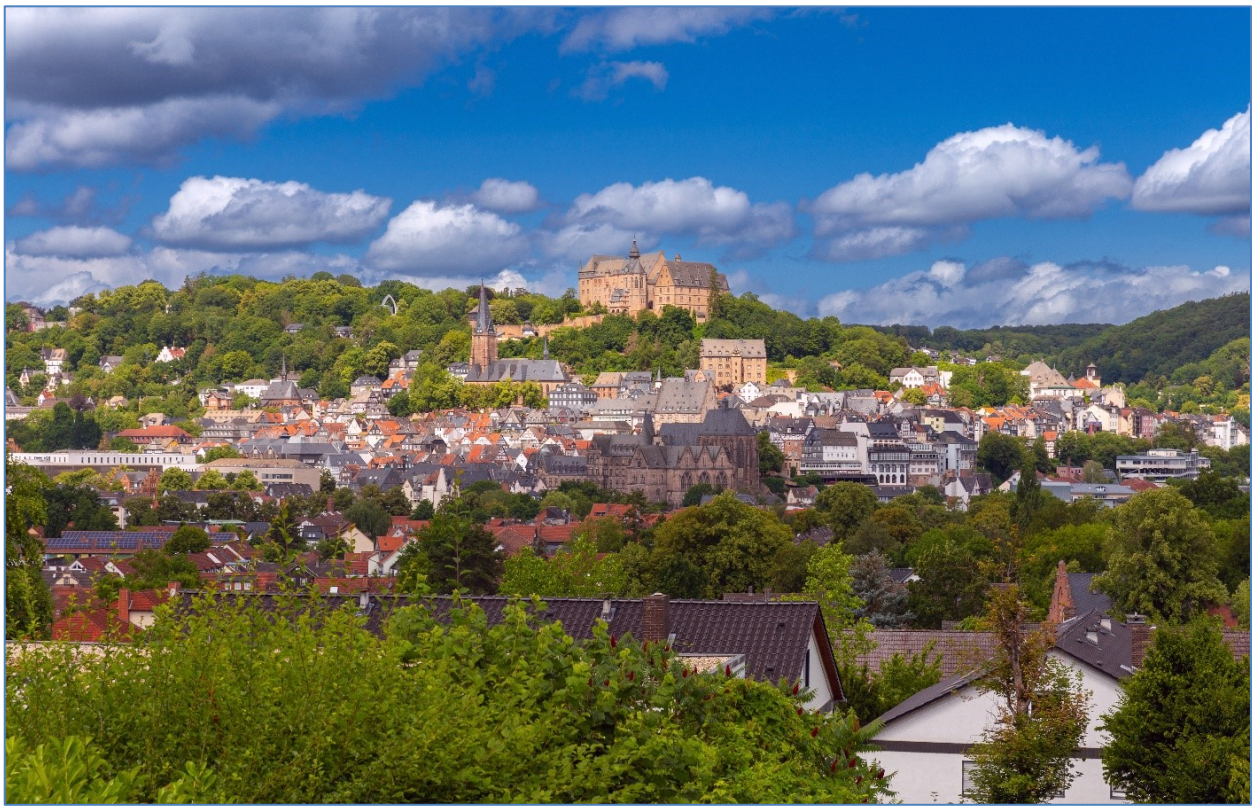
# 6th European NF-kappaB Workshop

Monday 02 September 2024 - Wednesday 04 September 2024

Vila Vita Rosenpark, Marburg

6th European NF-kappaB Workshop

# Book of Abstracts



## Venue

Vila Vita Rosenpark
Hotel & Residenz Rosenpark GmbH
Anneliese Pohl Allee 7-17
35037 Marburg, Germany
+49 (0) 6421 6005-0
info@vilavita.com
**All sessions will take place in the „Vivaldi“ room (basement floor of hotel)**

## Organisers

Michael Kracht
Rudolf Buchheim Institute of Pharmacology
Biomedical Research Center Seltersberg
Schubertstrasse 81
35392 Giessen
+49 (0)641 99-47600
michael.kracht@pharma.med.uni-giessen.de

Lienhard Schmitz
Institute for Biochemistry
Friedrichstrasse 24
35392 Giessen
+49 (0)641-99-47570
lienhard.schmitz@biochemie.med.uni-giessen.de

## Contact / Support

Orgalution GmbH
+49 (0)251 92870230
nfkb@orgalution.de

Heike Schubert/Office Rudolf Buchheim Institute
+49 (0)641-99-47601

Ines Höfliger/Office Institute for Biochemistry
+49 (0)641-99-47571

# Contents

# Book of Abstracts

# Talk 1 - Manolis Pasparakis

## IKK signalling in the regulation of RIPK1-mediated cell death and inflammation

**Author:** Manolis Pasparakis, Institute for Genetics, University of Cologne

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## Talk 2 - Jill Steels

# ABIN1 recruitment to the CBM signalosome facilitates A20-mediated regulation of T cell activation and is essential to maintain T cell homeostasis

**Authors:** Annelies Demeyer; Jill Steels[1]; Ioannis Skordos[1]; Jens Staal[1]; Kreike Marja[1]; Marie Lork; Rudi Beyaert[1]; Yasmine Driege

[1] *Unit of Molecular Signal Transduction in Inflammation, Department of Biomedical Molecular Biology, VIB-UGent Center for Inflammation Research, Ghent University, Ghent, Belgium*

**Corresponding Author:** jills@irc.vib-ugent.be

The CARD11-BCL10-MALT1 (CBM) complex is essential for T cell-mediated immune responses, yet the molecular mechanisms regulating CBM signaling are still largely unclear. We show that ABIN1 is recruited to MALT1 in activated T cells and that inducible MALT1/ABIN1 binding is dependent on CBM complex formation and HOIP. ABIN1 is known to negatively regulate NF-kB signaling by binding the NF-kB inhibitory protein A20. However, absence of ABIN1 or A20 had no major effect on NF-kB signaling in activated Jurkat cells. Cells lacking both ABIN1 and A20 showed hyperactivation of NF-kB, indicating ABIN1 to be partially redundant with A20. We show that T cell specific ABIN1 deficient mice have no spontaneous (immuno)phenotype compared to wild type mice, while T cell specific A20 deficient mice have reduced peripheral CD8+ T cells and NKT cells, increased thymic regulatory T cells, and elevated levels of activated effector T (Tef◎) cells. T cell specific A20/ABIN1 double deficient mice are characterized by a strong decrease in peripheral CD4+ and CD8+ T cells, and a further increase in activated Teff cells compared to absence of A20 only. Increased activation of Teff cells in A20/ABIN1 double knockout mice is prevented by additional deletion of MALT1 in MALT1/A20/ABIN1 triple knockout mice. We show that ABIN1 recruitment to the CBM complex facilitates A20's negative regulation of MALT1-dependent TCR-induced T cell activation, which is essential to maintain T cell homeostasis in mice.

# Talk 3 - Franziska Ober

## Deciphering the molecular mechanisms how CYLD counteracts constitutive and inducible NF-kB activation in T cells

**Author:** Franziska Ober[1] **Co-authors:** Bahareh Nemati Moud[1]; Kristina Herdt[1]; Antonia Keßler; Thomas Seeholzer; Daniel Krappmann[1]

[1] *Signaling and Immunity - Helmholtz Munich*

**Corresponding Author:** franziska.ober@helmholtz-munich.de

CYLD is a K63 and M1-specific deubiquitinase highly expressed in T cells. In the TNFR signaling pathway, CYLD interacts with LUBAC via SPATA2 to regulate ubiquitination. So far, the precise role of CYLD in T cell receptor (TCR) induced NF-kB activation and the impact of its cleavage by MALT1, a protease activated upon TCR stimulation, have not been defined. While ablation of CYLD in murine T cells has been suggested to promote chronic NF-kB activation, the underlying molecular mechanisms are poorly understood. To investigate how CYLD counteracts constitutive and inducible NF-kB activation in response to TCR and TNFR stimulation in T cells, we generated CYLD KO Jurkat T cells. These cells showed both constitutive and heightened inducible NF-kB activation and we observed that the catalytic activity of CYLD mitigates NF-kB activation. Further investigation revealed that constitutive NF-kB activation might act independently of tonic receptor signaling by TNFR and TCR and of SPATA2, which is however critical for recruiting CYLD to the TNFR complex and counteracting TNFa-triggered NF-kB signaling. We found that LUBAC and TAK1 mediate chronic NF-kB activation and that CYLD is constitutively recruited to LUBAC and TAK1 independently of SPATA2. Taken together, we show that constitutive NF-kB activation in CYLD KO T cells is mediated through LUBAC and TAK1 but is independent of tonic TCR or TNFR stimulation, revealing a new mechanism by which CYLD counteracts LUBAC activity.

