ORAL PRESENTATIONS

1

Intravital Imaging to Monitor Therapeutic Response in Moving Hypoxic Regions Resistant to PI3K Pathway Targeting in Pancreatic Cancer

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Pancreatic ductal adenocarcinoma (PDAC) is among the most deadly solid tumours. This is due to a generally late-stage diagnosis of a primarily treatment-refractory disease. Several large-scale sequencing and mass spectrometry approaches have identified key drivers of this disease and in doing so highlighted the vast heterogeneity of lower frequency mutations that make clinical trials of targeted agents in unselected patients increasingly futile. There is a clear need for improved biomarkers to guide effective targeted therapies, with biomarker-driven clinical trials for personalised medicine becoming increasingly common in several cancers. Interestingly, many of the aberrant signalling pathways in PDAC rely on downstream signal transduction through the mitogen-activated protein kinase and phosphoinositide 3-kinase (PI3K) pathways, which has led to the development of several approaches to target these key regulators, primarily as combination therapies. The following review discusses the trend of PDAC therapy towards molecular subtyping for biomarker-driven personalised therapies, highlighting the key pathways under investigation and their relationship to the PI3K pathway

2

Super-resolving presynaptic endocytic pathway dynamics: from plasma membrane binding to internalization in synaptic vesicles

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The capacity of neurons to communicate and store information in the brain critically depends on neurotransmission, a process which relies on the release of chemicals called neurotransmitters stored in small synaptic vesicles at the presynapse. Following their fusion with the presynaptic plasma membrane, synaptic vesicles are rapidly reformed by a process called endocytosis. Neurotoxins, such as Botulinum Neurotoxin type-A (BoNT/A) take advantage of this endocytic pathway to gain access into motor nerve terminals as part of its intoxication strategy to incapacitate nerve-muscle communication. The investigation of the endocytic pathway dynamics is severely restricted by the diffraction limit of light and, therefore, the recycling of synaptic vesicles, which are 45 nm in diameter, has been primarily studied with electrophysiology, low resolution fluorescence-based techniques and electron microscopy. To address this, we implemented a novel super-resolution technique called the subdiffractional tracking of internalized molecules (sdTIM), which is a pulse-chase based method for studying the single molecule mobility of endocytic vesicles. With 30-40 nm localization precision, we revealed that, once internalized, VAMP2-positive synaptic vesicles exhibit a markedly lower mobility than on the plasma membrane (imaged by uPAINT), an effect that was reversed upon restimulation in presynapses. Using HMM-Bayes modelling, we found that synaptic vesicles constantly oscillate between diffusive states and a combination of diffusive and transport states with opposite directionality. The plasma membrane binding of BoNT/A revealed a different mobility pattern than that of VAMP2 and upon internalization BoNT/A internalized into a distinct synaptic vesicle pool different from that of VAMP2-positive vesicles. Genetic inactivation of the BoNT/A ganglioside (GT1b) and co-receptor SV2 binding sites affects the plasma membrane binding, internalization and long range retrograde transport of the toxin. I will also discuss an sdTIM dual-colour imaging of recycling and signalling endosomes containing cholera toxin subunit-B.

1. Joensuu M, Martínez-Mármol R, Padmanabhan P, Glass NR, Durisic N, Pelekanos M, Mollazade M, Balistreri G, Amor R, Cooper-White JJ, Goodhill GJ and Meunier FA (2017): Visualizing endocytic recycling and trafficking in live neurons by subdiffractional tracking of internalized molecules. Nature Protocols, 12, 2590-2622.
2. Joensuu M, Padmanabhan P, Durisic N, Bademosi AT, Cooper-Williams E, Morrow IC, Harper CB, Jung W, Parton RG, Goodhill GJ, Papadopulos A and Meunier FA (2016) Subdiffractional tracking of internalized molecules reveals heterogeneous motion states of synaptic vesicles. The Journal of Cell Biology, 215, 277-292.
3. Joensuu M, Martínez-Mármol R, Padmanabhan P, Mollazade M and Meunier FA. Single molecule imaging of recycling synaptic vesicles in living nerve terminals by subdiffractional tracking of internalized molecules. Single Molecular Microscopy Neuromethods Series 2019. In review.

3

Dynamics of human stem cell colony proliferation *in vitro*

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With the advent of human Pluripotent Stem Cell (hPSC) technologies, particularly human induced Pluripotent Stem Cells (hiPSCs), there is now a real possibility to engineer countless different types of replacement tissues/cells in a personalized manner, for regenerative

medicine applications. However, for this to become reality we need to understand how human stem cells can be robustly and efficiently induced to take desired fates so that their products can be used clinically in a reliable and safe way.

In this talk I will describe how our group has recently been pioneering experimental and computational strategies to investigate quantitatively hPSC single-cell dynamics, in particular with the aims to clarify the role that cell biological features - like cell cycle progression, cell growth and size, cell migration or cell death – play in controlling overall hPSC proliferation in vitro, and how those features might in turn impact cell differentiation.

Our longer term goal is to clarify through this approach the mechanisms that give rise to efficiency, specificity and tumourigenic potential in hPSC differentiation, to optimize synthetic tissue design on a person by person basis.

4

Cancer associated fibroblast mediated remodelling of the extracellular matrix drives pancreatic cancer progression and metastasis

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Homeostasis of the extracellular matrix (ECM) is critical for correct organ and tissue function. Both the biochemical and biomechanical properties of the ECM contribute to modulating the behaviour of resident cells and are more than just passive bystanders. In tissue diseases such as cancer, the ECM undergoes significant change. These changes, driven by both tumour and stromal cells, feed into the pathological progression of the disease.

Genetically heterogeneous sub-populations of cancer cells are known to exist within tumours. Recent studies have also provided evidence that discrete subtypes of cancer associated fibroblasts (CAFs) also coexist within tumours. These CAFs are known to be able to both promote and restrain disease progression. Our recent work has shown that the specific mutational burden of cancer cells within a tumour, alters the paracrine education of local populations of CAFs within pancreatic ductal adenocarcinoma. These cancer cell educated CAFs have altered matrisomal secretomes, which leads to local remodelling of the tumour ECM. This remodelling underpins the generation of a pro-invasive and pro-metastatic environment for cancer cells, and also leads to alterations in the sensitivity of resident cancer cells to standard-of-care chemotherapy. Targeting this stromal remodelling is showing promise as a novel approach to normalise the tumour stroma and improve therapeutic outcomes.

Understanding at the molecular level how the changing ECM landscape facilitates tumour progression is an important step in the treatment of cancer.

5

Microtubule-Dependent Mechanosensing in Cell Migration and Cancer Invasion

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Metastatic melanoma is currently incurable and available therapies, although effective, result in resistance and recurrence. The majority of deaths are due to metastatic disease, highlighting the need for *'migrastatics'*, therapeutics which act to inhibit invasion. A recent paradigm shift positions the extracellular matrix as a key player in the metastatic cascade. Cell navigation of 3D matrix requires adaptive changes in cell and nuclear shape to fit matrix physical attributes in a process termed mechanosensing. This process incorporates dynamic remodelling of cell matrix adhesions and the cytoskeleton, to facilitate movement through confined spaces, via proteolytic matrix degradation or cell squeezing. Microtubules play a pivotal role in both of these processes. Our data show that the microtubule-binding proteins, CLASPs, are highly over-expressed in metastatic melanoma lines where they regulate the resistance of microtubule mechanical compression during melanoma invasion in 3D collagen matrices. Using high-resolution live-cell microscopy coupled to genetic alteration and substrate microfabrication, we have identified that patient-derived Melanoma cells utilise CLASP1 and CLASP2, for differing functions to drive 3D invasion. We report paralog specific depletion of CLASPs results in strikingly different 3D invasion phenotypes. Crucially, paralog specific depletion of CLASPs ablates the ability to inter-convert between adaptive invasion strategies by interfering with microtubule-dependent functions during 3D-invasion. Furthermore, pan-depletion of CLASPs within 1205Lu melanoma cells results in 3D migration stasis and reduced cell viability following conditions of 3D confinement, which we do not observe in 2D. These findings suggest that CLASPs function in melanoma cells to facilitate biomechanically regulated cellular processes of both invasion and survival in confined environments.

1. Ju R, Stehbens, S.J and Haass N.K. (2018) The Role of Melanoma Cell-Stroma Interaction in Cell Motility, Invasion and Metastasis. Frontiers in Medicine 5: 307.
2. Stehbens, S.J., … and Wittmann., T (2014) CLASPs link focal-adhesion-associated microtubule capture to localized exocytosis and adhesion site turnover. Nature Cell Biology 16(6): 558-570.

6

Multi-modal tracking of tumor metastasis

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Metastases are resistant to multiple therapies and are responsible for the large majority of cancer-related deaths. However, the molecular and cellular mechanisms driving metastasis formation remain to be elucidated and better described in a realistic *in vivo* context. Metastatic seeding is driven by cell-intrinsic and environmental cues, yet the contribution of biomechanics is poorly known. In addition, tumor extracellular vesicles have been shown to shape premetastatic niches and thereby favor metastastic colonization, but detailed information and mechanisms are missing. Moreover, new approaches need to be designed to specifically target and impair tumor progression. In the

past years, we have: 1. Developed a pioneer and unique intravital CLEM (Correlative Light and Electron Microscopy) technology that is perfectly suited for tackling key biological events driving tumor invasion and metastasis formation. 2. Identified new molecular players in the biogenesis and function of tumor exosomes. 3. identified the contribution of hemodynamic forces to tumor cell extravasation preceding metastasis formation.

7

Control of tissue growth and form via the Hippo pathway

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How animal tissues grow to the correct size and shape remains a fundamental unsolved problem in biology. Powerful genetic screens in the fruit fly *Drosophila* have uncovered key genes required for tissues to achieve their correct size and shape, but how these genes function together during development remains a mystery. A key example is the Hippo signalling pathway, whose genetic inactivation causes dramatic tissue overgrowth in *Drosophila*. It is understood that the Hippo pathway functions by inhibiting the action of a key transcriptional co-activator named Yorkie (Yki) in *Drosophila* (or YAP/TAZ in mammals), which normally binds to the Scalloped (Sd) transcription factor (or TEAD1-4 in mammals) to induce target genes such as *bantam* and *myc* to drive cell proliferation. However, it is still not understood how the Hippo pathway is physiologically regulated as tissues grow and then undergo morphogenetic change to achieve their final form. We seek to examine whether and how the Hippo pathway is regulated by fundamental inputs such as animal nutrition (as animal growth requires *nutrient intake*), genetic programs that coordinate cell behaviour through intercellular communication (including *morphogen gradients* and their downstream gene regulatory networks), and tissue mechanics (as *mechanical forces* are instrumental to both growth and morphogenesis). I will present new and unpublished data on the physiological regulation of Hippo signalling by these different inputs.

8

Variants in exons 5 and 6 of *ACTB* cause syndromic thrombocytopenia

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Publish consent withheld

9

SART3 genetic variants are a novel cause of gonadal dysgenesis and intellectual disability

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Human congenital disorders affecting brain and gonadal development are particularly distressing for patients and their families. Very little is known about what causes these rare disorders, and in most cases a genetic diagnosis cannot be made. We have identified three families where children with gonadal dysgenesis (46,XY females), intellectual disability (ID) and agenesis of the corpus callosum carry homozygous variants in the novel disease gene *SART3*. SART3 is important for spliceosome assembly and double-stranded DNA break repair, but a specific role for SART3 in brain and gonad development has not been established; particularly since studies on this gene have been hampered by mouse mutant lethality. We are now working to elucidate the role of *SART3* in embryogenesis. Using RNAi mediated knockdown in *Drosophila* we have found that the fly *SART3* homolog, *Rnp4f*, is required for development, and its knockdown in embryonic neurons causes severe CNS defects leading to lethality. However, knockdown in embryonic glia had no effect on embryogenesis. Interestingly, we found that this gene is also required for fly male fertility, and targeted knockdown in the testis triggers defects in meiosis. Together these data suggest that *SART3* plays a conserved role in neuronal and testicular development. To model the variants found in our patients we have created induced pluripotent stem (iPS) cell lines carrying these mutations. Preliminary results show that these mutant iPS cells fail to efficiently differentiate into neurons, and undergo apoptosis. In addition, using a novel in vitro method for differentiation of human stem cells to embryonic gonadal cells, we found that the patient *SART3* variant does indeed disrupt normal gonadal differentiation, perhaps through disrupted SART3 auto-regulation and defects in spliceosome assembly. This work identifies SART3 as a key regulator of neuronal and gonadal cell types in the developing embryo, and establishes SART3 as a novel cause of developmental disorders affecting these two tissues.

