DGZ wurden in 2023 zwei Ausgaben der „Cell News" veröffentlicht. Zudem wurden alle wichtigen Informationen über E-Mail und zusätzlich via X/Twitter verbreitet. Weiterhin berichtet Roland Wedlich-Söldner über Aktivitäten der DGZ auf politischer Ebene (Wissenschaftszeitvertragsgesetz, Tierversuche – EU-Initiative, Neubesetzung in der Zentralen Kommission für Biologische Sicherheit (ZKBS)) sowie über die internationalen DGZ-Tagung 2023 in Saarbrücken. Er stellt zudem den Stand der seit 2022 laufende Focus Workshop-Serie und der 2022 gestarteten neuen Webseite der DGZ dar. Die bislang nur schleppend erfolgte Eintragung von Mitgliederinformationen auf den persönlichen Seiten der DGZ und die Verwendung der interaktiven Karte wurden erneut thematisiert. Eine verstärkte Teilnahme der Mitglieder wird sich für das kommende Jahr erhofft.

3. Geschäfts- und Kassenbericht

Die Geschäftsführerin Gislene Pereira berichtet über die Finanzlage der DGZ im Geschäftsjahr 2022 und erläutert diese im Detail anhand der Einnahmen- und Ausgaben-Bilanzen und v.a. eingehend auf Einzelpositionen wie Einkünfte durch Mitgliedsbeiträge sowie Ausgaben für Büro- und Personalkosten, Konferenzunterstützung und Steuerberatung. Das Guthaben betrug zum 31.12.2022 EUR 68.613,24.

4. Bericht der beiden Kassenprüfer:innen

Die Einnahmen und Ausgaben im Geschäftsjahr 2022 waren durch die Kassenprüferin Prof. Dr. Maria Bohnert und den Rechnungsprüfer Prof. Dr. Ralph Gräf geprüft und für richtig befunden worden, es gab keine Beanstandungen. Die Prüfung der Unterlagen erfolgte digital, die Unterlagen wurden per E-Mail an die Kassenprüfer:innen zur Durchsicht und Überprüfung geschickt.

5. Entlastung des Vorstandes

Der Vorstand wird über online-Abstimmung (in Zoom) einstimmig – mit Enthaltungen der Vorstandsmitglieder – entlastet.

BILANZ 2022

EINNAHMEN	EUR	AUSGABEN	EUR
Mitgliedsbeiträge (abzgl. Retouren)	34.740,00	Bankkosten	811,53
Spenden, Preisgelder	15.500,00	Retoure Mitgliedsbeiträge	240,00
Zinsen	5,75	Spenden, Preisgelder	23.500,00
Cell News, Homepage (Werbeanzeigen, Firmen-Links)	5.176,50	Cell News	1.856,40
		Tagungen	57.670,11
Tagungen	57.670,11	Reisekosten	0,00
Überträge	4.000,00	Bürokosten/Gehalt Sekr.[1] Büromaterial, Homepage	45.847,28
Sonstige	33,32		
		Überträge	4.000,00
		Sonstige	5.327,68
Summe der Einnahmen:	117.145,68	Summe der Ausgaben:	139.253,00
Guthaben am 31.12.2021:	90.720,56	**Guthaben am 31.12.2022:**	**68.613,24**
Guthaben DGZ:	68.266,57	Guthaben DGZ:	50.341,92
Werner Risau Preis:	22.453,99	Werner Risau Preis:	18.271,32

Die Einnahmen und Ausgaben wurden von Kassenprüferin Julia Groß und Kassenprüfer Ralph Gräf geprüft und für richtig befunden.

6. Mitgliederversammlungen online über zoom

Die Mitglieder wurden befragt, ob zukünftige Mitgliederversammlungen auch weiterhin Online durchgeführt werden sollen. Die anwesenden Mitglieder stimmten einstimmig zu.

8. Sonstiges

Es erfolgte noch eine Diskussion über die Mitgliedschaftsgebühren (keine Zunahme der Austritte aufgrund von Erhöhung), die Zusammensetzung des neuen DFG-Panels für Zellbiologie (gemäß vorläufigem Endergebnis November 2023: 6 Mitglieder aus der DGZ-Liste, 2 Mitglieder aus Vorstand, 3 Workgroup-Sprecher), die Kooperation mit dem European Journal of Cell Biology (EJCB) (DEAL-Vertrag mit Elsevier als Voraussetzung, günstigere Publikation für Mitglieder) sowie das 2025 anstehende 50-jährige Jubiläum der DGZ (Jahrestagung in Heidelberg, Sonderausgabe mit EJCB, Focus workshop series über Zellbiologie in Deutschland).