## Talk 4 - André Filipe Carmo-Fernandes

# The atypical NF-κB modulator IκBNS is differently required for T follicular helper cell differentiation in spleen and Peyer's patches

**Author:** André Carmo-Fernandes **Co-authors:** Bettina Budeus; Daniel Todt; Ingo Schmitz

**Corresponding Author:** andre.carmofernandes@rub.de

Previously, our group has described a crucial role of the atypical NF-kB modulator IkBNS in Th1, Th17 and Treg cells. Its expression is induced upon cell stimulation, e.g. by TCR signaling. One CD4+ T cell subset that is repetitively being stimulated through TCR signaling is the T follicular helper cell (Tfh). We could confirm that the promoter activity of the gene *Nfkbid*, encoding IkBNS, is higher in CD4+ T follicular compared to non-follicular CD4+ cells. Because we did not observe any reduction of Tfh cells in Peyer's patches compared to spleen, we hypothesized that IkBNS is differently required for Tfh development in spleen upon an acute stimulus compared to Peyer's patches. To test our hypothesis, we used conditional knockout mice, where the *Nfkbid* gene is deleted in CD4+ cells. Mice were infected with Influenza A virus and organs were removed after 7 or 14 days of infection to analyze the adaptive immune response. We could observe that Tfh cell population decreased in the conditional knockout mice in the spleen, whereas in Peyer's patches no alteration was observed. Moreover, Tfh cells from conditional knockout mice were not able to help germinal center B cells to proliferate in the spleen. Acoordingly, conditional knockout mice had reduced antibody titers in the Bronchoalveolar lavage (BAL).
To decipher molecular mechanisms by which IkBNS drives Tfh differentiation, an integral genomics approach including scRNA-seq, bulk RNA-seq and Cut&Run will be employed.

## Talk 5 - Benedict Seddon

# Contrasting roles for NF-κB in the development and maintenance of different non-conventional T cell populations

**Authors:** Benedict Seddon; Cayman Williams; Farjana Islam

Non conventional T cells such as **γ**δ T cells and intraepithelial (IEL) *α*β T cells, play important roles in maintaining immunity at epithelial barriers and have anti-tumoural functions. If and how NF-κB regulates these non-conventional T cell populations is unknown. Here, we addressed this question using mouse genetics to specifically ablate expression of REL subunits or upstream activator of NF-κB, IKK1/2, in early T cell progenitors, prior to *α*β or **γ**δ T cell commitment in the thymus. While, thymic development of different **γ**δ T cell subsets appeared largely normal in the absence of cREL and/or RELA, peripheral lymphoid tissues and tissue site were almost entirely devoid of mature **γ**δ T cells. Additional deletion of Caspase8 did not restore the peripheral **γ**δ T cell compartment in IKK deficient mice, suggesting a requirement for NF-κB and IKK that is independent of extrinsic cell death pathways, in contrast to conventional T cells. *α*β IEL T cells develop in the thymus following strong agonist TCR selection signalling in immature CD4/CD8 double positive thymocytes. In contrast to **γ**δ or conventional *α*β T cells, ablation of cREL/RELA or IKK1/2 resulted in 5-10 fold increase in selected IEL precursors in the thymus. Analysing thymocytes lacking the negative NF-κB regulator, A20, revealed a decrease in IELs, confirming an unexpected negative regulatory function for NF-κB in IEL development. We will also present evidence that CD28 is responsible for triggering NF-κB.

## Talk 6 - Vinay Tergaonkar

# p52:ETS1: a novel NFkB factor essential for splenic GC formation

**Author:** Vinay Tergaonkar

It is established, that five members of NFκB family of transcription factors homo/hetero dimerise amongst themselves to bind DNA and regulate transcription. Our study challenges this paradigm by revealing the first in vivo role of NFκB subunit p52 in partnering with ETS1, a transcription factor outside its family, to form a hetero-tetramer and function as a co-factor. Through the generation and analysis of a knock-in mouse model (p52ki/ki) harbouring mutations in critical p52 residues required for its interaction with ETS1, while preserving interaction with its well-known NFκB partner RelB, we demonstrate the indispensability of p52:ETS1 complex in production of splenic germinal centre (GC) B cells and T cell-dependent antibody responses. Mechanistically, p52:ETS1 is the hitherto undiscovered master regulator of transcription factors, OCT1 and OBF1, critical for the GC program in a B cell intrinsic manner. Furthermore, we highlight distinct roles of p52:ETS1 from p52:RelB in regulating genes associated with specific human diseases, thereby proposing novel avenues for enhancing immune responses and treating immune disorders effectively through targeted interventions. In summary, we demonstrate for the first time that, NFκB subunits can function as tetramers formed with different transcription factor families, and activate transcription independently of DNA binding in vivo, and highlight major therapeutic implications for these novel findings.

# Talk 7 - Dimitri Thanos

## NF-kB memory coordinates the human antiviral stochastic gene expression program

**Author:** DIMITRIS THANOS[1]

[1] *BIOMEDICAL RESEARCH FOUNDATION ACADEMY OF ATHENS*

The stochastic human antiviral gene expression program begins with the expression of the IFN-b gene, where limiting amounts of the transcription factor NF-κB are captured by three DNA elements termed NRCs (NF-κB Reception Centers) in a small percentage of infected cells and are subsequently delivered to the IFN-b enhancer via stochastic interchromosomal interactions to trigger enhanceosome assembly and transcriptional activation. The transcription factor ThPOK binds cooperatively with NF-κB to NRCs and IFN-b and mediates their physical interaction via its oligomerization. Furthermore, the composite NRC-containing NF-κB/ThPOK elements mediate stochastic expression of dozens of additional virus inducible genes where all genes are coordinately expressed simultaneously only within a small percentage of cells. Each expressing cell organizes 3-5 NRC hubs in which all genes are recruited to receive NF-κB to initiate gene expression. ThPOK knock-down leads to NRC hub disassembly, causing sporadic and random expression of the genes. Thus, coordination of stochastic gene expression programs requires the assembly of higher-order chromatin structures containing all expressing genes to ensure their similar accessibility to critical factors. Importantly, virus induction causes high level expression of the p105 gene, thus generating high amounts of p50 homodimers whose subsequent binding to selected promoters creates a molecular "stamp" blocking their re-induction by NF-kB.

# Talk 8 - Argyris Papantonis

## Proinflammatory stimuli hijack the 3D regulatory landscape of human cells

**Author:** Argyris Papantonis[1]

[1] *University Medical Center Goettingen*

Human cells have evolved to respond to proinflammatory stimuli in a prompt and coordinated fashion. However, deployment of the regulatory interactions needed for the implementation of the proinflammatory gene expression program occur against the already active regulatory landscape of the cells. This then raises the question: how is this apparent regulatory conflict resolved? We previously showed that, alongside the de novo activation of proinflammatory enhancers, NF-κB also 'hijacks' thousands of already active enhancers in primary endothelial cells during the immediate early phase of the response. Some of these enhancers remain active and are presumably redirected to interact with new target genes, whereas others are decommissioned via the recruitment of negative regulators. Now, we use a capture-based 3C approach to map the changing 3D interactome of these hijacked enhancers during both the immediate-early and the late phases of the proinflammatory response to TNFα. We also apply single-cell RNA- and ATAC-seq as well as single-cell H3K27ac ChIP-seq at these two time points to show how endothelial cells remodel their gene expression profiles by altering their epigenomes in a concerted fashion with their 3D genomes. Together, this data exemplify how extracellular signaling cues impose a new epigenomic state onto the existing chromatin landscape of responding cells down to the single-cell level.

# Talk 9 - Vivien Ya-Fan Wang

## The regulation of transcriptional activation by NF-κB p52 homodimer and proto-oncogenic Bcl3

**Author:** Vivien Ya-Fan Wang[1]

[1] *University of Macau*

The binding of transcription factors (TFs) to their specific DNA response elements in the promoters/enhancers of target genes is the key event regulating gene transcription and consequent cellular events. The NF-κB family of TFs plays a critical role in diverse physiological processes. Our study focuses on the transcriptional regulation by one NF-κB family member, p52, and its specific co-factor, B-cell lymphoma 3 (Bcl3). Bcl3 is an oncoprotein, the constitutive nuclear presence of Bcl3 induces chronic inflammation and proliferation. Bcl3 is also extensively phosphorylated, it associates with NF-κB p52 homodimers to regulate transcription. Using the combination of structural and biochemical studies, we have shown 1) Bcl3 plays an essential role in enhancing p52:p52 homodimer population in cells which is an unique mechanism to p52 within NF-κB family. 2) Crystal structures of p52:p52 homodimer in complex with its natural κB DNA target site(s) revealed a widening of the DNA minor groove compared to all previously known structures of NF-κB-DNA complexes; further MD simulations studies provide new insights into allosteric control by closely related κB DNAs on NF-κB-dependent transcriptional specificity. 3) Phospho-modification mediated changes in Bcl3 regulate DNA accommodation by the Bcl3:(p52:p52) complex. Overall, our studies shed lights on the intricate structural changes driven by both DNA and protein conformation and dynamic states in modulating transcriptional activity.