10

Elevated canonical WNT signalling disrupts heart development and may underlie cases of human Heterotaxy

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Congenital heart disease (CHD) can occur in isolation or as part of a syndrome such as Heterotaxy, in which the laterality of internal organs is disrupted. Many cardiovascular abnormalities are associated with low heritability, hindering investigations into the underlying genetic causes of CHD. Heterotaxy, the most highly heritable cardiovascular abnormality, is frequently shown to arise from mutation of the ciliome. Mutation of the X-linked transcription factor ZIC3, a member of the Zic family of transcriptional regulators, is associated with both isolated CHDs and Heterotaxy but the cellular and molecular causes underlying *ZIC3*-associated Heterotaxy remain unknown.

A genetic screen for mutations that affect murine embryogenesis identified a novel null allele of *Zic3*, called katun (*Ka*). *Ka* mutant embryos exhibit Heterotaxy and also incompletely penetrant, partial (posterior) axis duplications and anterior truncation, with the latter two phenotypes redolent of elevated canonical WNT signalling. Previous work has shown that ZIC proteins can interact with TCF proteins to inhibit WNT/β-catenin mediated transcription in model systems. This raises the possibility that dysregulated WNT signalling may underlie some cases of Heterotaxy and CHD.

Using mouse genetics, we found that (i) ZIC3 loss-of-function leads to elevated WNT signalling and (ii) elevated WNT signalling is consistently associated with L-R axis and cardiac situs abnormalities in the absence of pronounced cilia defects. Detailed phenotyping of *Ka* mutant embryos showed that, during gastrulation, prospective definitive endoderm (DE) cells egress from the nascent mesoderm but fail to complete the mesoderm to epithelial transition and do not assemble basal basement membrane. Subsequently, DE cells do not completely clear from the emerging node, disturbing node morphogenesis and, presumably, impairing nodal flow resulting in L-R axis and cardiac situs abnormalities.

This work reveals that a specific dose of WNT activity is required for correct DE formation and is a pre-requisite for L-R axis establishment. Furthermore, it implicates genes involved in WNT signalling and DE formation as novel candidates for human CHD variants.

11

Cilia-dependent and independent mechanisms for left-right symmetry breaking among vertebrates

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Cilia play an essential role in left-right (L-R) symmetry breaking in many of vertebrates including the fish, frog and mouse. In the mouse embryo, for instance, rotational movement of motile cilia at the node generates leftward fluid flow, which is sensed by immotile cilia located at the periphery of the node. I will discuss 1) how motile cilia are correctly formed at the node and generate the unidirectional flow, 2) how the flow is sensed by immotile cilia, possible involvement of ciliary $Ca2^{+}$ in flow sensing, 3) how the fluid flow leads to degradation of *Cerl2/Dand5* mRNA, the readout of flow-induced signal. On the other hand, other animals such as chick do not required cilia for L-R symmetry breaking. I will discuss L-R symmetry breaking in reptiles: whether cilia are involved or not, and how key genes such as Nodal, Lefty and Cerl are expressed and regulated in reptile embryos. At the end, I will speculate evolutionary conservation of the symmetry-breaking mechanism among vertebrates.

12

Early events of articular cartilage differentiation

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Synovial joint is a hallmark feature of skeletal elements developed through the process of endochondral ossification. During endochondral ossification, mesenchymal cells initially condense to form a contiguous unit of cartilage. The first discontinuity in the otherwise uniform cartilage anlagen arises with the formation of the interzone. Cartilage interzone is a band of flattened tightly compacted cells which is molecularly and morphologically distinct from rest of the cartilage in the developing anlagen. It is at the site of the interzone that the cartilage anlagen segments and eventually leads to synovial joint formation. A synovial joint is a complex, membrane enclosed, fluid filled structure where two adjacent skeletal elements articulate. The articulating surface is covered by a cartilage, referred to as articular cartilage. Apart

from the thin articular cartilage layers, the entire cartilage anlagen is ultimately replaced by bone (transient cartilage). Interestingly, cartilage cells of the anlagen differentiate simultaneously into both articular and transient cartilage fates, right next to each other, under the influence and interplay of BMP and WNT signaling. My talk would focus on the earliest events of interzone specification and eventually throw light on how two different cartilage fates are attained simultaneously from a single set of progenitor.

13

No title provided

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No content provided.

14

Nervous system regionalization involves axial allocation prior to neural differentiation

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Neural induction in vertebrates generates a central nervous system that extends the rostral-caudal length of the body. The prevailing view is that neural cells are initially induced with anterior (forebrain) identity; caudalising signals then convert a proportion to posterior fates (spinal cord). To test this model, we used chromatin accessibility to define how cells adopt region-specific neural fates. Together with genetic and biochemical perturbations this identified a developmental time window in which genome-wide chromatin remodeling events preconfigure epiblast cells for neural induction. Contrary to the established model, this revealed that cells commit to a regional identity before acquiring neural identity. This “primary regionalization” allocates cells to anterior or posterior regions of the nervous system, explaining how cranial and spinal neurons are generated at appropriate axial positions. These findings prompt a revision to models of neural induction and support the proposed dual evolutionary origin of the vertebrate central nervous system.

15

Make do and make new: how zebrafish rapidly regenerates CNS injury

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Zebrafish have a remarkable capacity to regenerate following spinal cord injury. While many factors controlling neurogenesis have been identified, the cellular mechanisms regulating global neural regeneration are largely unknown. We used in vivo imaging to pin-point specific cells and signals that control CNS regeneration in zebrafish. Surprisingly, we identified two temporally and mechanistically distinct waves of cellular regeneration in the spinal cord. The initial wave of regeneration relying on cell migration of neural precursors to the lesion site, enabling rapid functional recovery, and the activation of quiescent neural stem and progenitor cells (NCSs). This is then followed by the second wave of regeneration which largely driven by regenerative neurogenesis. Neurogenesis compensates for both the loss of tissue at injury site as well as the cells depleted from proximal areas due to early migration. Furthermore, we find that inflammation and leukocytes play a critical role in differentially regulating cell recruitment and activation of NSCs after injury. The two waves of regeneration demonstrate how the zebrafish are able to rapidly regain motor function after complete ablation, but also gradually replenish lost tissue over time. Taken together, our data suggest that inflammation driven recruitment of neural precursors play an unanticipated role in neural repair.

16

Lineage specific requirement for RNA and DNA unwinding during lymphatic endothelial cell proliferation.

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Developing tissues undergo timely regulated cell divisions in order to form proportional organs. Cell division requires DNA and RNA synthesis, processes dependent on general cellular machinery. Yet, during development cell proliferation is highly regulated resulting in differently sized tissues. To date little is known about how these general factors guide these processes in a tissue-specific manner. Here we uncovered a new role for an RNA helicase and Topoisomerase during lymphatic development.

In a forward genetic screen to identify novel factors required for lymphangiogenesis, the *dead-box helicase 21* (*ddx21*) and *topoisomerase 3a* (*top3a)* have been identified, due to a loss of the thoracic duct. Phenotypic analysis of the lymphatic network using lymphatic transgenic

marker strains, revealed decreased numbers of lymphatic progenitors. The lymphatic phenotypes resulted from underlying defects in cell cycle progression of endothelial cells. Interestingly, rescue experiments for *ddx21* or *top3a* mutants with p53 knockdown completely restored lymphangiogenesis. Suggesting that cell cycle phenotypes induce p53-mediated cell death or senescence. As *vegfc/vegfr3* are the key growth factors regulating lymphatic endothelial cell proliferation, we examined if *ddx21* or *top3a* act in *vegfc/vegfr3* dependent manner. We found that upon ectopic overexpression of Vegfc, the cell proliferation is indeed dependent on *ddx21* or *top3a* identifying them as necessary downstream components of this signalling pathway.

These mutants offer unique insights into how an expansion of a new vasculature from a limited pool of progenitors is regulated. This study will further expand our knowledge of how general factors involved in basic cell machinery act in a tissue-specific manner.

17

Structural studies of phase-separating gene regulatory proteins and their role in membraneless organelles

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Paraspeckles are subnuclear bodies that form when a specific group of nuclear RNA binding proteins are brought into close proximity by binding the long noncoding RNA, NEAT1, through a process of liquid-liquid phase condensation. This process of paraspeckle formation is coordinated and dynamic, with a high nuclear concentration of soluble paraspeckle proteins 'poised' to condense onto NEAT1 as soon as it is transcribed.

Proteins that build paraspeckles are enriched in a type of intrinsically disordered domain termed the 'prion like domain' including FUS and HNRNPA1. We have been carrying out studies in vitro to characterise the biophysical properties of proteins required for paraspeckle formation in order to learn more about mechanisms involved. In addition to gel- and liquid-formation assays and crystallographic studies, we have also been using small-angle neutron scattering to seek structural detail on the essential paraspeckle protein HNRNPK which forms a variety of aggregates, droplets and fibrils when made recombinantly. These insights have helped us build a better understanding of the dynamics and molecular structure of paraspeckle components.

18

Inflammasome assembly and function

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Pattern recognition receptors (PRR) of the innate immune system mediate the first line of defence against infections and cellular stress signals. The inflammasome complexes initiated by a subset of PRRs form a platform for activation of procaspase-1. Active caspase-1 cleaves gasdermin D leading to membrane pore formation and rapid lytic death, and also processes precursors of inflammatory cytokines IL-1b and IL-18. The central role of IL-1b in inflammatory pathology has led to great interest in inflammasome regulation. PRRs such as NLRP3 that responds to diverse cellular stresses, and AIM2 that responds to cytosolic DNA, initiate recruitment of the adapter molecule ASC. Within minutes, all the ASC in the cell can polymerise into a single "speck" via formation of pyrin domain (PYD) filaments, and condensation of these via caspase activation domain (CARD) interactions. ASC in turn recruits procaspase-1 via CARD-CARD interactions. We demonstrated that there is a more complex web of caspase activation by inflammasomes, and procaspase-8 is recruited to cap the ASC PYD filament. Its activation can lead to inflammasome-dependent apoptosis in cell types not expressing caspase-1. In current work we are closely examining the relative timing of events in inflammasome activation, in order to elucidate the essential structural features for caspase activation.

19

Probing the self-association of RNA binding proteins and influence of nucleic acids

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Stress granules are important for cell survival, forming a protective mechanism for mRNA during periods of stress. Aberrant stress granule function is associated with neurodegenerative diseases in particular the motor neuron disease amyotrophic lateral sclerosis (ALS) and the dementia frontotemporal lobar degeneration (FTLD). Stress granules are a prominent example of a membraneless organelle which is formed, in part, by liquid-liquid phase separation. The fundamental principles by which liquid-liquid phase separation occurs is an area of active investigation. We are investigating the role of nucleic acids in modulating the self-association of RNA binding proteins present in stress granules using biophysical methods. These include the stress granule marker protein, TIA-1 and the prototypical phase separating protein Fused in Sarcoma (FUS) showing the differences in mechanism by which these deceptively similar RNA binding proteins can self-associate with potential implications for stress granule dynamics.

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Molecular basis of caveolar membrane coat complex assembly

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Caveolae are plasma membrane invaginations involved in multiple intracellular processes such as endocytosis, cell migration, vesicular transport and membrane tension sensing. Caveolae are known to protect cells under mechanical stress by providing an extra membrane

reservoir through caveolar flattening thus relieving mechanical tension at the plasma membrane. Their formation depends upon multiple interactions between membrane-embedded caveolins, lipids and cytosolic cavin proteins. Of the four cavin family members, only cavin1 is strictly required for caveola formation. The cavin proteins have a distinctive domain architecture possessing N- and C-terminal helical regions alternating with three disordered domains (DR1, DR2, DR3). A minimal N-terminal domain of the cavins, termed helical region 1 (HR1) is required and sufficient for cavin proteins homo- and hetero-oligomerization. Crystal structures of the mouse cavin1 and zebrafish cavin4a HR1 domains reveal highly conserved extended trimeric coiled-coil architectures, with inter subunit interactions that determine the specificity of cavin-cavin interactions (1). The C-terminal helical region contains an eleven residue repeat sequence exclusive to cavin1 (Undecad of cavin1 – UC1 domain) that is essential for its localisation to caveolae (2). The HR1 and UC1 domains possess a basic surface exposed patch of amino acids that interacts with specific membrane lipids such as poly-phosphoinositides and phosphatidylserine. The UC1 domain of cavin1 plays a crucial role in modulating the caveolar disassembly response in cells under mechanical stress (2). The HR1 and UC1 domains of cavin1 co-ordinate with the disordered domains to form large oligomers via a novel mechanism that promote membrane remodelling. Overall cavins possess an extensive membrane sculpting activity that results in a formation of membrane tubules *in vitro*. Lastly, we reveal that the rod like coiled-coil domain architecture of cavins form a filamentous coat on the surface of caveolae.