Prof. Dr. Roland Wedlich-Söldner
Präsident
Versammlungsleiter und Protokollführer

Prof. Dr. Gislene Pereira
Geschäftsführerin

Pressemitteilung
Gesellschaft für Biochemie und Molekularbiologie e.V. (GBM)
22. April 2024

Stellungnahme der unterzeichnenden Fachgesellschaften im Bereich der Natur- und Lebenswissenschaften zur geplanten Novelle des Wissenschaftszeitvertragsgesetzes

Eine Anpassung der Rahmenbedingungen zur Verbesserung der Karrierewege junger Wissenschaftler:innen innerhalb und außerhalb akademischer Einrichtungen, ist aus Sicht der Natur- und Lebenswissenschaften sehr zu begrüßen. Dazu zählt neben einer notwendigen Anpassung der Finanzierungs- und Personalstrukturen der wissenschaftlichen Einrichtungen auch die intensiv diskutierte Novelle des Wissenschaftszeitvertragsgesetzes (WissZeitVG). Das Bundeskabinett hat dazu in Anlehnung an den Referentenentwurf des BMBF vom 6. Juni 2023 kürzlich einen Gesetzesentwurf beschlossen, der jetzt in die parlamentarische Beratung geht. Der Entwurf sieht eine maximal 6-jährige Promotionsphase sowie eine darauffolgende maximal 4-jährige Postdoc-Phase vor. Darüber hinaus sollen zwei weitere Jahre der befristeten Anstellung mit verbindlichen Zielvereinbarungen für den Übergang auf eine Dauerstelle ermöglicht werden.

Die unterzeichnenden natur- und lebenswissenschaftlichen Fachgesellschaften begrüßen prinzipiell die Bestrebung eine bessere Planbarkeit und Verlässlichkeit wissenschaftlicher Karrieren zu ermöglichen, weisen jedoch auf folgende kritische Punkte hin:

1. Im Bereich der Natur- und Lebenswissenschaften sowie in der Biomedizin würde die geplante zeitliche Ausgestaltung der beiden Qualifizierungsphasen (max. 6 Jahre bis zur Promotion, [4+2] Jahre als Postdoc) grundsätzlich in einigen– aber nicht in allen -Fachgebieten nur sehr knapp - ausreichen, um die für eine Professur erforderlichen Projekterfolge in Form von umfangreichen Datensätzen, eigenverantwortlichen Publikationen, Lehrerfahrungen und eigenständig eingeworbenen Forschungsmitteln zu erarbeiten oder ein Experten-Profil für Karriereziele neben der Professur zu entwickeln und zu verfestigen. Da die angestrebte Novellierung des WissZeitVG keine zusätzlichen unbefristeten Stellen schaffen wird, ist in den meisten Fällen eine Begrenzung der Postdoc Qualifizierungsphase auf 4 Jahre zu erwarten. Gerade in experimentellen Fächern ist diese Zeit jedoch oftmals unzureichend und wird zu einer Abwanderung von Wissenschaftler:innen ins Ausland und zu einem dramatischen Verlust der Qualität der Forschung in den Natur- und Lebenswissenschaften und in der Biomedizin in Deutschland führen.

2. Eine weitere Öffnung der Tarifklausel, d.h. die Überlassung wichtiger Elemente der Befristungsregelungen zur Regelung durch das Tarifrecht, halten die unterzeichnenden Fachgesellschaften weder für sachgerecht noch für geboten. Befristungsregelungen in Flächentarifverträgen würden weder den unterschiedlichen wissenschaftlichen Einrichtungen noch den Karrierewegen junger Wissenschaftler:innen gerecht und würden zu einer Zersplitterung der rechtlichen und tariflichen Rahmenbedingungen führen. Eine nach Bundesländern differenzierte Ausgestaltung der Rahmenbedingungen für wissenschaftliche Karriereoptionen wäre kontraproduktiv und würde für ein Ungleichgewicht in der Forschung in den Bundesländern führen. Würde die gesamte Qualifizierungsbefristung in die Dispositionsfreiheit der Tarifpartner gelegt, wären die Folgen für den Wissenschaftsstandort Deutschland und für die Karrierewege junger Wissenschaftler:innen in den Naturwissenschaften und der Biomedizin schwerwiegend.