# Talk 10 - Binghua Zhang

## Identification of a conserved Short Linear Motif in NF-κB that regulates gene-selective transcription

**Authors:** Binghua Zhang[1]; Qiushi Liu[1]; Ruaidhrí Carmody[1]

[1] *University of Glasgow*

**Corresponding Author:** b.zhang.4@research.gla.ac.uk

The NF-κB family of transcription factors is a key mediator of inflammation and a critical determinant of human health and disease. NF-κB mediated transcription of genes that promote cell survival and proliferation, and the emerging importance of inflammation in diseases such as cancer, atherosclerosis and neurodegeneration, establishes NF-κB as a pathological factor of ever-increasing importance. Phosphorylation of NF-κB subunits is a key regulatory mechanism for the gene selective control of transcription. Here we describe a short linear motif (SLiM) enriched in sites of phosphorylation that is found in all NF-kB subunits. Our analysis shows that the SLiM is structurally conserved in all NF-κB subunits and contains both conserved and subunit-specific phosphorylation sites. We show that the mutation of phosphorylation sites in the SLiM of NF-κB subunits modifies target gene expression in a gene and subunit selective way. We propose that the NF-κB SLiM is a core regulatory site of NF-κB function that mediates interaction with key modulators of NF-κB-dependent transcriptional responses with the potential to be exploited for therapeutic benefit.

## Talk 11 - Sonia Rocha

# Unlocking NF-kappaB's Role in Hypoxia- Induced Gene Expression

**Authors:** Dilem Shakir[1]; Michael Batie[1]; Sonia Rocha[1]

[1] *University of Liverpool*

**Corresponding Author:** srocha@liverpool.ac.uk

Hypoxia, or reduced oxygen availability, frequently occurs during development and in various pathological states, significantly altering gene expression levels. Most hypoxia-induced genes are controlled by Hypoxia Inducible Factor (HIF). RNA-sequencing datasets reveal a conserved HIF and broader hypoxia signature across various tissues and cells. Hypoxia also induces NF-kB to control specific genes, including HIFs. However, whether NF-kB activation results in a conserved gene expression signature under hypoxic conditions remains unclear. To address this, we analyzed publicly available RNA-sequencing datasets and our own, focusing on NF-kB target genes and hallmark signatures in different cellular backgrounds exposed to hypoxia. Using a model cell system, we depleted individual Rel members of the NF-kB family to examine their contributions to the hypoxia-induced gene expression profile using RNA-seq. Our analysis revealed a distinct NF-kB hallmark signature induced by hypoxia across all analyzed cell types. However, the composition of this signature varies significantly between different cellular backgrounds, indicating cell type-specific responses. We will present our comprehensive analysis, highlighting the diverse and unexpected roles of NF-kB in the cellular response to hypoxia, providing new insights into the intricate regulatory networks governing cellular adaptation to low oxygen conditions.

## Talk 12 - Alexander Hoffmann

# The multi-dimer NFκB signaling system: a knowledge base to explore diverse biological contexts

**Author:** Alexander Hoffmann[1]

[1] *UCLA*

The nuclear factor κB (NFκB) system is critical for diverse biological functions in numerous cell types, including the inflammatory response, cell proliferation, survival, differentiation, and pathogen responses. Each cell type is characterized by a subset of the 15 possible NFκB dimers, and their activity is regulated in a stimulus-responsive manner. Here, I will describe a recently published synthesis of large amounts of biochemical knowledge that is comprised within a mathematical model of the multi-dimer NFκB system (Science Signaling vol. 16, eabo2838). This model accounts for the observed cell type-specific repertoires of NFκB dimers and their stimulus-specific activation and cross-talk. I will then apply the model to particular application: to elucidate why and how NFκB dysregulation worsens the prognosis of acute myeloid leukemia.

## Talk 13 - Verian Bader

# An NF-κB signaling platform is assembled at mitochondria upon TNF receptor activation

**Author:** Verian Bader[1] **Co-authors:** Zhixiao Wu[1]; Simran Goel[1]; M. Gerogina Herrera[1]; Tito Cali[2]; Marisa Brini[2]; Jörg Tatzelt[3]; Winklhofer Konstanze F.[4]

[1] *Department Molecular Cell Biology, Institute of Biochemistry and Pathobiochemistry, Ruhr University Bochum, Germany*

[2] *Centro Studi per la Neurodegenerazione (CESNE), University of Padova, Italy*

[3] *Department Biochemistry of Neurodegenerative Diseases, Institute of Biochemistry and Pathobiochemistry, Ruhr University Bochum, Germany, Cluster of Excellence RESOLV, Ruhr University Bochum, Germany*

[4] *Department Molecular Cell Biology, Institute of Biochemistry and Pathobiochemistry, Ruhr University Bochum, Germany, Cluster of Excellence RESOLV, Ruhr University Bochum, Germany*

**Corresponding Author:** verian.bader@rub.de

Mitochondria are increasingly recognized as cellular hubs to orchestrate signaling pathways that regulate metabolism, redox homeostasis, and cell fate decisions. Recent research revealed a role of mitochondria also in innate immune signaling, however, the mechanisms of how mitochondria regulate signal transduction are poorly understood. We found that the NF-κB pathway activated by TNF employs mitochondria as a signaling platform. TNF receptor activation induces the recruitment of HOIP, the catalytic component of the linear ubiquitin chain assembly complex (LUBAC), and the NF-κB essential modifier NEMO to the outer mitochondrial membrane, where M1- and K63-linked ubiquitin chains are generated. NF-κB is locally activated and escorted to the nucleus by mitochondria, leading to an increase in mitochondria-nucleus contact sites in a HOIP-dependent manner. Notably, NEMO undergoes M1-ubiquitin-dependent phase separation in vitro and forms condensates in cells, which is a prerequisite for efficient NF-κB signaling. Our study revealed two advantages of mitochondrial signaling platforms: First, the possibility of signal amplification by virtue of the large mitochondrial surface and the presence of regulatory signaling components at the outer mitochondrial membrane. Second, mitochondria can act as vehicles to transport activated transcription factors from the cell periphery to the nucleus.

# Talk 14 - Fakun Cao

## NF-kB information processing is encoded within IL-1 signaling condensates

**Author:** Fakun Cao[1] **Co-authors:** Binaya Paudyal; Elke Ziska; Arjun Narayanan; Marcus Taylor

[1] *MPIIB Berlin*

**Corresponding Author:** cao@mpiib-berlin.mpg.de

Cells distinguish and process biological, chemical, and physical information in a dynamic environment to make precise responses. How the spatiotemporal dynamics of signaling proteins build information flow remains unclear. Here, we uncovered the mechanisms by which the NF-kB transcription factor receives and transmits information from the receptor in IL-1 innate immune signaling. By live cell imaging of endogenous signaling proteins, we found that IL-1 signaling proteins from MyD88 down to NF-kB are organized into a condensate at the plasma membrane. Within the condensate, MyD88 and NF-kB have a lifecycle of assembly and disassembly. The amounts of MyD88 (input) and NF-kB (output) are positively correlated, suggesting information transmission is embedded within the condensate. Additionally, FRAP analysis shows all signaling components are strongly associated with the condensates, indicating the compositional changes within the condensates drive information transmission. To understand how the internal compositional changes drive information processing, we used phase portrait analysis and found that the condensate lifecycle has three phases: a MyD88-rich early growth phase, the incorporation of NF-kB, and finally a disassembly phase. In summary, we demonstrated that the dynamic assembly and disassembly of condensates drive information processing in IL-1 signaling networks. This work reveals a physical and spatial model for how biochemical networks can process information.

## Talk 15 - Jana Wolf

# Elucidating the temporal regulation of IKK/ NF-κB activation upon genotoxic stress by computational modeling

**Authors:** Claus Scheidereit; Fabian Konrath[1]; Jana Wolf

[1] *MDC Berlin*

NF-κB family members are critical for the regulation of important cellular processes such as proliferation and survival. Based on the stimuli and involved components, one can distinguish three pathway branches: canonical, non-canonical and genotoxic NF-κB signaling. In all three signaling pathways, members of the IKK complex play pivotal roles in the regulation of NF-κB activity. In the last decades, numerous computational models of the canonical NF-κB signalling branch have been developed and studied which greatly contributed to the understanding of the regulation and dynamics of the pathway. Here, we introduce a first computational model describing IKK/ NF-κB activation in response to DNA double strand breaks. The model reproduces quantitative time course data of various genotoxic IKK/ NF-κB signaling pathway components and enabled the analysis of the regulation of IKK activity in a time-resolved manner. The analysis shows that the impact of individual pathway processes on IKK activity varies with the amount of DNA damage. Moreover, it demonstrates a critical role of the sensor protein PARP-1 in the IKK activity regulation. Overall, our results add detailed mechanistic insights into the regulation of IKK/ NF-κB signaling upon genotoxic stress.

# Talk 16 - Robin Plevin

## A novel "Non-canonical" IL-1beta- stimulated NFkappaB signalling axis - implications for nuclear p52 translocation

**Author:** Robin Plevin[1] **Co-authors:** Kirsty Tinto[1]; Mohammed Farhan[1]; Margaret Cunningham[1]; Kathryn Macintosh[1]

[1] *University of Strathclyde*

**Corresponding Author:** r.plevin@strath.ac.uk

NFkappaB2 (p100) phosphorylation is a key event in the activation of the non-canonical pathway. In numerous cell types, LTX or LIGHT ligand stimulated a delayed, IKK alpha/NIK-dependent phosphorylation and processing of p100 and formation of cellular p52. Surprisingly, we found that IL-1beta (IL-1) stimulated a rapid, transient phosphorylation of p100 without concomitant processing. Phosphorylation was abrogated in IKK alpha CRISPR cells, using IKK alpha siRNA or a novel first-in-class selective IKK alpha inhibitor, SU1261. In contrast, inhibition/knockdown of IKK beta was without effect on p100. Furthermore, we found that pre-treatment with a NIK inhibitor had no effect on IL-1 induced phosphorylation, rather the IL-1 response was significantly inhibited by TAK-1 siRNA, or a TAK-1 inhibitor, suggesting that p100 phosphorylation is regulated by IKK alpha from within the NEMO/IKK alpha/IKK beta complex.