1. Kovtun O, Tillu VA, Jung W, Leneva N, Ariotti N, Chaudhary N, Mandyam RA, Ferguson C, Morgan GP, Johnston WA, Harrop SJ, Alexandrov K, Parton RG, Collins BM. Structural insights into the organization of the cavin membrane coat complex. Dev Cell. 2014 Nov 24;31(4):405-19.
2. Tillu VA, Lim YW, Kovtun O, Mureev S, Ferguson C, Bastiani M, McMahon KA, Lo HP, Hall TE, Alexandrov K,Collins BM, Parton RG (2018). A variable undecad repeat domain in cavin1 regulates caveola formation and stability. EMBO reports 19, e45775.

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Nuclear filamentous actin functions in the replication stress response

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Replication stress is the main driver of genome instability in early cancer development and is recognized as a hallmark of cancer. Actin is a cytoskeletal protein that polymerizes from monomeric to filamentous form (F-actin) to provide cells with mechanical support, transport pathways, and a driving force for movement. While actin is traditionally considered a cytoplasmic protein, nuclear actin polymerization was recently identified to contribute to double strand break repair.

Here we used a suite of imaging tools to identify that nuclear F-actin plays a prominent role in the replication stress response in human cells. Consistent with replication stress, live and fixed imaging assays revealed that pharmacological inhibition of actin polymerization resulted in S-phase elongation, altered replication dynamics, and chromosome segregation errors. Using live and super resolution imaging we also found that replication stress resulted in ATR, IPMK and mTOR-dependent nuclear F-actin, which altered nuclear architecture by promoting nuclear ellipticity and expanding the nuclear volume to counteract membrane deformation induced by replication stress. Additionally, we developed novel analytical tools for live cell-imaging that revealed nuclear F-actin promoted the mobility of stalled replication foci within the nuclear volume. This incresed motility included the directed movement of stalled replication foci along actin fibres to the nuclear periphery. Inhibiting ATR, mTOR or actin polymerization, suppressed F-actin dependent alteration of nuclear architecture in response to replication stress and prevented the restart of stalled replication forks. Finally, inhibiting actin polymerization slowed resolution of ATR-dependent DNA damage response signalling in response to replication stress.

Cumulatively, these data reveal a novel pathway regulated by ATR and mTOR, where F-actin dependent forces shape nuclear architecture in response to replication stress to maintain genome stability. A current pre-print of the manuscript describing these data is availble on bioRxiv at: https://doi.org/10.1101/451708

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Mechanical cell competition: mechanisms and implications for epithelial biology

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Cell competition is a form of cell interaction that allows fitter cells (called 'winners') to kill less fit cells (called 'losers') and colonize tissues in their stead. The goal of the research we carry out in my group is to understand cell competition, its mechanism of action and how it modulates cell colonization in tissues and organs.

Our group has recently discovered that mammalian cells can compete using mechanical insults. In mechanical cell competition, loser cells have intrinsic hypersensitivity to cell density and crowding from winner cells kills them by apoptosis. A key player in mechanical cell competition is p53, which we find to be necessary and sufficient for cells to become hypersensitive to cell compaction and behave as mechanical losers. Since p53 is upregulated by a wealth of cellular stress conditions, this suggests that mechanical cell competition may be a widespread mechanism to eliminate damaged cells from tissues. Our recent unpublished work has identified the first physiological context where mechanical cell competition may play a role, i.e. in collective cell migration and epithelial wound healing.

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Optimizing drug combinations for cancer treatment through integrative network modelling

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The advent of targeted drugs, e.g. kinase inhibitors, has revolutionized cancer treatment; but resistance to these drugs as monotherapies remains a major problem preventing their full clinical impact. Resistance is caused in part by the ability of cancer signalling networks to dynamically adapt and rewire in response to single-drug treatment, ultimately evading the drug effect. Consequently, combination therapies are being actively investigated to combat single-agent resistance. However, given the vast number of possible target (drug) combinations but clinical trials are slow and expensive, how can we rationally predict and prioritize optimal drug combinations? Further, due to the plasticity of cancer signalling networks increasing evidence suggests that sequential drug combinations, where the first drug 'primes' the network for effective inhibition by the second drug, may be superior than concurrent combinations where both are administered simultaneously. Yet, under which contexts such sequential combination therapies may be favoured is poorly understood.

I will describe how we try to integrate computational network modelling with experimental analysis to address these important questions. This integrative approach has enabled us to predict and prioritise synergistic drug combinations targeting a multi-pathway RTK signalling network in triple-negative breast cancer (TNBC), an aggressive form of breast cancer with no current targeted treatment. Analysis of clinical data and patient-specific model simulations further allowed us to stratify the patients for optimal benefit from the combinatorial treatment. I will also present new results where we have developed a computational framework to identify, among thousands of possible network topologies, those conferring better sensitivity to sequential over concurrent drug combinations. This analysis yields a design table highlighting a finite set of circuits susceptible to sequential treatments that are enriched with feedback loops, which provide a useful framework to guide future application of sequential targeted therapies.

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Heterogeneity in single-cell apoptotic signalling promotes chemoresistance in neuroblastoma

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The emergence of chemoresistance is a major clinical problem for almost all tumour types where chemotherapy remains the frontline treatment regime. A number of theories have been proposed to describe the single-cell dynamics of chemotherapy response and the eventual expansion of resistant clones. These usually involve the selection of an existing resistant stem cell population, a low frequency somatic mutation or the de novo acquisition of new somatic mutations. In contrast to these predominantly genetic mechanisms, we have now utilised mathematical modelling and longitudinal single-cell imaging to demonstrate that the survival and propagation of a single cell clone can arise merely through the inherently noisy process of gene expression and the non-linear behaviour of signalling pathways.

High-risk neuroblastoma is an aggressive, childhood tumour with no clinically successful targeted therapies and high rates of chemoresistance. Approximately 15% of high-risk neuroblastoma patients do not respond to treatment with chemotherapy, and a further 40-50% of patients will relapse following an initial response. We have previously demonstrated that in silico, patient-specific modelling of apoptotic signalling can stratify neuroblastoma patient cohorts and provide robust biomarkers of patient survival (Fey et al., 2015, Science Signaling). We now show that application of this model to single-cell populations also predicts the presence of a potentially transient chemoresistant cell population, which cannot activate a sufficient drug-induced signalling response to reach an in-built apoptotic threshold.

Using kinase activity biosensors and high-throughput imaging we have now confirmed the existence of these innately chemoresistant neuroblastoma cells. We also demonstrate that rationalised therapeutic strategies aimed at lowering this apoptotic threshold can overcome this stochastic single-cell chemoresistance in both cell line and PDX models of primary and relapsed neuroblastoma.

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Mechanisms of dysregulated cytokine receptor signalling in cancer

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Cytokine receptor signalling regulates a diverse range of cellular and physiological properties including cell proliferation, differentiation, inflammation, metabolism, and growth. The major signalling pathway regulated by cytokine receptors is the JAK-STAT pathway and dysregulation of this signalling is common in cancer, particularly in haematological cancers. Our previous studies centred on understanding how cytokine binding transmitted a signal through the transmembrane domain to the associated JAK kinases. These studies highlighted

the critical structural nature of the transmembrane and juxtamembrane regions of cytokine receptors. Mutations in the interleukin-7 receptor alpha chain (IL-7Ra), thymic stromal lymphopoietin protein receptor (TSLPR), and thrombopoietin receptor (TPOR/MPL) are common in the transmembrane and juxtamembrane region in leukaemia and myeloproliferative neoplasms. We have recently defined the mechanism of how mutations in the IL-7Ra that introduce positive charged amino acids at the extracellular transmembrane boundary contribute to enhanced JAK-STAT signalling and T-cell leukaemia. We have also investigated the mechanism of different clinical activating mutants in the transmembrane domain of TPOR and find differential strengths of STAT and Erk1/2 signalling. Negative regulation of JAK signalling is also an important feature regulating cytokine receptor signalling with mutations in LNK (a negative regulator of JAK2) also identified in proliferative haematological disorders. We have investigated the molecular interactions of LNK with JAK2 and TPOR and their role in regulating signalling. In addition, we have studied cytokine receptor oligomerisation and membrane dynamics at the single molecule level using single-step photobleaching and live-cell single-particle tracking with photoactivated localization microscopy (spt-PALM), as this new knowledge is critical for our understanding of the mechanism of receptor activation and regulation at the cell membrane. Our studies will provide novel insights into cytokine receptor activation and signalling, and an increased understanding of diseases caused by dysregulated JAK-STAT signalling.

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Pre-assembled GPCR signalling complexes mediate distinct cellular responses to ultra-low ligand concentrations

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G protein–coupled receptors (GPCRs) are the largest class of cell surface signalling proteins, participate in nearly all physiological processes, and are the targets of 30% of marketed drugs. Typically, nanomolar to micromolar concentrations of ligand are used to activate GPCRs in experimental systems. We detected GPCR responses to a wide range of ligand concentrations, from attomolar to millimolar, by measuring GPCR-stimulated production of cAMP with high spatial and temporal resolution. Mathematical modelling showed that femtomolar concentrations of ligand activated, on average, 40% of the cells in a population provided that a cell was activated by one to two binding events. Furthermore, activation of the endogenous beta2-adrenoceptor and muscarinic acetylcholine M3 receptor by femtomolar concentrations of ligand in cell lines and human cardiac fibroblasts caused sustained increases in nuclear translocation of extracellular signal–regulated kinase (ERK) and cytosolic protein kinase C (PKC) activity, respectively. These responses were spatially and temporally distinct from those that occurred in response to higher concentrations of ligand and resulted in a distinct cellular proteomic profile. This highly sensitive signalling depended on the GPCRs forming pre-assembled, higher-order signalling complexes at the plasma membrane. Recognising that GPCRs respond to ultra-low concentrations of neurotransmitters and hormones challenges established paradigms of drug action and provides a previously unappreciated aspect of GPCR activation that is quite distinct from that typically observed with higher ligand concentrations.

1. Civciristov et al. 2018 Science Signaling 11:eaan1188

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Inflammasome signalling in the host defense against infectious disease

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Recognition of pathogens by the host cell is of paramount importance for the initiation of an immune response and clearance of the pathogen. Intracellular and cytosolic bacteria must secure entry into the host cytoplasm in order to engage activation of cytosolic immune sensors and the inflammasome. However, microbial ligands from extracellular pathogens are also detected by cytosolic innate immune sensors. We discuss our latest findings on the role of inflammasomes and innate immune regulatory proteins in the recognition of bacteria. We also highlight novel anti-microbial host defense system mediating cytosolic release of bacterial ligands for sensing by pattern-recognition receptors and inflammasomes.

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Activation of mitochondrial apoptotic signalling to trigger inflammation

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Apoptotic cell death is classically regarded as immunologically silent. The mitochondrial or “intrinsic” apoptotic pathway is an evolutionarily conserved, BCL-2 family regulated, process that promotes the death and phagocytic clearance of stressed, damaged or infected cells. BAX and BAK-mediated mitochondrial membrane damage is essential for mitochondrial apoptosis, and is required for the downstream activation of apoptotic effector caspase activity. Consequently, the study of BAX/BAK regulation in cancer cells has resulted in the development of rationally designed BH3-mimetics compounds that trigger BAX/BAK to induce cancer cell death. However, research into the consequences of activating mitochondrial apoptosis in innate immune cells, such as macrophages, is less well studied. Using a range of targeted BH3-mimetic compounds, and other cancer chemotherapeutics, we have discovered the key pro-survival BCL-2 family members required to prevent spontaneous BAX/BAK apoptotic cell death in macrophages. Unexpectedly, we also observed that BAX/BAK signaling in macrophages triggers the activation of inflammasome-associated caspase, caspase-1, resulting in the activation and release of the pro-inflammatory cytokine, interleukin-1b (IL-1b). Using a panel of mice deficient in apoptotic signaling machinery, inflammasome sensor proteins or inflammatory caspases, we have defined a novel pathway by which BAX/BAK can cause inflammation via NLRP3 inflammasome activation. We believe this molecular pathway may underpin observations linking chemotherapeutic and cellular stress responses to inflammasome and IL-1b-driven inflammation.

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Viral MLKL homologs subvert necroptotic cell death by sequestering cellular RIPK3

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Cell death via the necroptosis pathway has been implicated in many human pathologies and is widely considered to have evolved as an innate immunity mechanism. Recent data have revealed rapid evolution of the pathway, with important mechanistic differences apparent between mouse and human pathways. At the heart of these interspecies differences are co-evolved signaling cassettes comprising the protein kinase, RIPK3, and the terminal effector in the necroptosis pathway, the pseudokinase MLKL.