3. Für die wissenschaftliche Arbeit in den Natur- und Lebenswissenschaften ist die Möglichkeit, weiterhin im Kontext von Drittmittelprojekten befristen zu dürfen - ohne zeitliche Obergrenzen oder eine Limitierung der Anzahl drittmittelbasierter Verträge - essentiell.

4. Die geplante Mindestvertragslaufzeit von 3 Jahren für den Erstvertrag von Doktoranden:innen ist generell sehr zu begrüßen. Wir weisen darauf hin, dass eine flächendeckende Umsetzung einer solchen Mindestvertragslaufzeit die wissenschaftlichen Einrichtungen vor zusätzliche Herausforderungen stellt. So müssten finanzielle Resourcen aus den Haushalten der Einrichtungen bereitgestellt werden, um zu gewährleisten, dass verfügbare Drittmittel mit Restlaufzeiten von weniger als 3 Jahren sachgerecht eingesetzt werden können.

5. Die vorgesehene Mindestvertragslaufzeit von einem Jahr für studienbegleitende Beschäftigungen ist aufgrund der inhärenten Organisation universitärer Praktika und Kurse in den Naturwissenschaften und in der Biomedizin in vielen Fällen nicht durchführbar, so dass eine Beeinträchtigung der universitären Lehre zu befürchten ist. Wir empfehlen daher, begründete Ausnahmen von der Mindestvertragslaufzeit zuzulassen.

Um die mit der Novellierung des WissZeitVG angestrebte verbesserte Planbarkeit wissenschaftlicher Karrierewege zu erreichen, ist aus Sicht der unterzeichnenden Fachgesellschaften im Bereich der Natur- und Lebenswissenschaften die Schaffung zusätzlicher unbefristeter Stellen erforderlich. Dies ist nur durch eine deutliche Aufstockung der dauerhaften Mittel zur Grundfinanzierung der Hochschulen und außeruniversitären Einrichtungen zu erreichen. Die unterzeichnenden Fachgesellschaften regen daher an eine diesbezügliche Diskussion mit den Vertretern des Bundes und der Länder alsbald zu führen.

Gesellschaft für Biochemie und Molekularbiologie (GBM)
Anatomische Gesellschaft (AG)
Deutsche Gesellschaft für Experimentelle und Klinische Pharmakologie und Toxikologie (DGPT)
Deutsche Gesellschaft für Extrazelluläre Vesikel (GSEV)
Deutsche Gesellschaft für Immunologie (DGfI)
Deutsche Gesellschaft für Medizinische Psychologie (DGMP)
Deutsche Gesellschaft für Zellbiologie (DGZ)
Deutsche Pharmazeutische Gesellschaft (DPhG)
Deutsche Physiologische Gesellschaft (DPG)
Gesellschaft Deutscher Chemiker (GDCh)
Gesellschaft für Entwicklungsbiologie (GfE)
Gesellschaft für Genetik (GfG)
Gesellschaft für Mikroskopie und Bildanalyse (GerBI-GMB)
Gesellschaft für Virologie (GfV)
Neurowissenschaftliche Gesellschaft (NWG)
Signal Transduction Society (STS)
Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM)

Die unterzeichnenden Fachgesellschaften vertreten mehr als 55.000 Mitglieder in den Natur- und Lebenswissenschaften sowie in der Biomedizin.

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Information zur GBM
Die Gesellschaft für Biochemie und Molekularbiologie (GBM) ist die größte biowissenschaftliche Fachgesellschaft Deutschlands. Sie bietet ihren rund 5.000 Mitgliedern aus Hochschulen, Forschungsinstituten und Industrie nach außen eine starke Interessenvertretung gegenüber Politik, Wirtschaft und Öffentlichkeit, sowie Gelegenheiten zu internationaler Kooperation. Nach innen eröffnet die GBM zahlreiche Möglichkeiten der Vernetzung und der Förderung der wissenschaftlichen Karriere.

DGZ Focus Workshops 2024

Zoom, last Tuesday of a month, 12:00 CEST

Zoom-ID: 961 7810 6979

Passcode: DGZ_FW

(for questions contact Sandra Iden, sandra.iden@uks.eu)

April 30, 2024	Cell Adhesion and Extracellular Matrix Carsten Grashoff, Sara Wickström
May 28, 2024	Cellular and Organismal Proteostasis Thorsten Hoppe, Jörg Höhfeld
June 25, 2024	Physics of the Cell Alf Honigmann, Leonhard Möckl

New schedule for third round of Focus Workshops is in preparation.

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