IL-1 also stimulated a rapid translocation of p52 to the nucleus (30-60 min). This NIK-independent translocation was partially inhibited by IKK alpha CRISPR, siRNA but not SU1261, suggesting that IKK alpha-dependent phosphorylation p100 is not responsible for p52 nuclear translocation. Rather p52 translocation was sensitive to IKK beta inhibition/deletion suggesting the potential for p65 to interact with cellular p52 and co-translocate to the nucleus.

Overall, we identify two novel features of IL-1beta-stimulated NFkappaB signalling in cells.

# Talk 17 - Michael Kracht

## (Feedback) control of p65/RELA target gene expression revealed by proximity interactomics

**Authors:** Michael Kracht[1, 6, 7]; Lisa Leib[1]; Jana Juli[1]; Stefanie Wirth[1]; Hendrik Weiser[1]; M. Lienhard Schmitz[2]; Jasmin Priester[1]; Johanna Meier-Soelch[1]; Uwe Linne[3]; Simon Hanel[1]; Emmanuel Addo[1]; Jochen Wilhelm[4, 6, 7]; Axel Weber[1]; Marek Bartkuhn[5, 7]; Christin Mayr-Buro[1]; Liane Jurida[1]

[1] *Rudolf Buchheim Institute of Pharmacology, Justus Liebig University, Giessen, Germany*

[2] *Institute of Biochemistry, Justus Liebig University, Giessen, Germany*

[3] *Mass Spectrometry Facility of the Department of Chemistry, Philipps University, Marburg, Germany*

[4] *Institute for Lung Health, Justus Liebig University Giessen, Giessen, Germany*

[5] *Biomedical Informatics and Systems Medicine, Science Unit for Basic and Clinical Medicine, Institute for Lung Health, Justus Liebig University Giessen, Giessen, Germany*

[6] *German Center for Lung Research (DZL) and Universities of Giessen and Marburg Lung Center (UGMLC)*

[7] *Member of the Excellence Cluster Cardio-Pulmonary Institute (CPI), Giessen, Germany*

**Corresponding Author:** michael.kracht@pharma.med.uni-giessen.de

The proinflammatory cytokine interleukin-1 (IL-1) plays a pivotal role in the transcriptional induction of p65 / RELA target genes. At the peak of transcription, IL-1-induced transcripts become transiently unstable through an unknown pathway involving the 5′ exonuclease XRN1. Consequently, cells lacking XRN1 display markedly elevated mRNA stability of canonical NF-κB target, but these effects are mitigated at the mRNA steady-state level, suggesting the existence of a transcriptional buffering mechanism. Proximity labelling was used to determine the protein interactomes of p65 / RELA and XRN1 in intact cells. This strategy revealed over 350 RELA interactors from untreated and IL-1α-stimulated cells, including 172 transcription factors (47% of all interactors) and over 50 epigenetic regulators belonging to different classes of chromatin remodelling complexes. In contrast, the XRN1 interactome was found to be significantly enriched in multiple proteins involved in deadenylation-mediated mRNA decay and RNA metabolism. However, a small number of interactors were shared between both data sets, and thus comprise candidate proteins that mediate feedback regulation between transcription and mRNA decay in the NF-κB pathway. The remarkably large and dynamic, high-resolution interactomes of RELA and XRN1 shed light on the connectivity of the nuclear and cytosolic parts of the gene expression pathway and provide a new framework for explaining how p65 / RELA cooperativity determines gene expression patterns.

## Talk 18 - Savas Tay

# Understanding NF-kB signaling using microfluidic live cell analysis, single cell proteomics and computational modeling

**Author:** Savas Tay, University of Chicago

"No abstract available"

# Talk 19 - Jonathan Storm

## NF-κB in Proliferation and Survival of Human Cancer Stem Cells from Various Tissues

**Author:** Jonathan Storm[1] **Co-authors:** Diana Pschick[1]; Katja Nowak[1]; Barbara Kaltschmidt[1]; Christian Kaltschmidt[1]

[1] *Institute for Cell Biology, University of Bielefeld*

Cancer stem cells (CSCs) are crucial for tumor initiation, invasiveness, metastasis, and recurrence across various cancers. Our group recently isolated and characterized CSCs from different tissues, including non-small cell lung cancer (NSCLC), prostate cancer, and glioblastoma. Using full-length cDNA nanopore sequencing, we identified overexpression of NF-κB target genes, a significant enrichment of the GO term 'NF-κB binding', and ubiquitous expression of the RelA subunit as characteristics of CSCs. Immunocytochemical staining of various CSCs confirmed widespread RelA expression, though basal nuclear localization levels varied. On the other hand, RelB and cRel expressions were heterogeneous. In prostate CSCs, TNFα stimulation reduced survival, particularly in populations with impaired RelA translocation. In contrast, TNFα enhanced survival in glioblastoma and NSCLC CSCs. NF-κB inhibition with PDTC effectively reduced cell viability and countered the cytoprotective effects of TNFα. However, non-cancer human cardiac stem cells were significantly more sensitive to PDTC, indicating low selectivity for cancer cells. Our findings highlight the promise of cancer-specific treatment strategies targeting NF-κB pathways in CSCs to enhance therapeutic efficacy and minimize adverse effects.

# Talk 20 - Kirsty Tinto

## Bad to the bone: An RNA-sequencing study identifying a role for IKK alpha in osteosarcoma cell division and progression

**Author:** Kirsty Tinto[1] **Co-authors:** Marco Bonfanti[2]; Lily Tosh[3]; Margaret Cunningham[3]; Robin Plevin[2]

[1] *University of Strathclyde*

[2] *Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde*

[3] *Strathclyde Institute of Pharmacy and Biomedical Sciences*

**Corresponding Author:** kirsty.tinto@strath.ac.uk

Osteosarcoma (OS) is the most prevalent primary bone tumour and is diagnosed in one to three individuals per million people each year. Given the lack of improvement to OS treatment and prognosis in the last 30 years, research at a molecular level is imperative to enable discovery of therapeutic drug targets for OS. Our present study utilises short-read RNA-sequencing techniques to a human OS cell line, U2OS with and without IKK alpha deletion by CRISPR-Cas9 knockdown.

Our findings highlight that without any stimulation, IKK alpha knockdown significantly decreases expression of cell division genes associated with OS progression, including AURKA, AURKB, TPX2, BIRC5, GTSE1, E2F2, FOXM1 and SPC24 ($p<0.0001$). Interestingly, IKK alpha knockdown increased osteoclast-associated receptor (OSCAR) gene induction ($p<0.0001$), which is a central receptor in bone degradation processes. These effects on OSCAR were mimicked by RT-qPCR, and at protein expression level by immunofluorescence techniques, and SiRNA and subcellular fractionation followed by western blotting. Increased osteoclast activity is associated with decreased OS-derived metastasis, and hence this implies a role for IKK alpha in promoting metastasis. Additionally, IKK alpha knockdown reduced CXCL5 and GAS7 gene expression following IL-1beta (10 ng/mL) stimulation for 8 hours.

Overall, IKK alpha appears to play a role in OS proliferation and progression and could be potentially pharmacologically targeted in OS.

# Talk 21 - Mélanie Favre-Juilland

## LMP1-induced MALT1 activity contributes to EBV-mediated B-cell transformation

**Author:** Mélanie Favre-Juilland[1] **Co-authors:** Margot Thome[1]; Sylvia Rothenberge[2]; Laurence Romy[1]

[1] *UNIL*

[2] *CHUV*

**Corresponding Author:** melanie.favrejuilland@unil.ch

Epstein-Barr virus (EBV), a human gamma-herpes virus affecting >95% the adult population, can infect and immortalize B lymphocytes in vitro and in vivo, causing lymphoma. EBV latency proteins contribute to lymphomagenesis by activating the transcription factor NF-kB. Constitutive NF-kB activation sustains EBV latent infection, favoring viral persistence in vivo.

Here we identify the protease MALT1, a key driver of NF-kB activation in lymphocytes, as an essential component of EBV latency and proliferation of EBV-induced B-cell lymphomas. Some EBV-positive cell lines exhibited constitutive MALT1 activity. In these lines, treatment with a MALT1 inhibitor or LMP1 silencing abrogated MALT1-dependent substrate cleavage and decreased proliferation. MALT1 silencing also decreased LMP1-driven NF-kB activation. BTK or PKC. inhibition did not inhibit MALT1 protease in EBV-infected B-cell lymphoma lines, indicating that MALT1-activation was BTK and PKC independent. Consistent with this conclusion, LMP1 directly interacted with MALT1 in 293T cells. Finally, we found that MALT1 protease inhibition reduced tumor burden in an EBV-infected lymphoma cell line mouse xenograft model. We have thus established a key role for MALT1 protease in LMP1-mediated cellular transformation and provided a rationale for inhibiting MALT1 protease to treat EBV-infected B-cell lymphomas.