We propose that pathogen-encoded proteins targeting the pathway, such as orthopoxvirus MLKL (vMLKL) orthologs, have driven this rapid evolutionary divergence. Expression of vMLKL in human and mouse cell lines led to abrogation of necroptotic cell death and hallmarks of necroptosis, such as cellular MLKL phosphorylation and cytokine release, without impacting apoptosis. vMLKL comprises solely a pseudokinase domain and lacks the N-terminal executioner domain present in cellular MLKL. The befits its function as a mimic of host MLKL to inhibit necroptosis by binding the RIPK3 kinase domain to thwart both necrosome assembly and engagement and phosphorylation of host MLKL. These data support the idea that the ancestral origins of necroptosis lie in host defense with subsequent evolutionary loss of necroptotic effectors from a subset of animal genomes having been driven by pathogen subversion of the pathway.

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Noncanonical inflammasome signalling elicits gasdermin-D dependent neutrophil extracellular traps

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Neutrophil extrusion of neutrophil extracellular traps (NETs) and concomitant cell death (NETosis) provides host defense against extracellular pathogens, while macrophage death by pyroptosis enables defense against intracellular pathogens. Here we report the surprising discovery that GSDMD connects these cell death modalities. We show that neutrophil exposure to cytosolic lipopolysaccharide or cytosolic Gram-negative bacteria (*Salmonella ΔsifA*, *C. rodentium*) activates non-canonical (caspase-4/11) inflammasome signaling and triggers GSDMD-dependent neutrophil pyroptosis. Remarkably, GSDMD-dependent death induces neutrophils to extrude antimicrobial NETs. Caspase-11 and GSDMD are required for neutrophil plasma membrane rupture during the final stage of NET extrusion. Unexpectedly, caspase-11 and GSDMD are also required for early features of NETosis, including nuclear delobulation and DNA expansion; this is mediated by the coordinate actions of caspase-11 and GSDMD in mediating nuclear membrane permeabilization and histone degradation. *In vivo* application of DNase I to dissolve NETs during murine *Salmonella ΔsifA* challenge increases bacterial burden in wild-type but not *Casp11*$^{-/-}$and *Gsdmd*$^{-/-}$mice. We thus reveal that neutrophils deploy a novel inflammasome- and GSDMD-dependent NETosis mechanism against cytosolic intruders, to ensnare bacteria upon the NET and prevent cytosolic infection. The discovery of caspase-11-induced NET extrusion defines a new nuclear function for GSDMD, with broad relevance for cell death, as well as infectious, inflammatory and autoimmune diseases.

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Mitochondrial dynamics control macrophage inflammatory and antimicrobial responses during bacterial infections

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Although mitochondria are well-known for their role in energy production, these organelles also have a number of specialized roles in immune responses. Emerging evidence suggests that mitochondrial dynamics (fusion/fission) also influence both cell metabolism and inflammation. Here we show that this process plays an essential role in macrophage responses against bacterial pathogens.

Using both pharmacological and genetic approaches, we show that Toll-like receptor (TLR)-mediated activation of macrophages results in increased mitochondrial fission, and that this response is functionally linked to both inflammatory outputs and host-protective antimicrobial responses in these cells. We also demonstrate key roles for Dynamin-1-like protein (Dnm1l/Drp1) and mitofusin 1 (Mfn1) in mitochondrial dynamics in the context of these responses in macrophages. Furthermore, we find that infection of mouse macrophages with the Gram-negative bacterial pathogen *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) delivers a robust TLR response but does not result in increased mitochondrial fission, suggesting that this pathogen may antagonize this cellular response as a host evasion strategy. Accordingly, skewing towards mitochondrial fission by silencing *Mfn1* enhances clearance of *S.* Typhimurium by mouse macrophages. Mfn1 is active in its deacetylated form, the generation of which is catalysed by the cytoplasmic enzyme histone deacetylase (HDAC) 6. We show that HDAC6 promotes mitochondrial fusion in macrophages and constrains the capacity of these cells to clear intracellular *S.* Typhimurium. Genetic (knock-out or knock-down) or pharmacological (tubastatin A) targeting of HDAC6 promotes mitochondrial fission in *S.* Typhimurium-infected macrophages and reduces bacterial loads within these cells. Furthermore, treating macrophages with mitochondrial fusion-promoting compounds (M1 and/or mdivi1), as well as silencing *Drp1*, antagonized the antimicrobial effect of tubastatin A, confirming the key role of mitochondrial fission in macrophage antimicrobial responses. Mechanistically, mitochondrial fission enhances several antimicrobial pathways in macrophages, including mitochondrial reactive oxygen species generation, and is efficient against a number of different bacterial pathogens including multi-drug-resistant *S.* Typhimurium, *M. tuberculosis* and uropathogenic *Escherichia coli*. Finally, the antimicrobial effect of mitochondrial fission was confirmed *in vivo*; Tubastatin A dramatically reduced bacterial dissemination to the spleen and liver after challenge of C57BL/6 mice with *S.* Typhimurium.

Collectively, our findings demonstrate a central role for mitochondrial dynamics in functional responses of macrophages to bacterial challenge. They also suggest that, through the modulation of mitochondrial dynamics and the reprogramming of innate immune host defence pathways, HDAC6 inhibitors may have applications for the treatment of acute and/or chronic bacterial infections.

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The EMBO Keynote Lecture - Guided Mechanochemical Self-Organization

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Turing systems have been invoked to explain examples of pattern formation in development. On the one hand, spontaneous pattern formation in Turing systems relies on feedback to amplify the developing pattern. However, in cells and tissues patterns generally do not form spontaneously, but are under control of upstream pathways that provide molecular guiding cues. The relationship between guiding cues and feedback amplification in controlled biological pattern formation remains unclear. On the other hand, the feedback structures that enable pattern formation can rely on an intertwined coupling between mechanical events such as force generation and flows, and regulation. The physical basis that underlies such types of pattern formation are not well understood. In this talk I will report on our recent findings on how the *C. elegans* zygote undergoes polarization, and on how the red flour beetle *Tribolium castaneum* undergoes gastrulation. In both cases I will highlight the role of force generation and flows, how such mechanical processes contribute to pattern formation. I will also discuss how such modules of mechanochemical feedback are guided by external and upstream cues, to achieve the appropriate structure at the right place and the right time.

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Bacterial infection and cell intrinsic immunity

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Many bacterial pathogens have acquired the capacity to attach to and/or replicate inside human cells by avoiding cell intrinsic innate immune pathways. The subversion of host cell signaling by pathogens frequently depends on the ability to transport virulence proteins, called effector proteins, into the infected cell via specialised protein secretion systems. We work on a range of virulence effectors from pathogenic bacteria that interfere with host innate immune signalling pathways and block inflammation and cell death. In this way effector proteins can be used as tools to understand the innate responses important for control of the pathogen. We recently defined EspL as a T3SS cysteine protease effector that degrades the signalling proteins, RIPK1 and RIPK3 in the RHIM domain thereby inhibiting necroptosis and inflammation. More recently, we have worked to identify host substrates of EspL homologues found in *Shigella*, termed OspD2 and OspD3, which are also cysteine proteases. Using the proteomic approach, ProteoMap, we discovered that both OspD2 and OspD3 targeted

components of the type I and type III interferon signalling pathway and blocked type I interferon signalling. EspL from EPEC also had activity in this pathway. This suggests that type I and/or type III interferon induced proteins mediate host defense against *Shigella* and EPEC infection. The activities of EspL and its homologues define a new family of bacterial effector proteins found in a range of bacteria and reveal the mechanisms by which gastrointestinal pathogens directly target inflammatory and cell death signalling pathways.

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Only Regulation of NMDA receptor trafficking by sorting nexin 27

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NMDA receptors (NMDARs) are ionotropic glutamate receptors that mediate the flux of calcium into the postsynaptic compartment that underpins multiple forms of synaptic plasticity, learning and memory. Mice lacking functional NMDARs exhibit no long-term potentiation (LTP) in the hippocampus and display impairment in spatial memory. The majority of synaptic NMDAR currents is mediated by the GluN2A-containing heteromeric NMDARs. Although the cytoplasmic C-terminal tail of GluN2A is crucial for function, the molecular mechanisms underlying activity-dependent trafficking of GluN2A remains elusive. Recently, we identified a member of the sorting nexin (SNX) family of proteins, SNX27, as a GluN2A C-terminal interacting partner. Mutations of *SNX2*7 gene is linked to intellectual disability, epilepsy and growth retardation. Mice lacking SNX27 display impairments in glutamatergic neurotransmission and LTP, as well as deficits in learning and memory. Here we report that SNX27 plays an important role in regulating the basal and activity-dependent forward trafficking of GluN2A-containing NMDARs in primary hippocampal neurons. SNX27 directly binds to GluN2A C-terminal tail through its postsynaptic density 95/discs large/zona occludens (PDZ) domain. Interestingly, their interaction can be modulated by the phosphorylation of GluN2A Ser-1459 residue by the Ca^{2+}/calmodulin-dependent kinase II (CaMKII), which is enhanced by glycine stimulation that mimics LTP *in vitro*. Overexpression of GluN2A S1459A phosphorylation-deficient mutant significantly reduces GluN2A surface expression and activity-dependent insertion of NMDARs, whereas GluN2A S1459D phospho-mimetic mutant enhances basal GluN2A surface expression and occludes the glycine-induced enrichment of GluN2A in hippocampal neurons. Altogether, our study provides the first molecular link between GluN2A, SNX27 and CaMKII in controlling NMDAR surface expression in mammalian central neurons.

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Regulation of Toll-like Receptor Signalling in Inflammation: Roles for transmembrane adaptors and SRC family kinases.

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Toll-like receptors (TLRs) are activated by pathogen or danger signatures for signalling and initiation of innate immune or inflammatory responses. TLR-induced signalling pathways program the release of inflammatory cytokines and other mediators under the influence of signalling adaptors, cross-talk receptors, GTPases, kinases, phospholipids and membrane compartments. As a direct, non-TIR binding partner of TLRs, the transmembrane adaptor SCIMP scaffolds Src kinases that phosphorylate TLRs and drive selective, proinflammatory cytokine outputs. The expression, phosphorylation and trafficking of SCIMP itself are regulated within pathogen-activated environments to orchestrate TLR regulation, coupled with other cell surface proteins. The roles of SCIMP as a member of the transmembrane TRAP adaptor family will be presented along with its potential for regulating inflammation in homeostasis and disease.

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New Participants and Pathways in Immune Receptor Trafficking.

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Expression of immune receptors at the cell surface is a key determinant of immunity. Understanding the molecular mechanisms that govern receptor expression patterns will enable exploitation of receptors in settings of immunotherapy or vaccination. Here, we have aimed to analyse the molecular mechanisms and immune consequences of immune receptor trafficking. One mechanism by which receptor expression is controlled is ubiquitination. Ubiquitination regulates the cell surface display of MHC II (major antigen presenting molecule) and CD86 (co-stimulatory molecule). First, we have identified the ubiquitin codes (ubiquitin chain linkage types) associated with MHC II isolated from primary murine immune cells. Second, we have identified a new role for MHC II ubiquitination in the regulation of dendritic cell homeostasis that involves a surprising intersection between innate and adaptive immunity. Third, we have performed a genome-wide CRISPR/Cas9 screen to identify molecular participants in ubiquitin-mediated immune trafficking. We identified ubiquitin like 3 (Ubl3), a previously uncharacterised mammalian protein, as a novel regulator of MHC II and CD86 trafficking. We describe new roles for Ubl3 in dendritic cells, B cells and thymic epithelium with *Ubl3*-deficient mice. Finally, we have analysed the trafficking of dendritic cell receptors that are of significant interest for antibody-targeted vaccination, including C-type lectin DEC-205. We have conducted CRISPR/Cas9 screens to identify new genes that regulate both the expression and the internalisation of DEC-205 from the cell surface. In summary, our analysis provides significant advances to understanding how immune receptors are trafficked inside cells with important consequences for immunity.

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Functional assays for determining the pathogenicity of *ATP7A* variants using primary fibroblasts and patient derived induced pluripotent stem cells.

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Mutations in the *ATP7A* gene cause X-linked hereditary distal motor neuropathy (dHMNX). To date, p.T994I and p.P1386S in this copper (Cu) transporting ATPase are the only confirmed mutations causing dHMNX. Next generation sequencing is increasing the rapid detection of variants in genes known to cause disease, however the absence and size of additional families, make it challenging to determine the pathogenic or benign status of these identified variants of unknown significance (VUS). We have recently identified new variants in *ATP7A* in patients with progressive peripheral neuropathy, suggesting further genetic heterogeneity of dHMNX. Two of these new variants, p.E840V and p.M1311V, are located at highly conserved amino acids within domains of ATP7A that are critical for the catalytic cycle of the copper transporter. We have also identified three additional variants (p.R703H, p.Y760C and p.A768G) that cluster within close proximity of the known dHMNX mutations p.T994I and p.P1386S in the 3D structure of ATP7A, strongly suggesting these variants may cause disease by sharing a common pathomechanism.