## Talk 22 - Bakhtiar Yamini

# p52 tyrosine phosphorylation and glioblastoma growth

**Authors:** Bakhtiar Yamini[1]; Riley Driscoll; Giovanna Bernal; Longtao Wu

[1] *University of Chicago*

**Corresponding Author:** byamini@surgery.bsd.uchicago.edu

The role of p52 in glioblastoma growth and pathogenicity is examined in animal models and human samples. Mass spectrometric analysis of p52 post translational modification reveals tyrosine phosphorylation as a novel alteration. Unbiased MS/MS analysis identifies Src kinase as the enzyme that promotes p52 site-specific phosphorylation. Crispr/Cas9 technology is used to examine the functional role of the p52 tyrosine phosphorylation site. This work unveils new insight into p52 modification and its role in GBM.

## Talk 23 - Manfred Fliegauf

# Pathogenic NFKB1 and NFKB2 variants cause a diversity of biochemical p105/p50 and p100/p52 defects and disturb the NF-κB signaling system at multiple levels

**Authors:** Bodo Grimbacher; Manfred Fliegauf

Germline heterozygous *NFKB1* and *NFKB2* mutations constitute the largest subgroup of the monogenic antibody deficiencies in humans and typically occur with highly variable immune disease phenotypes, uncorrelated with the genotype. Some distinctive features indicate dampened immune responses and inefficient signaling, while others indicate overactive NF-κB.
We test mutant NF-κB proteins *in vitro* for stability and localization, target gene binding and activation under simulated NF-κB on/off conditions, accomplished by co-overexpression of pathway components.
Most *NFKB1* mutations are early or internal truncations, with non-functional protein remnants leading to p105/p50 insufficiency. Central truncations skip the p105 precursor and predict direct expression of p50-like proteins, while the role of the sporadic late truncations is unknown. Missense variants within the RHD can cause protein decay or LOF effects, indicating etiologies other than insufficiency. When residing in the C-terminal half, missense variants affect p105-specific functions.
Most *NFKB2* mutations render p100 unprocessible, sustain its inhibitory activity on the NF-κB system, and shorten the source for p52. Precursor-skipping and particularly haploinsufficiency variants cause milder phenotypes. Further p52 damages descend from various missense changes.
The imbalanced availability of NF-κB molecules – caused by a diversity of protein defects – results in a multitude of signaling errors.

## Talk 24 - Sophia Marie Heimann

# NFKB1 mutations in patients - Towards a clinical score, the best treatment, and a genotype-phenotype correlation

**Authors:** Andrés Caballero; Bodo Grimbacher; Sophia Marie Heimann; Katharina Thoma; Manfred Fliegauf; Pia Hassunah

**Corresponding Author:** sophia.heimann@uniklinik-freiburg.de

Review of 52 papers on NFKB1 patients. Data harmonization with GenIA. Identification of 42 characteristic organ manifestations of NFKB1 insufficiency. Selection of 42 items for an NFKB1 Disease Activity Score (NFKB1-DAS). 21 less relevant items removed by leave-one-out analyses. A longitudinal study was conducted on 32 NFKB1 patients, collecting clinical/laboratory parameters, quality- of-life (QoL) data and medication histories. The NFKB1-DAS and three QoL scores were calculated. Immunomodulatory medications were evaluated.

We identified >420 individuals with pathogenic NFKB1 variants, including affected and unaffected family members. 75% of the carriers were affected, 26% were asymptomatic. We observed distinct phenotypic trends upon clustering according to specific protein defects and calculated the age-dependent penetrance for each type. Leave-one-out analyses identified 21 most relevant score parameters constituting the NFKB1-DAS. 32 patients showed a decrease of the score in 9 patients, while 19 patients had an increasing DAS. 24 of 32 patients showed improvement in at least one score item. No medication was found to consistently decrease disease activity of individual item scores across all patients.

Genotype-phenotype correlations show whether the disease heterogeneity is reflected in the diversity of protein defects. Our NFKB1-DAS is capable of measuring disease activity.

## Talk 25 - Lesley Stark

# Proteomic analysis reveals exercise has a differential impact on colonic epithelial cell pathways in the P50 null model of inflammaging, compared to wild type mice

**Authors:** Qingxuan Qian[1]; Hazel Thoms[1]; Laura Greaves[2]; Fiona Oakley[2]; Alex Von Kriegsheim[1]; Lesley Stark[1]

[1] *University of Edinburgh*

[2] *University of Newcastle*

Advanced age is a major risk factor for colorectal cancer, while physical activity can reduce this risk. However, the mechanisms behind exercise's prevention of colon cancer and the influence of age remain unclear. We used wild-type (WT) mice and an age-related cancer model (nfkb1-/- (P50 null) mouse) to explore the effects of 3 months of moderate exercise on colonic tissue signalling pathways. We firstly focused on TIF-IA, which accumulates with age and is involved in age-related inflammation. Exercise unexpectedly increased TIF-IA in WT mice colons, aligning with related data showing low TIF-IA correlates with poor colon cancer survival. Basal TIF-IA levels were higher in P50 null mice, but exercise minimally affected levels of the protein. Proteomic analysis of colonic tissue by mass spectrometry showed exercise had a greater impact on the WT proteome (205/5364 significant changes) than on the P50 null proteome (46/6272 significant changes). Gene ontology analysis indicated that in WT mice, exercise significantly altered metabolic processes, while in P50 null mice, it affected cell adhesion and plasma membrane processes. These findings suggest exercise has a reduced and different impact on pathways in a model of inflammaging compared to WT mice, warranting further investigation.

# Talk 26 - Marieke Carels

# CARD14-induced cellular signaling in intestinal motility

**Authors:** Aigerim Aidarova[1]; Marieke Carels[1] **Co-authors:** Rudi Beyaert[1]; Inna Afonina[1]; Ellen De Paepe[2]; Lynn Vanhaecke[2]; Mira Haegman[1]; Steven Timmermans[3]; Rita de Cassia de Oliveira Collaco[4]; Frank Bosmans[4]; Javier Aguilera-Lizarraga[5]; Yasmine Driege[1]; Claude Libert[3]; Guy Boeckxstaens[5]; Joan Manils[6]; Steven C. Ley[7]; Tom Van de Wiele[8]; Eline Van Damme[8]

[1] *Center for Inflammation Research, Unit of Molecular Signal Transduction in Inflammation, VIB, Ghent, Belgium & Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium*

[2] *Department of Translational Physiology, Infectiology and Public Health, Ghent University, Ghent, Belgium*

[3] *Center for Inflammation Research, Unit of Molecular Signal Transduction in Inflammation, VIB, Ghent, Belgium & Center for Inflammation Research, Unit of Mouse Genetics and Inflammation, VIB, Ghent, Belgium*

[4] *Department of Basic and Applied Medical Sciences, Ghent University, Ghent, Belgium*

[5] *Department of Chronic Diseases, Metabolism, and Ageing, KU Leuven, Leuven, Belgium*

[6] *Immunity, Inflammation and Cancer Group, Oncobell Program, Bellvitge Biomedical Research Institute, Barcelona, Spain & Serra Húnter Programme, Immunology Unit, Department of Pathology and Experimental Therapy, School of Medicine, University of Barcelona, Barcelona, Spain*

[7] *Institute of Immunity & Transplantation, University College London, NW3 2PP, UK*

[8] *Center for Microbial Ecology and Technology (CMET), Faculty of Bioscience Engineering, Ghent University, Ghent University, Ghent, Belgium*

**Corresponding Author:** mariekec@irc.vib-ugent.be

Chronic constipation is a common worldwide health problem, yet the underlying etiology of this condition is still poorly defined. Consequently, common treatment options are restricted to diet, lifestyle changes or use of osmotic laxatives, which frequently produce unsatisfactory results, prompting the need to gain better mechanistic insight into the regulation of gut motility. However, preclinical studies in this field are hampered by the lack of well-described mouse models and often rely on the administration of non-specific drugs delaying intestinal transit. We have made the surprising observation that mice expressing the gain-of-function CARD14(E138A) NF-kB signaling protein specifically in intestinal epithelial cells suffer from slow intestinal transit, dry stool and increased risk for prolapse development. We report disturbances in gut hormone production and defects in Paneth cell function in these mice. Furthermore, we observe microbial dysbiosis and changes in the stool metabolome, that possibly contribute to slow intestinal transit. Using CARD14(E138A)IEC mice as a novel model of slow gut transit, we expect to uncover novel molecular and cellular mechanisms that mediate slow gut transit.

# Talk 27 - Lienhard Schmitz

## Human NF-κB knockouts

**Author:** M. Lienhard Schmitz[1]

[1] *Institute of Biochemistry, University of Giessen (Germany)*

**Corresponding Author:** lienhard.schmitz@biochemie.med.uni-giessen.de

Our understanding of the physiological role of NF-kB has significantly progressed through studies in model organisms such as mice. The analysis of loss-of-function and gain-of-function mutants has provided strong evidence for NF-kB's crucial role in immune signaling and the response to harmful conditions. In humans, the discovery of mutations in various NF-kB components and their impact on disease development has leveraged our knowledge of NF-kB's (patho)physiological functions. Recent deep sequencing studies of human exomes and genomes have facilitated the identification of essential and of redundant genes in humans. I have combined data sets from various papers, including a recently published study describing exome sequencing of nearly one million people, identifying a total of 6,356 different non-essential genes. Some of these knockouts also include NF-kB components or members of the upstream signaling cascades. Since the NF-kB system is also heavily regulated by post-translational modifications, the key modification sites were examined for their susceptibility to mutations. While missense mutations are found for most modification sites, a limited number of the modified amino acids is never changed, which suggests the functional relevance of these modifications in human NF-kB signaling. These findings about redundant NF-kB proteins and non-essential posttranslational modifications could also be valuable for the development of NF-kB-targeting therapeutics, which has so far been unsuccessful.

# Talk 28 - Michael Naumann

## ADP-heptose-induced and CYLD- dependent NF-kB activation in Helicobacter pylori infection

**Author:** Michael Naumann[1] **Co-author:** Michelle Lim[2]

[1] *Otto von Guericke University*

[2] *Otto von Guericke University*

**Corresponding Author:** naumann@med.ovgu.de

The human microbial pathogen Helicobacter pylori is a risk factor for the development of gastric diseases including cancer. Interestingly, the lipopolysaccharide metabolite ADP-D-glycero-b-D-manno-heptose (ADP-Hep) triggers a variety of cellular processes in the host, including the activation of NF-kB. We have investigated the interplay of different deubiquitinylases (DUBs), e.g. A20, USP15 and USP48, in classical and alternative NF-kB signaling in the context of gastric pathology. Our recent unexpected data show that CYLD in a catalytically inactive form is required for the promotion of NF-kB activation in H. pylori infection.

# Talk 29 - Pinghui Feng

## A nucleotide synthetic enzyme reprograms RelA to fuel aerobic glycolysis and nucleotide synthesis in cancer and viral infection

**Author:** Pinghui Feng[1]

[1] *University of Southern California*

**Corresponding Author:** pinghuif@usc.edu

In studying herpesvirus immune evasion, we discovered important regulatory roles of protein deamination mediated by glutamine amidotransferases (GATs). GATs are genuine metabolic enzymes and six of them catalyze the de novo nucleotide synthesis. Our recent study demonstrates that cancer cells and viruses activate a glutamine amidotransferase, carbamoyl phosphate synthetase, aspartate transcarbamoylase and dihydroorotase (CAD), to couple immune evasion to elevated nucleotide synthesis. Specifically, activated CAD deamidates RelA, which shunts RelA from mediating an inflammatory response to aerobic glycolysis. Deamidated RelA fails to activate classical NF-kB-responsive genes, rather upregulates the expression of an array of glycolytic enzymes to fuel aerobic glycolysis and cell proliferation or viral replication. Naturally occurring cancer mutations within the human Rela gene predispose RelA to CAD-mediated deamidation, fueling aerobic glycolysis and cell proliferation. Equally interestingly, SARS-CoV-2 deploys NSP9 to activate CAD that inhibits inflammatory response while promoting aerobic glycolysis and nucleotide synthesis. Finally, depletion and pharmacological inhibition of CAD impede the proliferation of cancer cells and the replication of SARS-CoV-2, via restoring inflammatory response and blocking nucleotide synthesis. These results uncover new functions of a nucleotide synthetic enzyme and RelA, a pivotal transcription factor underpinning diverse biological processes.

# Talk 30 - Emma Teixeiro

## IKK2/NFkB signaling controls lung residentCD8+ T cell memory during influenza infection

**Authors:** Dezzarae Luera[1]; Emma Teixeiro[2]

[1] *Department of Molecular Microbiology and Immunology. School of Medicine. Next Gen Presision Medicine Bldg. University of Missouri.*

[2] *NextGen Precision Bldg/ School of Medicine/ Dept. Molecular Microbiology and Immunology/University of Missouri*

CD8+ T cell tissue resident memory (TRM) cells are especially suited to control pathogen spread at mucosal sites. However, their maintenance in lung is shortlived. TCR-dependent NFkB signaling is crucial for T cell memory but how and when NFkB signaling modulates tissue resident and circulating T cell memory during the immune response is unknown. Here, we find that enhancing NFkB signaling in T cells once memory to influenza is established, increases prosurvival Bcl-2 and CD122 levels thus boosting lung CD8+ TRM maintenance. By contrast, enhancing NFkB signals during the contraction phase of the response leads to a defect in CD8+ TRM differentiation without impairing recirculating memory subsets. Specifically, inducible activation of NFkB via constitutive active IKK2 or TNF interferes with TGFβ signaling, resulting in defects of lung CD8+ TRM imprinting molecules CD69, CD103, Runx3 and Eomes. Conversely, inhibiting NFkB signals not only recovers but improves the transcriptional signature and generation of lung CD8+ TRM. Thus, NFkB signaling is a critical regulator of tissue resident memory, whose levels can be tuned at specific times during infection to boost lung CD8+ TRM.

# Talk 31 - Rabina Giri

## Immunomodulation of NF-κB by gut bacteria in Inflammatory Bowel Disease

**Author:** Rabina Giri

Inflammatory Bowel Disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract. NF-κB represents a key inflammatory pathway that contributes significantly to pathogenesis of IBD. Current IBD therapies either directly or indirectly target NF-κB. Additionally, the gut microbiome, the composition of which is altered in IBD, has been implicated as a significant driver of disease activity.

We investigated alterations in microbial immunomodulatory function (MIF) in IBD patients with respect to NF-κB pathway. Faecal waters from IBD patients lacked suppressive modulatory effect on TNF mediated NF-κB signalling. We identified bacterial strain Enterococcus Faecalis AHG0090 with NF-κB suppressive activity which ameliorated colitis and ileal disease in murine models via direct downregulation of IL-23 p19 production by macrophages through inhibition of NF-κB - c-Rel translocation. A second strain Clostridium boltea AHG0001ameliorated spontaneous colitis by inhibiting both canonical and non-canonical NF-κB signalling. Synthesized analogues of the bioactive potently inhibit NF-κB target genes with efficacy on 3 orthogonal colitis model.

Overall, our NF-κB assay system allow for rapid identification of bacteria producing immunomodulatory bioactives. Characterising the MIF in individual patients may enable a precision medicine approach, by identifying pathways, such as NF-κB, to target with existing medical therapy as well as identifying targets for novel therapies.

# Talk 32 - Dhairya Rajguru

## Activation of NFκB upon herpes simplex virus 1 (HSV1) infection in human cells

**Author:** Dhairya Rajguru[1] **Co-authors:** Emilia Repossi[1]; Ishamel Blankson[1]; Christoph Borner[2]

[1] *Institute of Molecular Medicine and Cell Research, University of Freiburg*

[2] *Institute of Molecular Medicine and Cell Research, University of Freiburg, Stefan Meier Str. 17, D-79104 Freiburg*

**Corresponding Author:** dhairya.rajguru@mol-med.uni-freiburg.de

The transcription factor NFκB regulates cell processes like cell survival, proliferation, inflammation and innate immune responses to pathogens. Herpes simplex virus 1 (HSV1) has evolved strategies to activate NFκB signaling in order to favor cell survival, effective virus reproduction and escape host antiviral responses. It is known that HSV1 enters host cells by binding to the cell surface receptor HVEM/TNFRSF14, a member of the TNF receptor family. We postulate that this binding may activate the NFκB pathway similarly as TNF[g acting on TNF receptor 1 (TNFR1). However, molecular mechanisms of NFκB signaling in HSV1-infected epithelial cells are not well studied. Human larynx carcinoma HEp-2 and colorectal carcinoma HT-29 cells were infected with HSV1 strain F (MOI of 10) for up to 24 h. In both cell lines HSV1 infection triggers NFκB activation starting at 8 h post infection as evidenced by enhanced JNK1/2 and IKKα/β phosphorylation, IκBα degradation and NFκB p65 phosphorylation. No caspase-3 activation was detected indicating that the cells survived the HSV1 infection. In contrast to HSV1, TNF-α treatment triggered NFκB activation within the first hour of treatment. In summary, our results reveal a delayed activation of NFκB signaling upon HSV1 infection contrary to the known rapid activation with canonical TNFR signaling. This indicates that NFκB activation by HSV1 may not be via complex I formation on its HVEM/TNFRSF14 but rather by an indirect non-canonical mechanism.

## Talk 33 - Liwen Liu

# Individual NF-kB family members regulate the response of mouse gastric organoids to Helicobacter infection and Th1 cytokines

**Author:** Liwen Liu[1] **Co-authors:** Mushfique Alam[1]; Carrie Duckworth[1]; Mark Pritchard[1]

[1] *ISMIB, University of Liverpool*

**Corresponding Author:** liwen.liu@liverpool.ac.uk

Helicobacter pylori (H. pylori) infection is the primary risk factor for gastric cancer. Studies using transgenic mice have shown that classical and alternative NF-κB pathways regulate Helicobacter-induced gastric pathology. However, it is unclear whether these effects are due to NF-κB signalling in epithelial or immune compartments. We have therefore investigated whether NF-κB family members regulate the direct effects of Helicobacter and Th1 cytokines upon gastric epithelial cells.