Our investigations using patient fibroblasts and an Atp7a conditional knock in mouse model for dHMNX have shown defective retrograde trafficking of mutant ATP7A leading to Cu dysregulation. We have systematically assessed this phenotype on patient fibroblasts. Our results indicate altered Cu induced trafficking of ATP7A can be used to assess pathogenicity of newly identified variants in *ATP7A* and establishes an important functional assay for validating the pathogenic status of VUS in those cases where genetic evidence is limited.

To investigate how defective ATP7A trafficking leads to degeneration of motor neurons we have generated a line of induced pluripotent stem cells by re-programming fibroblasts from a dHMNX patient with the ATP7A p.T994I mutation. The patient motor neurons will be an ideal neuronal system to model axonal degeneration and to investigate how defective trafficking of ATP7A leads to motor neuron death in dHMNX and other neurodegenerative diseases in which Cu dysregulation occurs.

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Different insults to long bone growth lead to distinct scaling effects in developing mice

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How do organs achieve species-specific proportions despite remarkable differences in absolute size? The regulation of organ size and internal proportions during development is crucial for the proper functioning of our bodies, and yet it remains as one of the outstanding mysteries in developmental biology [1]. Limbs are well suited to study this topic, as they are dispensable and can be extensively manipulated without affecting embryonic viability. Moreover, by altering growth unilaterally, it is possible to maintain an internal control, which allows for intra-individual comparisons. We have developed new mouse models that enable induction of transient growth insults in the left but not the right hindlimb [2]. With this approach, we have recently shown that mosaic cell arrest in the left embryonic growth plate (the region that drives long-bone growth) does not lead to changes in bone length or width, due to the activation of local and systemic compensatory mechanisms [3]. We have now performed similar experiments with different insults: mosaic cell death in the whole limb mesenchyme, and overexpression of Connective Tissue Growth Factor (CTGF) in the growth plate. In both models, growth plate architecture is transiently disrupted and a severe limb asymmetry is generated, revealing that the efficacy of compensatory mechanisms depends on the insult type. Moreover, while internal bone proportions are preserved in the cell-death and cell-arrest models, the scaling mechanism of CTGF-overexpressing bones is disrupted. These models therefore provide a unique opportunity to study organ growth and scaling. I will discuss the extrinsic and intrinsic mechanisms potentially underlying the observed phenotypes.

1. A. Rosello-Diez and A. L. Joyner. Regulation of Long Bone Growth in Vertebrates; It Is Time to Catch Up. Endocr Rev 36, 646-680 (2015).
2. A. Rosello-Diez, D. Stephen and A. L. Joyner. Altered paracrine signaling from the injured knee joint impairs postnatal long bone growth. Elife 6, (2017).
3. A. Rosello-Diez, L. Madisen, S. Bastide, H. Zeng and A. L. Joyner. Cell-nonautonomous local and systemic responses to cell arrest enable long-bone catch-up growth in developing mice. PLoS Biol 16, e2005086 (2018).

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A GATA2 bound enhancer responsible for regulation of Prox1 and control of lymphatic endothelial cell identity.

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Transcriptional enhancer elements are responsible for orchestrating the temporal and spatial control over gene expression that is crucial for programming cell identity during development. Here, we describe a novel enhancer element important for regulating *Prox1* expression in lymphatic endothelial cells. This evolutionarily conserved enhancer is bound by key lymphatic transcriptional regulators including GATA2, FOXC2, NFATC1 and PROX1. CRISPR-Cas9 genome editing of this enhancer element revealed that deletion of only 5 nucleotides encompassing the GATA2 binding site has a dramatic impact on lymphatic vascular development; mice homozygous for this deletion die soon after birth exhibiting profound lymphatic vascular defects. Lymphatic endothelial cells in enhancer mutant mice exhibit reduced levels of genes characteristic of lymphatic endothelial cell identity and acquire characteristics of hemogenic endothelium, including the capacity to generate hematopoietic cells. These data reveal the first transcriptional enhancer element important for regulating *Prox1* expression and lymphatic endothelial cell identity and suggest that *Prox1* is important for repressing hemogenic cell identity in the lymphatic endothelium.

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Nephron progenitor commitment is a stochastic process influenced by cell migration

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Progenitor self-renewal and differentiation is often regulated by spatially restricted cues within a tissue microenvironment. This model broadly applies to the nephrogenic niche of the developing mouse kidney where spatially restricted signals within the tips of the ureteric epithelium are thought to pattern coarse subdomains in surrounding clouds of mesenchymal nephron progenitor cells. These subdomains are assumed to reflect a linear progression in fate from an uninduced nephron progenitor state, through progressive stages of commitment, to an early epithelial nephron. While this is the dominant model in the field, our previous work identified a substantial amount of cell movement within and between nephron progenitor niches, including large cell movements from the 'uninduced' region to the site of nephron commitment and *vice versa*. As such, our results of a swarming nephron progenitor population were at odds with the current linear, domain-based model of nephron progenitor regulation.

How then does progenitor cell migration impact on regionally induced commitment within the nephrogenic niche? Using lineage tracing and live imaging we identify a subset of cells that are induced to express *Wnt4*, an early marker of nephron commitment, but migrate back into the progenitor population where they accumulate over time. Single cell RNA-Seq and computational modelling of returning cells reveals that nephron progenitors can traverse the transcriptional hierarchy between self-renewal and commitment in either direction. Our observation of cells which receive a trigger to initiate *Wnt4* expression but fail to commit may indicate a requirement for prolonged induction or a second, as yet undefined, consolidating trigger. These results demonstrate that nephron progenitor commitment is a stochastic process influenced by cell migration, which regulates exposure to local inductive cues. The demonstrated plasticity in nephron progenitor fate may enable robust regulation of nephron formation as niches remodel and grow during kidney development.

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The alternative splicing regulator Nova2 constrains vascular Erk signalling to limit specification of the lymphatic lineage

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Publish consent withheld

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The role of the Nedd4 ubiquitin ligase during lymphatic development.

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The lymphatic vasculature is a crucial component of the cardiovascular system, with vital roles in tissue homeostasis, immune cell trafficking and absorption of lipids from the digestive system. Although signalling events important for the development of the blood vasculature system have been thoroughly investigated, less is known about the signalling pathways involved in development of the lymphatic vasculature. Our

work has revealed that the ubiquitin ligase Nedd4 is crucial for morphogenesis of the lymphatic vasculature during mouse embryogenesis; *Nedd4*$^{-/-}$ embryos exhibiting strikingly mis-patterned dermal lymphatic vessels. Furthermore, conditional deletion of Nedd4 from lymphatic endothelial cells using the *Prox1CreERT2* mouse line results in aberrant dermal lymphatic vessel patterning, demonstrating a cell autonomous role for Nedd4 in lymphatic development. Ubiquitination regulates the function of an array of proteins by controlling their stability, subcellular localisation or degradation. Here, we provide evidence demonstrating that Nedd4 regulates lymphatic vascular development in a cell autonomous manner by controlling the abundance and trafficking of VEGF receptors, leading to reduced signalling in response to VEGF-C. Furthermore, we demonstrate that Nedd4 regulates the remodelling of lymphatic endothelial cell adherens junctions; in the absence of Nedd4, the failure of junctional remodelling results in decreased capacity of lymphatic endothelial cells to migrate in response to VEGF-C. Current work aims to identify the substrates of Nedd4 responsible for regulating lymphangiogenesis.

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Apoptotic foci in mitochondrial permeabilization during apoptosis

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Apoptotic cell death is essential for development, immune function or tissue homeostasis, and it is often deregulated in disease. Mitochondrial outer membrane permeabilization (MOMP) is central for apoptosis execution and plays a key role in its inflammatory outcome. Knowing the architecture of the macromolecular machineries mediating MOMP is crucial for understanding their function and for the clinical use of apoptosis. Our recent work reveals that Bax dimers form distinct line, arc and ring assemblies at specific apoptotic foci to mediate MOMP. However, the molecular structure and mechanisms controlling the spatiotemporal formation and range of action of the apoptotic foci are missing. Although it is widely accepted that Bax and Bak function and molecular mechanism at apoptotic foci largely overlap, there is limited evidence how Bak works. Here I present our latest discoveries in the molecular similitudes and differences between Bax and Bak in apoptosis.

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Genetic mosaicism of the mitochondrial genome.

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With life expectancies increasing around the world, neurodegenerative disorders and other late-onset inflictions represent an enormous disease burden. A cellular hallmark of these diseases is a loss of mitochondrial function. Mitochondria are the organelles within our cells that convert the calories we eat into the primary energy substrate of the cell, ATP. These organelles harbour their own genome (mtDNA), which is essential for mitochondrial function but prone to mutation and molecular lesion. A gradual build of mtDNA damage during time has been proposed to contribute to the progressive nature of late-onset diseases. Moreover, mtDNA mutations are transmissible to the next generation and can cause a range of devastating metabolic disorders called mitochondrial diseases. Using novel approaches to purify cell-specific mitochondria across large populations of animals, we have discovered that mutations in mtDNA follow a stereotyped pattern of distribution in different tissue types. Our results suggest that certain cells are prone to propagating mitochondrial mutations more than others, which may help to explain the mosaic pattern of tissue and organ dysfunction in patients carrying mtDNA mutations. We have identified that mitochondrial autophagy (mitophagy) driven by the PINK1-parkin axis determines the stereotyped patterns of mosaicism within somatic tissues and organs. Interestingly, we also discovered that germ cells harboured the highest level of mtDNA mutations – a surprising finding considering that these mitochondria are transmitted to the next generation. I will present our latest findings on this phenomenon as well as discuss the results of a genome-wide genetic screen designed to discover molecules that can counteract the effects of mtDNA damage, which inevitably accumulates over our lifetimes.

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The importance of fidelity in mitochondrial gene expression for cell and organ function

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Mitochondria have their own unique gene expression system that has diverged dramatically since the original endosymbiotic event that created eukaryotes. The fidelity of gene expression is crucial in prokaryotes and for the nuclear-encoded proteins of eukaryotes, however little is known about its role in mitochondria and its effects on metabolism. We have generated mouse models to understand the importance of mitochondrial gene expression for cell function in mammals. We have found diverse tissue-specific pathologies that activate different stress signalling pathways, alter metabolism and manifest in diverse disease phenotypes. Our findings highlight the importance of mitochondrial function in many different diseases and their myriad roles in normal cellular biology.

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Metabolic characteristics of ageing and function in CD8+ T cells

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My research focuses on understanding the key drivers of effective CD8+ T cell immunity, in particular on how the abundance and quality of antigen-specific T cells in the preimmune repertoire impacts on primary immune responses. More recently, our work has investigated how

ageing undermines primary CD8 T cell responses, in part, through direct effects on naïve CD8 T cells that alter their phenotype and decrease their functionality. To understand the molecular basis of these defects, we have assessed functional, metabolic and transcriptional differences across various subsets of naive CD8 T cells from young and aged mice and humans. Understanding characteristics that drive or delimit effective T cell responses permits the optimization or recovery of T cell function via strategies that target these mechanisms.

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The not so "Mighty"- chondrion: Designing therapies for diabetes complications.

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Mitochondria, our cellular power stations have the unenviable task of producing most of our body's transportable energy, ATP amongst other important tasks. Kidneys are fuel hungry organs second only to the heart in mitochondrial number and oxygen consumption. In diabetes, both delivery of fuels for ATP production and mitochondrial function are compromised and are postulated to contribute to the development and progression of diabetic kidney disease. Although early mitochondrial dysfunction appears early n diabetes it may be reversible. Indeed, this may be the factor that discerns those 1/3 of individuals with diabetes who ultimately go on to develop kidney and cardiovascular disease from those who don't, which is an unsolved mystery in diabetes complications. This talk will highlight why mitochondria are key for kidney function and how this could be targeted to prevent the development of diabetic kidney and cardiovascular disease.

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Mechanobiology of cytotoxic T- and tumour cell interactions

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Cytotoxic T lymphocytes (CTLs) are specialised immune cells that search for and eliminate tumour cells. Burgeoning immunotherapies against cancers attempt to harness the capacity of these T cells to navigate various barriers and organs to reach the tumour and then effectively engage and kill their targets, yet little is known about the cellular forces that underpin their movements and interactions.