Gastric organoids (gastroids) derived from wild-type C57BL/6, NF-κB1-/-, NF-κB2-/-, c-Rel-/- and Rel-B-/- mice were treated with TNF, IFN-γ or IL-1β in vitro. 2D monolayer models derived from these gastroids were infected with H. pylori or H. felis to replicate in vivo conditions.

Wild-type gastroids showed significant area reduction and increased apoptosis 72 hrs following TNF and IFN-γ, but not IL-1β. NF-κB1-/- and c-Rel-/- gastroids responded similarly. However, NF-κB2-/- and Rel-B-/- gastroids were resistant to TNF-induced changes, but responded to IFN-γ similarly to wild-type.

Infection with H. pylori or H. felis increased apoptosis and reduced proliferation in wild-type, NF-κB1-/- and c-Rel-/- monolayers, but NF-κB2-/- and Rel-B-/- monolayers were resistant.

Our results validate previous findings about the regulation of Helicobacter induced gastric pathology by NF-κB family members and suggest that these effects are at least partly mediated by signalling in gastric epithelial cells.

## Talk 34 - Teresa Faupel

# NEMO deletion causes brain endothelial cell death

**Authors:** Teresa Faupel[1]; Ümit Özorhan; Josephine Lampe; Raoul Strasburger; Phillip Ehrich; Markus Schwaninger

[1] *Universität zu Lübeck Institut für Experimentelle und Klinische Pharmakologie und Toxikologie*

The cerebral small vessel disease (CSVD) Incontinentia pigmenti (IP) is caused by a mutation in the IKBKG gene, coding the NF-κB essential modulator (NEMO) protein. One-third of patients suffering from IP show, apart from abnormalities of the skin, neurological symptoms including seizures, and intellectual disability as a lack of NEMO in endothelial cells causes the blood-brain barrier (BBB) to become leaky. Mice with a conditional knockout of Ikbkg in brain endothelial cells (NEMObeKO) are characterized by the rarefaction of brain microvessels, cerebral hypoperfusion, a disrupted BBB, epileptic seizures and the formation of string vessels (empty basement membrane tubes) due to the death of brain endothelial cells. The mechanism of how the brain endothelial cells undergo cell death is still not understood, hence we investigate the involved cell death pathway (necroptosis/ferroptosis). By applying pharmacological or genetic tools, we found that receptor-interacting serine/threonine-protein kinases (RIPK) 1 and 3 mediate string vessel formation in NEMObeKO mice. We saw that RIPK inhibition can partially ameliorate the NEMObeKO phenotype. Combining immunohistochemistry and single-cell RNA sequencing or proteomics data gave further insight into the involved cell death mechanisms. Our data point to new ways to treat endothelial cell death in IP and possibly also other CSVD such as Alzheimer's disease, stroke, COVID-19 or aging.

## Talk 35 - Lluís Espinosa

# Separation-of-function mutants reveal the NF-KB-independent involvement of IKB$\alpha$ in the regulation of intestinal stemness

**Authors:** Anna Bigas; Daniel Alvarez-Villanueva; Laura Sole Font; Lluís Espinosa; Daniel Floor; María Maqueda

We have previously shown that the NF-KB inhibitor IKBA binds the chromatin together with PRC2 to confer responsiveness to PRC2 targets in the presence of inflammatory cues. This alternative function has been elusive in both physiological and disease conditions because of the predominant role of IKBA as a negative regulator of NF-KB.

Here, we uniquely characterize the specific residues of IKBA that allow the generation of separation-of-function (SOF) mutants that are defective for either NF-KB-related (SOF deltaNF-KB) or chromatin-related (SOF deltaH2A/H4) activities. Expression of IKBA SOF deltaNF-KB, but not SOF deltaH2A/H4, is sufficient to negatively regulate a specific stemness program in intestinal cells, thereby rescuing the differentiation block imposed by IKBA deficiency. By ChIP assay, we demonstrated that IIKBA binds to several stemness genes that are transcriptionally repressed upon IKBA SOF deltaNF-KB induction.

Our data suggest that SOF mutants provide an exclusive tool for studying IKBA functions in physiology and disease.

# Talk 36 - Nele Czaniera

# Role of NF-κB in neuronal differentiation and fate determination

**Authors:** Nele Czaniera[1]; Wiebke Schulten[1]; Christian Kaltschmidt[1]; Barbara Kaltschmidt[1]

[1] *Institute of Cell Biology, University of Bielefeld, Universitaetsstr. 25, 33615 Bielefeld*

**Corresponding Author:** nele.czaniera@uni-bielefeld.de

In the mammalian nervous system, the transcription factor NF-κB regulates many cellular processes, including cell fate determination. For example, in mouse embryonic stem cells (mESC) the inhibition of the NF-κB kinase complex (IKK) has been shown to alter cell fate during neural crest formation. While an IKK-activation results in a mesodermal differentiation, the inhibition shifts it towards the neuroectoderm. Further downstream differentiation processes are also regulated by NF-κB. For instance, it has been shown in human neural stem cells (hNSC) that inhibition of the c-Rel subunit leads to a neurogenesis to gliogenesis fate-shift. During neuronal differentiation, c-Rel inhibitor Pentoxifylline treated hNSCs differentiated into oligodendrocytes at the expense of neurons. In addition, a putative role of c-Rel was observed in human induced pluripotent stem cells (hiPSC). Here, we present an increased activity of c-Rel until ~day 14, which then decreased and remained at a constant level from day 20 onwards in induced neurons. Next, we will analyse a putative fate shift after inhibition of c-Rel. Finally, we are interested in loss of function mutations upstream of NF-κB signaling which were identified in Alzheimer disease (AD) patients. Therefore, we aim to understand the role of AD-mutations affecting NF-κB in neuronal differentiation of hiPSCs.

## Talk 37 - Bernd Baumann

# Role of NF-kB/Notch crosstalk signaling in brain aging

**Authors:** Hina Zarrin; Qingwen Yang; Burak Yildiz; Nada Korayem; Tabea Hein; Franz Oswald; Bernd Baumann

**Corresponding Author:** bernd.baumann@uni-ulm.de

Inflammaging is a progressive, sterile low-grade inflammatory process which in the CNS is mainly driven by glial cells, and which is associated with cellular senescence and neurodegeneration. The NF-kB and Notch signaling pathways are both proposed to regulate brain inflammaging and the development of senescence-associated secretory phenotype, SASP, however the underlying molecular mechanisms and their mode of interaction are not well-defined. Both pathways are differentially active upon aging and are also interlinked on multiple levels including specific overlapping NF-kB/Notch DNA-binding sites, which we determined and named crosstalk (CT) sites. Using various bioinformatic tools, we identified specific genes either associated with brain aging or SASP that possess CT sites in their promotor regions occupied by NF-kB and Notch/RBPJ factors. These CT site carrying genes are differential expressed upon aging in the cortex of transgenic mouse models allowing modulation of Notch or NF-kB signaling.
To test the function of CT sites on the regulation of gene expression, we performed extensive reporter gene studies which revealed that NF-kB driven activation of CT-reporter genes gets suppressed in the presence of Notch signaling. Importantly, we also identified a direct physical interaction between specific Notch and NF-kB pathway components. Based on our data, we hypothesize that NF-kB/Notch crosstalk signaling plays an important role in brain aging.

## Participants

| Last Name | First Name | Email Address | Institute / Company | Country |
|---|---|---|---|---|
| **Alvarez Villanueva** | Daniel | dalvarez@researchmar.net | Hospital del Mar Research Institute | Spain |
| **Antoshkina** | Irina | irina.antoshkina@helmholtz-munich.de | Helmholtz Munich | Germany |
| **Bader** | Verian | verian.bader@rub.de | Ruhr-Universität Bochum | Germany |
| **Baud** | Véronique | veronique.baud@inserm.fr | INSERM-Université Paris Cité | France |
| **Baumann** | Bernd | bernd.baumann@uni-ulm.de | Institute of Physiological Chemistry | Germany |
| **Becher** | Jonas | jonas.becher@med.uni-giessen.de | JLU Giessen | Germany |
| **Beuerlein** | Knut | knut.beuerlein@pharma.med.uni-giessen.de | Rudolf Buchheim Institute of Pharmacology, Giessen | Germany |
| **Borggrefe** | Tilman | tilman.borggrefe@biochemie.med.uni-giessen.de | University of Giessen, Department of Biochemistry | Germany |
| **Cao** | Fakun | cao@mpiib-berlin.mpg.de | Max Planck Institute for Infection Biology | Germany |
| **Campos** | Luiza | luiza.c.campos@ucl.ac.uk | UCL | United Kingdom |
| **Carels** | Marieke | mariekec@irc.vib-ugent.be | VIB-UGent | Belgium |
| **Carmo Fernandes** | André | andre.carmofernandes@rub.de | Ruhr-Universität Bochum | Germany |
| **Carmody** | Ruaidhri | ruaidhri.carmody@glasgow.ac.uk | University of Glasgow | United Kingdom |
| **Czaniera** | Nele | nele.czaniera@uni-bielefeld.de | Bielefeld University | Germany |
| **Dreute** | Jan | jan.dreute@biochemie.med.uni-giessen.de | JLU Gießen, AG Schmitz | Germany |
| **Espinosa** | Lluis | lespinosa@researchmar.net | Hospital del Mar Research Institute | Spain |
| **Faupel** | Teresa | teresa.faupel@uni-luebeck.de | Universität zu Lübeck Institut für Experimentelle und Klinische Pharmakologie und Toxikologie | Germany |
| **Favre** | Mélanie | melanie.favrejuilland@unil.ch | University of Lausanne | Switzerland |
| **Feng** | Pinghui | pinghuif@usc.edu | University of Southern California | United States |