Following cognate antigen recognition, a CTL grasps its target cell and forms a mechanically dynamic synapse at their interface, through which the contents of cytotoxic granules are released to effect target cell death. Here, we employ an integrative and multidisciplinary method encompassing biophysical instruments (optical tweezers, dual micropipette aspiration), advanced live-cell microscopy, image analysis and computational modelling to uncover the intricate mechanobiology of T-cell mediated tumour rejection.

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Live imaging of non-centrosomal microtubule dynamics controlling early mammalian development

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The spatial and temporal configuration of the microtubule cytoskeleton is pivotal for tissue integrity and function. Whereas in proliferating, undifferentiated somatic cells, the centrosome serves as the major microtubule organizing center (MTOC), most differentiated cells, for instance epithelial cells, neurons and muscles, adopt a polarized non-centrosomal microtubule architecture. However, the regulation of microtubule growth and dynamics during the formation of the inner pluripotent cell mass of the early mammalian embryo lacking centrosomes remained elusive.

Using live imaging, I discovered a new form of non-centrosomal microtubule organization creating a shared asymmetric microtubule network between every pair of sister cell across the preimplantation embryo. Contrary to centrosome-containing cells, the cytokinetic bridge does not undergo stereotypical abscission after cell division in the mouse embryo. Instead, it transforms into a non-centrosomal MTOC by accumulating the microtubule minus-end protein CAMSAP3 and promoting microtubule nucleation. I show that the microtubules emanating from this MTOC direct the intracellular transport of membrane polarity proteins such as E-cadherin which is required for the formation of the pluripotent inner cell mass. Moreover, in line with the absence of centrosomes, the mitotic spindles of the early embryo are anastral. However, a distinct polar microtubule network establishes at the end of cell division causing the exclusion of F-actin from the apical cortex of all outer cells of the embryo. The resulting apical actin rings expand towards cell-cell junctions, zipper and seal the embryo to allow the formation of the blastocyst.

Together, live imaging reveals a novel mechanism how non-centrosomal microtubules are organized and function during early mammalian embryogenesis.

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The spindle shows its moves: polarising the oocyte for extreme asymmetric division

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The spindle is the cellular machinery comprised of microtubules that pulls chromosomes apart at anaphase. Following anaphase-onset, the spindle midzone that forms between separating chromosomes signals to the overlying cortex to direct the site of furrowing and consequently, the division plane, thereby delivering half the chromosomes to each daughter cell. In the case of meiotic divisions in female reproductive cells, one daughter cell is very large (the oocyte) and the other is very small (the polar body). Understanding how oocytes achieve these asymmetric divisions is an area of intense interest since this process enables unwanted chromosomes to be ejected while retaining critical

cytoplasmic reserves for supporting the embryo after fertilisation. One important step that has been extensively investigated is pre-anaphase migration of the spindle to the oocyte cortex to place the division plane in an off-centre position. We recently showed that this pre-anaphase spindle displacement, while sufficient for symmetry breaking, is not sufficient for achieving extreme asymmetry. We identified a second phase of migration occurring after anaphase-onset that is critical for producing the smallest polar bodies. During this second phase, the spindle migrates into the membrane to create a protrusion that subsequently becomes the polar body. We have been studying how this post-anaphase-onset phase of spindle migration comes about, how it is linked with cell-cycle regulation, how furrowing is delayed despite a spindle midzone presence during post-anaphase-onset migration and how this delay is influenced by spindle migration speed and spindle size.

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Cyclic resetting of RhoA residence kinetics regulates cell contractility

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Contractile signaling in the cell requires that the small GTPase RhoA recruit and engage downstream effectors at the plasma membrane. We now show that such signaling is conditioned by an intrinsically labile association of active, GTP-RhoA with the membrane. Effective signaling requires the scaffold anillin, which antagonizes the membrane-dissociation of GTP-RhoA independently of the canonical actions of GAPs and GDI. Anillin recruits GTP-RhoA by direct, reversible binding, and also locally concentrates PIP2 in the membrane. The latter functions to transiently enhance the membrane retention of free GTP-RhoA when it dissociates from anillin. Therefore cycles, where active RhoA first binds cortical anillin and then is retained by PIP2 even after disengagement, allow the dissociation kinetics of GTP-RhoA to be repeatedly 'reset', increasing its availability to recruit effectors. This demonstrates a new pathway for regulating contractile RhoA signalling and a more general model of scaffolding via the control of cortical dwell times. Further to this, we identified Myosin II as an anchor to concentrate Anillin at the cortex, thereby facilitating a positive feedback loop that can amplify signaling from RhoA for effective contractility.

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Defining the earliest cues driving apical-basal polarity establishment: the tumor suppressor protein Scribble regulates supermolecular assembly and positioning of cell-cell adherens junctions.

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Epithelial tissue function requires a robust, reciprocal interaction between cell-cell contacts and the apical-basal polarity axis, but the precise nature of this interplay is not well understood. *Drosophila* cellularization provides a superb model for examining junction formation as polarity is first established. Work from our lab and others identified Bazooka/Par3 as a key polarity cue required for adherens junction formation during cellularization. Canoe/Afadin, which links adherens junctions to the underlying actin cytoskeleton, and its regulator Rap1, act upstream of Bazooka. We recently found that Rap1 acts at multiple levels during polarity establishment, facilitating retention of Canoe at the membrane, and also directing correct apical positioning and organization of Canoe into supermolecular assemblies at tricellular junctions. We show that the Rap1 GEF Dizzy directs a subset of these events. Our data reveal that regulation of apical-basal polarity establishment by Rap1 requires a diverse set of inputs that specify unique aspects of Canoe's behavior on the polarizing membrane. In the current model, Rap1/Canoe, Bazooka and adherens junctions initiate polarity, and subsequently other protein complexes are recruited to elaborate on the polarity program. Our latest findings indicate this linear hierarchy is significantly oversimplified. Instead, proteins of the basolateral Scribble polarity module are required at the onset of polarity establishment to spatially restrict Canoe to apical ends of polarizing cells. Strikingly, mislocalized Canoe puncta continue to colocalize with adherens junction proteins. Thus Scribble is critical for the supermolecular assembly of smaller cadherin-catenin complexes into mature spot junctions and the positioning of these junctions apically. Intriguingly, overexpressing Canoe in a *scribble* RNAi background altered architecture of the mislocalized Canoe puncta, with assembly of more contiguous structures that may represent enhanced clustering. These data form part of a larger scale effort in our lab to define the full network of proteins and mechanisms by which individual cadherin-catenin complexes are assembled to form apical adherens junctions, of which serve to direct elaboration of apical-basal polarity.

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Cryo-EM and correlative imaging of DNA-binding proteins

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In higher eukaryotes, all cells contain the same DNA sequence but different cell types carry out vastly different functions. This ability is regulated by a dramatic reorganization of the genome during cell differentiation, whereby packaging of DNA into a compact chromatin structure, termed heterochromatin, results in the transcriptional silencing of specific genes. Conversely, transcribed regions of the genome are maintained in an open chromatin structure termed euchromatin. Compacting of DNA into heterochromatin is a complex process, which involves the function of many factors that regulate and fine-tune the accessibility of the transcriptional machinery to the DNA. In my talk, I will discuss how recently developed electron microscopy (EM) methods can be applied to visualize chromatin in isolation and inside the nucleus. Firstly, single particle cryo-EM analysis for high-resolution structure determination of reconstituted chromatin complexes. Secondly, electron tomography combined with correlative light and electron microscopy (CLEM) of cells. Together, these imaging techniques provide complimentary structural information about how DNA-binding proteins regulate gene expression.

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A new entry pathway for *Plasmodium vivax* into reticulocytes

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Malaria parasites are exquisitely adapted for survival within human red blood cells. Understanding how malaria parasites enter human red blood cells is essential for the development of strategies to inhibit blood stage infection. *Plasmodium vivax* preferentially invades reticulocytes, which are young red blood cells. Successful entry depends on the specific interactions between *P. vivax* reticulocyte-binding protein 2b (PvRBP2b) and transferrin receptor 1 (TfR1). The PvRBP2b-TfR1 invasion pathway is essential for *P. vivax* entry into human reticulocytes, as TfR1-deficient erythroid cells are refractory to invasion by *P. vivax,* and anti-PvRBP2b monoclonal antibodies inhibit reticulocyte-binding and block *P. vivax* invasion in field isolates. We present a high-resolution cryo-EM structure of a ternary complex with PvRBP2b bound to human TfR1 and transferrin which provides a detailed view of the critical interaction surfaces. While the interaction domain of PvRBP2b is subject to balancing selection, mutational analyses show that PvRBP2b residues involved in complex formation are conserved, providing a promising design strategy to engineer antigens that may be strain-transcendent against *P. vivax* infection. We are able to demonstrate that PvRBP2b binds to TfR1 using residues that are specific to human TfR1. These functional analyses of TfR1 highlight how *P. vivax* has hijacked TfR1, which is an essential housekeeping protein, by binding to sites that govern host specificity without affecting its cellular function of transporting iron. Crystal structures of PvRBP2b in complex with antibody fragments have definitively mapped the inhibitory epitopes and define different structural modes of inhibition. Our results establish a structural framework for understanding how *P. vivax* reticulocyte-binding protein engages its receptor and the molecular mechanism of inhibitory antibodies, providing important information for the design of novel vaccine candidates.

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Modelling skeletal muscle T-tubule formation in zebrafish

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The skeletal muscle T-tubule system, a specialized membrane domain essential for coordinated muscle contraction, shows dysmorphology in a number of genetically inherited muscle diseases. However, in the absence of genetically tractable systems the mechanisms involved in T-tubule formation are unknown. Here, we have used the optically transparent zebrafish system to probe T-tubule development in vivo. By combining live imaging with three-dimensional electron microscopy, quantitative overexpression screening and genetic knockout models, we define a new endocytic capture model involving i) formation of dynamic endocytic tubules at transient nucleation sites on the sarcolemma ii) stabilization by myofibrils/sarcoplasmic reticulum and iii) delivery of membrane from the recycling endosome and Golgi complex.

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Intersectin-1 interacts with the golgin, GCC88, to couple the actin network and Golgi architecture

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Aim: The Golgi apparatus is a dynamic organelle that, in vertebrates, is organized into a continuous ribbon-like structure known as the Golgi ribbon. The conversion of individual Golgi stacks into a ribbon structure is a highly regulated process which relies on a balance between the actin and microtubule network. While the involvement of the microtubule network has been well defined, the role of the actin network is poorly characterised. Our previous studies have shown that a membrane tether/golgin known as GCC88, located at the *trans*-Golgi network (TGN), modulates the architecture of the Golgi ribbon in a dose-dependent manner. Increased levels of GCC88 led to the loss of the Golgi ribbon whereas GCC88 depletion resulted in the formation of longer Golgi ribbons[1]. The aim of the current study was to identify the underlying mechanism for GCC88-mediated re-organization of the Golgi ribbon.

Methods: To delineate the role of GCC88 in the modulation of the Golgi ribbon, candidate interactors were identified through an *in vivo* enzymatic labelling approach known as BioID. We have also generated a HeLa cell line referred to as HeLa-B6 that stably expresses a GFP-GCC88 chimera at ~ 2-fold increase compared with endogenous GCC88. HeLa-B6 cells are characterised by the loss of the Golgi ribbon and dispersal of Golgi mini-stacks.

Results: We have shown that GCC88-mediated dispersal of the Golgi ribbon is an actin-dependent process and involves non-muscle myosin-IIA. We have identified the long isoform of Intersectin-1 (ITSN-1), which has GEF activity for the Rho GTPase Cdc42, as a novel interaction partner of GCC88 and demonstrated its localisation at the TGN and involvement in GCC88-mediated loss of the Golgi ribbon. We have also shown that the ITSN-1-GCC88 interaction is important in other models of Golgi fragmentation such as alterations to membrane flux and in a neurodegenerative model.

Conclusion: Our study shows that GCC88 is involved in linking the TGN to the actin cytoskeleton through the interaction with ITSN-1. This interaction regulates the balance between a compact Golgi ribbon and dispersed mini-stacks and is a relevant pathway to pathophysiological conditions.

1. Gosavi, P, Houghton, FJ, McMillan, PJ, Hanssen, E and Gleeson, PA (2018) J Cell Sci 131, jcs211987

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Understanding ubiquitin signals through deubiquitination

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Protein ubiquitination, as its name implies, is ubiquitous, and is emerging as a major regulator of protein homeostasis and cell signalling. Deregulation of protein ubiquitination is involved in a variety of human diseases, including cancer, inflammation and neurodegeneration. The last decade has shown that a plethora of distinct ubiquitin signals exist. My lab tries to understand the complex 'ubiquitin code' by focussing on the different ubiquitin signals themselves, in order to eventually link the specificity in the system back to physiological functions.

Deubiquitinases are emerging as key players in the regulation of the ubiquitin code. They hold huge therapeutic potential for their role in stabilising proteins – inhibiting the DUB that stabilises an oncogene should destabilise the oncogene. While we understand DUBs structurally and mechanistically in great detail, a complete picture of biological roles for DUBs is largely missing.

In my seminar, I will discuss our latest work on DUBs, focussing on the important inflammatory regulator OTULIN, its roles in disease and emerging roles in trafficking. OTULIN regulates exclusively a lowly abundant ubiquitin chain type, Met1-linked or 'linear' ubiquitin chains, and its involvement in new biological pathways suggests unappreciated roles for linear chains outside inflammation. Secondly, I will discuss two closely related USP deubiquitinases, USP25 and USP28. The latter stabilises the key-oncogene c-myc, and holds some promise as a drug target. Our structures of USP25 and USP28 indeed reveal a route to inhibition due to mechanisms of oligomerisation that regulate activity. Characterisation of adaptor regions in USP25 may indicate new biological functions that require careful follow-up studies.

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The essential role of protein phosphatase 2A in tumour suppression and embryonic development

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PPP2R2A encodes the B55α regulatory subunit of PP2A, a family of serine/threonine phosphatases that regulate multiple signalling pathways involved in cell proliferation, survival, migration and inflammation. *PPP2R2A* is a proposed tumour suppressor gene, with recurrent loss detected in a range of cancers, including breast, lung and prostate, however the functional role of B55α loss is not known. Here we show that molecular inhibition of B55α enhances the proliferation, colony formation, anchorage independent growth and *in vivo* tumour growth of human breast cancer cells. B55α knockdown also enhanced migration and invasion, in association with increased expression of focal adhesion kinase and epithelial-to-mesenchymal transition proteins. Comparative phosphoproteomics identified known and novel proteins regulated by PP2A-B55α, and revealed altered mTOR, RhoA, MAPK, and HIPPO signalling pathways. Of clinical importance, PP2A-B55α knockdown induced resistance to the ER-antagonist, tamoxifen, and the HER2/EGFR inhibitor, lapatinib. However, B55α-knockdown cells were sensitive to a range of PP2A activating drugs. Indeed, PP2A activators sensitized resistant cells to tamoxifen and lapatanib, and inhibited tumour growth and metastases in orthotopic xenografts, suggesting a possible therapeutic option. To further examine the functional role of PP2A-B55α, we generated PP2A-B55α (*Ppp2r2a*) knockout mice. As with many tumour suppressor genes, constitutive knockout of *Ppp2r2a* was embryonic lethal, with embryos dying during late development, post 14.5 days p.c. *Ppp2r2a*$^{-/-}$ embryos displayed neural tube defects (exencephaly and spina bifida), limb defects (non-divergent digits), and impaired epidermal barrier formation. In contrast, mice with mammary specific *Pppr2ra* deletion were viable, and displayed significantly greater mammary gland branching, supporting a role for PP2A-B55α in mammary gland morphogenesis. Together this work demonstrates the importance of PP2A-B55α in normal development and as a tumour suppressor in breast cancer, and suggests that targeting PP2A activation is a potential therapeutic strategy for poor outcome patients.

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Repurposing kinase regulatory mechanics towards ubiquitination

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The Tribbles family of pseudokinases recruit substrates—including metabolic proteins and transcription factors—to the COP1 ubiquitin ligase for ubiquitin-mediated protein degradation. CCAAT-enhancer binding protein (C/EBP) family transcription factors are a particularly relevant class of Tribbles-COP1 substrate in adipocyte and myeloid development, and acute myeloid leukaemia. We have solved several crystal structures of TRIB1, including an autoinhibited state and the TRIB1 pseudokinase in complex with the Tribbles recognition degron from C/EBPα. The structures show that TRIB1 undergoes a significant conformational change relative to its substrate-free structure to bind C/EBPα in a pseudo-substrate-like manner. Importantly, substrate-induced rearrangement of TRIB1 engages an allosteric network that links substrate recruitment, release of the COP1 binding-motif of TRIB1 and formation of the cognate COP1-TRIB1 ubiquitin E3 ligase. Such a cooperative model is consistent with biochemical experiments and molecular dynamics simulations. These findings offer a view of pseudokinase regulation with striking parallels to bona fide kinase regulation—via the activation loop and αC-helix—and raise the possibility of small molecules or binding proteins that target either the activation loop-in or loop-out conformations of Tribbles pseudokinases.

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The tandem SH2 domains of ZAP70 allow regulation of bond lifetimes that are sensitive to the affinity of TCR-pMHC interactions.

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T cells are activated when their T cell receptors (TCR) interact with antigens in the form of agonist peptides presented in the context of a major histocompatibility complex (MHC). T cells must remain quiescent when presented with highly abundant low affinity self peptide MHC interactions. To achieve this it is generally accepted that a kinetic proof-reading mechanism exists to filter out noise induced by short-lived binding events, but the molecular mechanism remains ill-defined. Zeta-chain-associated protein kinase 70 (ZAP70) plays a critical role in the proximal signaling cascade downstream of T cell receptor activation, where it is recruited to phosphorylated immunotyrosine-based activation motifs (ITAMs) in the TCR. ZAP70 contains tandem Src homology 2 (SH2) domains that bind with high specificity and affinity to the two phospho-tyrosines in ITAMs, but such stable interactions would be incompatible with a role in the kinetic proof-reading mechanism. To mediate this role the duration of ZAP70 recruitment to pMHC-engaged TCR should depend on the lifetime of the TCR-pMHC interaction.

Using total internal reflection microscopy and single particle tracking we assessed the lifetime of a ZAP70 at the membrane in ILA TCR-expressing Jurkat T cells activated with four different altered peptide ligands of differing affinities. As our hypothesis suggests the membrane lifetime of individual ZAP70 molecules correlated with the lifetime of TCR-pMHC interactions. We next directly measured the binding kinetics of ZAP70 on phosphorylated ITAMs using surface plasmon resonance and microscopy. The results of kinetic measurements on mono- and bi-phosphorylated ITAMs support a model in which ZAP70 rapidly cycles between monovalent and bivalent binding states. In monovalent states one tyrosine is available for dephosphorylation by phosphatases, which would prevent ZAP70 from cycling into the bivalent conformation and thus shorten the overall complex lifetime. In support of this model inclusion of a protein tyrosine phosphatase accelerated ZAP70-ITAM dissociation. Taken together, these results indicate that tandem SH2 domains allow bond lifetimes with ITAMs to be regulated by the local activity of phosphatases in a manner that a monovalent interaction would not be. We propose that this allows ZAP70 membrane recruitment to be sensitive to TCR-pMHC binding lifetimes and thus allows it to function as a step in the kinetic proofreading mechanism. Given the prevalence of proteins with multiple SH2 domains this mechanism may function generally in many signalling reactions that require rapid kinetic tuning that is sensitive to the local balance of kinase and phosphatase activities.

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RagC phosphorylation autoregulates mTOR complex 1

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The mechanistic (or mammalian) target of rapamycin complex1 (mTORC1) controls cell growth, proliferation and metabolism in response to diverse stimuli. Two major parallel pathways are implicated in mTORC1 regulation including a growth factor-responsive pathway mediated via TSC2/Rheb, and an amino acid-responsive pathway mediated via the Rag GTPases. Here we identify and characterize three highly conserved growth factor-responsive phosphorylation sites on RagC, a component of the Rag heterodimer, implicating cross talk between amino acid and growth factor mediated regulation of mTORC1. We find that RagC phosphorylation is associated with destabilization of mTORC1 and is essential for both growth factor and amino acid-induced mTORC1 activation. Functionally, RagC phosphorylation suppresses starvation-induced autophagy, and genetic studies in Drosophila reveal that RagC phosphorylation plays an essential role in regulation of cell growth. Finally, we identify mTORC1 as the upstream kinase of RagC on S21. Our data highlight the importance of RagC phosphorylation in its function, and identify a previously unappreciated auto regulatory mechanism of mTORC1 activity.

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Regulation of autophagy-dependent cell death

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Defective lysosome homeostasis during autophagy causes skeletal muscle disease

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Skeletal muscle is heavily reliant upon the cytoprotective functions of autophagy and its inhibition causes disease in mice and humans. During autophagy lysosomes are heavily consumed, via their fusion with autophagosomes, before being replenished to steady state levels. *In vitro* cellular studies have shown that if lysosomes are depleted, autophagy becomes progressively suppressed. Two alternate pathways regulate lysosome repopulation during autophagy; TFEB/TFE3-dependent lysosome biogenesis and autophagic lysosome reformation (ALR). The microphthalmia-associated transcription factors (MiTF) TFEB/TFE3 are master regulators of the lysosomal system, controlling the expression of nearly all genes required for *de novo* lysosome formation. ALR recycles existing autophagic membranes to reform lysosomes. In skeletal muscle basal autophagy is high, and is further increased as an adaptive response to exercise or fasting. As such, we predicted that muscle would have a high demand for lysosome generation to maintain continued autophagic flux. Here we report for the first time that defective lysosome homeostasis in muscle causes marked inhibition of the autophagy pathway and severe muscle disease. We define a novel mechanism for regulating lysosome production during autophagy through the spatiotemporal regulation of key phosphoinositides. Our study further identifies how lysosomes are maintained in muscle *in vivo* and how failure of this process causes disease. This represents a new disease pathway in muscle which we predict will have broader implications for other disorders where autophagy is important including neurodegenerative conditions.

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ORP1L regulates cholesterol exit from late endosomes and lysosomes: a role for phosphatidylinositol biphosphates

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Phosphatidylinositol phosphates (PIPs) and cholesterol are known to regulate the function of late endosomes and lysosomes (LELs). ORP1L, a putative lipid sensor/transporter, specifically localizes to LELs. Here, we show that the highly conserved lipid-binding domain of ORP1, ORP1-ORD, can transport sterols between membranes only in the presence of $PI(4,5)P_2$ or $PI(3,4)P_2$. Surprisingly however, ORP1-ORD can't transport any PIPs *in vitro*. In cells, both ORP1L and $PI(3,4)P_2$ were required for the efficient removal of cholesterol from LELs. The crystal structures of ORP1-ORD in complex with cholesterol or $PI(4,5)P_2$ reveal that the N-terminal loop and the lid of ORP1-ORD play important roles in ORD oligomerization and function, and that the 5- or 3-phosphate group of $PI(4,5)P_2$ or $PI(3,4)P_2$ may facilitate ORP1-mediated cholesterol transport by keeping the lid of ORP1-ORD open. We further demonstrate that ORP1L can regulate mTORC1 signaling and autophagosome maturation by maintaining lysosomal lipid homeostasis. Thus, our work unveils a novel mechanism by which PIPs may regulate OSBP/ORPs-mediated transport of major lipid species such as cholesterol.

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Regulation of mitophagy receptors BNIP3 and BNIP3L by SCF ubiquitin ligases

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Mitochondria are essential organelles that control energy synthesis, cell signalling, and apoptosis. Due to their important function in cell metabolism, the quantity and quality of mitochondria must be regulated tightly, through mitochondrial fusion and fission, biogenesis, and degradation. Damaged mitochondria are recycled and degraded through mitophagy, a specific form of selective autophagy. Mitophagy receptors BNIP3(BCL2/adenovirus E1B 19 kDa interacting protein 3) and its homologue BNIP3L (also known as NIX) are mitophagy receptors which tag defective mitochondria and act in concert with the autophagy machinery to engulf damaged mitochondria in the autophagosome double membrane. We have data demonstrating that BNIP3 and BNIP3L are regulated by the Skp1-Cul1-F-box protein (SCF) family of ubiquitin ligases. The regulatory pathways mediating the degradation of BNIP3 and BNIP3L, as well as the consequence of BNIP3 and NIX stabilisation on cellular mitophagy pathways, will be discussed.

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Anterograde trafficking and Golgi sorting of the membrane cargo β-secretase (BACE1) and the production of amyloid peptides in Alzheimer disease

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Aims: Perturbation of membrane trafficking can lead to neurodegenerative diseases such as Alzheimer's disease (AD) which is the most common form of neurodegeneration in aging populations. Accumulation of amyloid plaques in the brain is a hallmark of AD and are formed by the aggregation of amyloid β peptides (Aβ) generated after proteolytic processing of the amyloid precursor protein (APP) by the protease beta secretase (BACE1). Defining the dynamic intracellular trafficking of newly synthesized BACE1 and APP, especially in neurons is required to understand the regulation of Aβ production, development of AD and design of novel therapeutics.