## Participants

| | | | | |
|---|---|---|---|---|
| **Fliegauf** | Manfred | manfred.fliegauf@uniklinik-freiburg.de | Institute for Immunodeficiency, Center for Chronic Immunodeficiency, Medical Center - University of Freiburg | Germany |
| **Gay** | Nick | njg11@cam.ac.uk | University of Cambridge | United Kingdom |
| **Giri** | Rabina | rabina.giri@mater.uq.edu.au | Mater Research | Australia |
| **Gonther** | Sina | sina.gonther@uksh.de | Universitätsklinikum Schleswig-Holstein, Dermatologie Allergologie und Venerologie | Germany |
| **Grimbacher** | Bodo | bodo.grimbacher@uniklinik-freiburg.de | Medical Center - University of Freiburg, Institute for Immunodeficiency | Germany |
| **Hanel** | Simon | simon.hanel@pharma.med.uni-giessen.de | Rudolf Buchheim Institute of Pharmacology, Giessen | Germany |
| **Haußner** | Erik | erik.haussner@uk-halle.de | Medical Faculty/Martin-Luther University Halle | Germany |
| **Heimann** | Sophia Marie | sophia.heimann@uniklinik-freiburg.de | Uniklinik Freiburg - ZTZ | Germany |
| **Hennessy** | Meagan | m.hennessy@liverpool.ac.uk | University of Liverpool | United Kingdom |
| **Hoffmann** | Alexander | ahoffmann@ucla.edu | UCLA | United States |
| **Hudoba** | Liza | l.hudoba@ucl.ac.uk | UCL IIT | United Kingdom |
| **Jurida** | Liane | liane.m.jurida@pharma.med.uni-giessen.de | Rudolf Buchheim Institute of Pharmacology, Giessen | Germany |
| **Kaltschmidt** | Barbara | barbara.kaltschmidt@uni-bielefeld.de | Institute for Cell Biology, University of Bielefeld, Germany | Germany |
| **Kaltschmidt** | Christian | c.kaltschmidt@uni-bielefeld.de | Universität Bielefeld | Germany |
| **Keysberg** | Leonie | leoniesophia.keysberg@med.uni-duesseldorf.de | Clinic for Gastroenterology, Hepatology and Infectious Diseases, Universitätsklinikum Düsseldorf | Germany |
| **Konrath** | Fabian | fabian.konrath@mdc-berlin.de | Max-Delbrueck-Center Berlin | Germany |
| **Kracht** | Michael | michael.kracht@pharma.med.uni-giessen.de | Justus-Liebig-University Giessen, Faculty of Medicine, Rudolf Buchheim Institute of Pharmacology | Germany |

## Participants

| | | | | |
|---|---|---|---|---|
| **Krappmann** | Daniel | daniel.krappmann@helmholtz-munich.de | Signaling and Translation / Helmholtz Center Munich | Germany |
| **Ley** | Steven | steven.ley@ucl.ac.uk | Institute of Immunity & Transplantation, University College London | United Kingdom |
| **Liu** | Kuangzheng | liu.kuangzheng@uni-ulm.de | Urologische Universitätsklinik | Germany |
| **Liu** | Liwen | liwen.liu@liverpool.ac.uk | University of Liverpool | United Kingdom |
| **Meier-Sölch** | Johanna | johanna.meier-soelch@pharma.med.uni-giessen.de | Rudolf Buchheim Institute of Pharmacology, Giessen | Germany |
| **Montoto Fernández** | Ángela | amontoto@researchmar.net | Hospital del mar research institute | Spain |
| **Mouhandes** | Bayan | bayan.mouhandes83@gmail.com | Uniklinik Würzburg | Germany |
| **Naumann** | Michael | naumann@med.ovgu.de | Otto von Guericke University, Institute of Experimental Internal Medicine | Germany |
| **Norbäck** | Hanna | hanna.norback@stud.ki.se | EMBL Heidelberg | Germany |
| **Ober** | Franziska | franziska.ober@helmholtz-munich.de | Signaling and Translation / Helmholtz Munich | Germany |
| **Papantonis** | Argyris | argyris.papantonis@med.uni-goettingen.de | University Medical Center Goettingen | Germany |
| **Pasparakis** | Manolis | pasparakis@uni-koeln.de | Institute for Genetics, University of Cologne | Germany |
| **Peppler** | Helke | helke.peppler@pharma.med.uni-giessen.de | Rudolf Buchheim Institute of Pharmacology, Giessen | Germany |
| **Perkins** | Neil | neil.perkins@ncl.ac.uk | Newcastle University | United Kingdom |
| **Pfisterer** | Maximilian | maximilian.pfisterer@biochemie.med.uni-giessen.de | Institute of Biochemistry, JLU Gießen | Germany |
| **Plevin** | Robin | r.plevin@strath.ac.uk | University of Strathclyde | United Kingdom |
| **Pogge von Strandmann** | Elke | poggevon@staff.uni-marburg.de | Philipps-University Marburg | Germany |
| **Pschik** | Diana | diana.pschik@uni-bielefeld.de | Institute for Cell Biology, University of Bielefeld | Germany |

## Participants

| | | | | |
|---|---|---|---|---|
| **Rajguru** | Dhairya | dhairya.rajguru@mol-med.uni-freiburg.de | Institute of Molecular Medicine and Cell Research, Albert Ludwig University of Freiburg | Germany |
| **Repossi** | Emilia | emilia.repossi@mol-med.uni-freiburg.de | Institute of Molecular Medicine and cell Research, University of Freiburg | Germany |
| **Rocha** | Sonia | srocha@liverpool.ac.uk | University of Liverpool | United Kingdom |
| **Saul** | Vera | vera.saul@biochemie.med.uni-giessen.de | Justus-Liebig-University Gießen, Institute of Biochemistry | Germany |
| **Schmidt** | Franziska | franziska.schmidt@pharma.med.uni-giessen.de | Rudolf Buchheim Institute of Pharmacology, Giessen | Germany |
| **Schmidt** | Lena | lena.schmidt@biochemie.med.uni-giessen.de | Institute of Biochemistry, University of Giessen | Germany |
| **Schmitz** | Ingo | ingo.schmitz@rub.de | Molecular Immunology / Ruhr University Bochum | Germany |
| **Schmitz** | Lienhard | lienhard.schmitz@biochemie.med.uni-giessen.de | Justus-Liebig-University Giessen, Faculty of Medicine, Institute for Biochemistry | Germany |
| **Schulten** | Wiebke | wiebke.schulten@uni-bielefeld.de | Bielefeld University | Germany |
| **Seddon** | Benedict | benedict.seddon@ucl.ac.uk | University College London | United Kingdom |
| **Sen** | Ranjan | senranja@grc.nia.nih.gov | National Institutes of Health | United States |
| **Shikh Shaban** | M. Samer | mohammed.s.shaban@pharma.med.uni-giessen.de | Rudolf Buchheim Institute of Pharmacology, Giessen | Germany |
| **Solé Font** | Laura | lsole@researchmar.net | Centro de Investigacion Biomedica En Red (Ciber) | Spain |
| **Stark** | Lesley | lesley.stark@ed.ac.uk | University of Edinburgh | United Kingdom |
| **Steels** | Jill | jills@irc.vib-ugent.be | VIB-UGent | Belgium |
| **Storm** | Jonathan | jonathan.storm@uni-bielefeld.de | Institute for Cell Biology, University of Bielefeld | Germany |
| **Tay** | Savas | tays@uchicago.edu | University of Chicago | United States |
| **Teixeiro** | Emma | teixeiropernase@missouri.edu | School of Medicine/ Dept. Molecular Microbiology and Immunology/University of Missouri | United States |

## Participants

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|---|---|---|---|---|
| **Tergaonkar** | Vinay | vinayt@imcb.a-star.edu.sg | IMCB, Singapore | Singapore |
| **Thanos** | Dimitris | thanos@bioacademy.gr | Biomedical Research Foundation Academy of Athens | Greece |
| **Tinto** | Kirsty | kirsty.tinto@strath.ac.uk | University of Strathclyde | United Kingdom |
| **Turner** | David | david.turner@liverpool.ac.uk | University of Liverpool | United Kingdom |
| **Wang** | Vivien Ya-Fan | vivienwang@um.edu.mo | University of Macau | Macao SAR China |
| **Weber** | Axel | axel.weber@pharma.med.uni-giessen.de | Rudolf Buchheim Institute of Pharmacology, Giessen | Germany |
| **Wolf** | Jana | jana.wolf@mdc-berlin.de | Max-Delbrueck-Center for Molecular Medicine | Germany |
| **Yamini** | Bakhtiar | byamini@bsd.uchicago.edu | University of Chicago | United States |
| **Zhang** | Binghua | b.zhang.4@research.gla.ac.uk | University of Glasgow | United Kingdom |