Method: We are investigating the anterograde trafficking of newly synthesized BACE1 and the identity of trafficking machinery using the Retention Using Selective Hooks (RUSH) system (Boncompain et al, 2012). The RUSH system allows us to synchronize and analyse in real time the trafficking of BACE1 in fixed and live cultured cells and primary mouse neurons. We have combined the RUSH system with the knockdown of Golgi adaptors and the use of TIRF microscopy.

Results: We found that BACE1 is transported to the plasma membrane directly after its exit from the TGN. Subsequently, BACE1 is trafficked to the early endosomes then the recycling endosomes compartments. Significantly, we observed that the depletion of AP1, but not AP4, impairs the Golgi exit of BACE1 and its arrival at the plasma membrane. The retention of BACE1 in the Golgi leads to an increase of Aβ production. On the other hand, the Golgi exit of APP in absence of AP1 is not affected.

Conclusion: Our results show that BACE1 and APP trafficking from the Golgi apparatus is tightly regulated by different adaptor proteins, resulting in the partitioning of BACE1 and APP and the regulation of APP processing. Alterations in their anterograde trafficking can lead to a dramatic increase in Aβ production.

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Molecular mechanisms of autophagosome formation

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Autophagy is a conserved lysosome-mediated degradative pathway required for cell survival, homeostasis and human health. Autophagy is carried out by membranes called autophagosomes which capture cytosolic cargo such as damaged mitochondria and deliver it to lysosomes. Autophagosome formation is a complex process that requires a cohort of autophagy proteins (ATG proteins) acting in a coordinated manner. This core machinery drives the formation of the phagophore, the nucleating membrane, and the nascent autophagosome. Under amino acid starvation this coordinated response is initiated by the transmembrane protein ATG9 and the serine/threonine ULK1/2 kinase. ATG9 trafficking and ULK1/2 activation sense signals which drive the initiation of the phagophore. Following initiation, the Class III PI3 kinase, and the PI3P effector WIPI2 mediate recruitment of ATG12-5-16L1, and the ATG8 family of proteins. ATG8s are required for cargo selection and maturation of the autophagosome. In an effort to understand the formation and maturation of autophagosomes we are studying the trafficking and function of ATG9, substrates of the ULK1/2 complex, and the selectivity of the ATG8 proteins. Our recent results will be presented which provide new insight into these processes.

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New mechanisms of autoinflammatory and neuroinflammatory disease.

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Mutations in innate immune genes can cause autoinflammatory disease. A particular focus in this area has been the inflammasome protein complex which generates the cytokines IL-1b and IL-18. Specifically, we found mutations that activate the Pyrin inflammasome in a dominantly inherited skin disease we called PAAND, leading to successful therapy by blocking IL-1b. Additionally, the team identified novel mutations in the NLRC4 inflammasome that trigger IL-18 production with early onset inflammatory bowel disease.

A separate class of autoinflammatory diseases are associated with increased production of type I IFN, for example with mutations that activate the cGAS/Sting cytoplasmic DNA sensor system. We have now implicated this same system in the progression of amyotrophic lateral sclerosis (ALS). It turns out that TDP-43, which is normally a nuclear protein, mislocalises to mitochondria in patients with ALS, releasing mitochondrial DNA and triggering neuroinflammation.

POSTER PRESENTATIONS

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Characterisation of the heterogeneous cell subpopulations of primary prostate epithelial cell cultures derived from patient tissue by label-free ptychography, in order to develop a pre-clinical drug-testing model

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Despite several drugs showing promise after testing with standard *in vitro* assays, including extensive cell line panels, many tumours fail to respond to these drugs at the clinical trial stage. Thus, despite huge monetary investment in trials, the inability of pre-clinical models to predict success is hampering efforts to progress cancer treatment and to personalize cancer chemotherapy. Cell lines have been the work-horse of cancer research for decades, however they do not represent tumour heterogeneity or patient variability. There is a need for a better model to carry out pre-clinical testing in order to give greater chance of success in clinical trials, which would in turn mean benefit for more patients and an overall reduction of wasted funds.

To address this need for more clinically relevant models, the use of primary cell cultures derived from patient tumours is becoming more desirableand more common. We have previously shown that there are resistant subpopulations of cells within patient-derived prostate tumour cultures. Thus, to develop a successful treatment, all cell types must be targeted, and so specific combination treatments are likely to be more successful than monotherapies.

Here, we present the use of ptychography, a label-free imaging technique, to characterise primary prostate epithelial cultures derived from patient tumour tissue. This technique allows segmentation and extraction of individual cell metrics from time-lapse data. These metrics include cell number, area, thickness, dry mass, duration in mitosis, position, speed, orientation, directionality, Euclidean distance, meandering index (Euclidean distance/ total track length) and more. The cells were analysed as a mixed population and also as three separate populations, including transit amplifying cells (TA) ($\alpha2\beta1integrin^{hi}/CD133^{-}$) and committed basal cells (CB) ($\alpha2\beta1integrin^{lo}$). Alongside this novel imaging technique we used fluorescent labelling of surface markers to confirm cell identity.

Our aim is to use ptychography to carry out real-time analysis of cell response to drug treatments, using docetaxel as the standard of care treatment and comparator. A full characterisation of all types of patient- derived tumour cells, alongside analysis of their response to current and novel drugs will allow assessment of detailed biological effects of the drugs tested as well as identification of resistant cells. This could lead to patient cells becoming part of the drug development pipeline, which will ultimately result in targeted and patient stratified therapies that take into account intra- and inter-tumour heterogeneity.

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Coronary Vascular Progenitor Cells Require Paracrine Signalling from Cardiomyocytes for their Endothelial Cell Fate Determination

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Aim: Endothelial cells participate in the development of many organs, including the heart. Despite advances in our understanding of how cardiac coronary vasculature is formed in the embryo, translating this knowledge into protocols for coronary vasculature repair has proved difficult. Kang et al., have identified a novel population of cardiac vascular progenitor cells ($PDGFRA^{+}$/*Nestin*-$GFP^{+}/CD31^{-}$) (VPCs) in the developing heart (unpublished data). These progenitor cells can form vessel like structures when they transplanted subcutaneously and generated mature endothelial cells. Characterizing the molecular regulation of VPCs can help in developing strategies to improve tissue regeneration following myocardial infarction. The aim of this study was to examine the effect of cardiomyocyte cells (CMs) on the endothelial fate of *Nestin* expressing VPCs.

Methods: We used a 3D microfluidic device to investigate, signalling of CMs on the VPCs on its vascular fate determination. Cells were cultured in separate microchannels with different co-culture conditions in the presence of a specific density of hydrogel that allows cells to migrate between two channels. We used live cell imaging and confocal microscopy to study the interactions of VPCs and CMs

Results: The live cell imaging indicated that the migrating cells are mostly VPCs that tend to migrate toward the CMs. This happened in both mixed and separate co-culture conditions where cells are in direct or indirect contact with each other. Statistical analysis of quantitative key indicators of cell migration showed that VPCs were significantly attracted towards the CMs paracrine signalling

Conclusions: This study demonstrates that the CMs determine the fate of VPCs fate towards endothelium. This model of microfluidic approach helps us to understand cell-cell interactions in cell fate determination and that can model coronary vasculature development on-a-chip.

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In vivo imaging of Rac1 targeting in metastatic breast cancer

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Small GTPases such as Rac1 enable cells to migrate during development as well as metastasize during cancer progression. More specific, time-resolved monitoring of key drivers of survival and metastasis in e.g. mammary cancer such as small GTPases could be done in an *in vivo* setting with the use of FRET-biosensor mice to track protein activity and the effect of therapeutic intervention.

Here, we describe the generation and characterization of a FRET-biosensor mouse to examine Rac1[1] activity in an *in vivo* setting in two mouse models of mammary cancer. Using time-correlated single photon counting (TCSPC) multiphoton microscopy allowed for the imaging of this signalling biosensor in tissues and live mice by the application of optical windows[2]. Elevated levels of Rac1 activity was observed in the polyoma-middle-T-antigen (PyMT) and Her2-driven breast cancer models. This activity was further upregulated at the invasive borders of these tumours. Two inhibitors of Rac1 activity were evaluated in 2D and 3D *in vitro* settings and NSC-23766 identified to successfully inhibit invasion of PyMT cells in a 3D context. Shear stress analysis revealed decreased invasion and survival of cells after treatment with NSC-23766. Finally, longitudinal imaging of the inhibition of Rac1 activity live *in vivo* was achieved by employing optical windows implanted on top of developed tumours. The therapeutic response was further correlated live to the extra-cellular matrix and to the local vasculature. This allowed for the tailoring of targeted intervention in a spatiotemporal manner. Chronic treatment of a cohort of PyMT mice starting at the onset of tumour development until endpoint further revealed a significant reduction in metastatic burden in these animals.

In conclusion, the development and use of the FRET biosensor mice represents a strong resource in understanding tissue context specific signalling events during migration and drug target validation *in vivo*, identifying Rac1 as a strong therapeutic target in metastatic breast cancer.

1. Johnsson, A.-K.E., Dai, Y., Nobis, M., et al. (2014) The Rac-FRET mouse reveals tight spatiotemporal control of Rac activity in primary cells and tissues. Cell Reports. 6, 1153–1164
2. Ritsma, L., Steller, E.J.A., Ellenbroek, S.I.J., et al. (2013) Surgical implantation of an abdominal imaging window for intravital microscopy. Nature Protocols. 8, 583–594

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Predicting cancer spread before it happens – implications for anti-invasive intervention

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E-cadherin-mediated cell-cell junctions play a prominent role in maintaining epithelial architecture. Their dysregulation in cancer can lead to the collapse of tumour epithelia and subsequent invasion and metastasis. Recent evidence suggests that, apart from modulating E-cadherin expression, cells are able to mobilise E-cadherin within their cell-cell junctions upon migration and invasion. We have developed new tools to assess the spatiotemporal dynamics of epithelial tumour cell-cell junctions to study the earliest stages of invasion and metastasis.

Methods:

Here, we have generated an E-cadherin-GFP mouse, which enables intravital quantification of E-cadherin clustering and mobility to provide insight into tumour cell-cell junction strength and integrity in intact tissues and tumours.

Results: We reveal that:

(1) E-cadherin mobility and clustering become de-regulated in invasive and metastatic tumours compared to healthy tissues and non-invasive pancreatic tumours.

(2) These subcellular aberrations in E-cadherin dynamics can be targeted with anti-invasive treatment to re-stabilise cell-cell junctions and to reduce cancer invasiveness.

Discussion: We suggest that these techniques can be used as:

(1) **novel tools** to fundamentally expand our understanding of cell-cell junction dynamics *in vivo* in native microenvironments

(2) **novel pre-clinical drug-screening platform** to predict cancer spread and to estimate the efficacy of anti-invasive treatment.

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Proteome aggregation patterns under proteostasis stress as signatures for understanding Huntington's Disease

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Protein homeostasis (proteostasis) is regulated by an extensive quality control network governing protein synthesis, folding, transport and degradation. When proteostasis becomes unbalanced protein misfolding and aggregation is observed. Critically, inappropriate protein aggregation and proteostasis imbalance are two of the central pathological features of common neurodegenerative diseases including Alzheimer, Parkinson, Huntington, and motor neuron diseases. However, it remains unclear to which proteins in the proteome become preferentially altered in aggregation state in disease and how these patterns relate to mechanisms of proteostasis imbalance.

To investigate this problem, we devised a quantitative proteomic workflow for cell culture samples that have been fractionated into soluble and insoluble fractions after treatment with distinct pharmacological stresses on proteostasis including Hsp90 inhibition, and Hsp70 inhibition, proteasome inhibition, ER stress induction, and oxidative stress. We find that by and large these treatments yield distinct subproteomes that are altered in solubility, which produce molecular signatures for the type of stress invoked. We also compared these findings to the aggregation of the Huntingtin exon 1 protein (Htt) in a Huntington Disease cell model. For this we examined cells expressing mutant Htt that had been first separated into populations lacking Huntingtin aggregates from those with aggregates using our previously developed flow cytometry-based Pulse Shape Analysis. We found soluble mutant Htt induced a cluster of nuclear and mitochondrial proteins to change their solubility. When Htt aggregated, proteins involved in quality control including protein refolding and ER-associated degradation became more insoluble. These data indicated that mutant Htt aggregation led to a cascade of proteome solubility changes that involve a mixture of the stress signatures, notably those associated with ER stress, oxidative stress and impaired proteostasis.

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Revealing the function of Nr6a1 in axial elongation and lineage choice

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In the elongating mouse embryo, posteriorly located neuromesodermal progenitors (NMPs) have the ability to either self-renew or give rise to the precursors of the spinal cord and paraxial mesoderm. The balance between proliferation and differentiation of NMPs must be coordinated with the process of somitogenesis which generates the segmented body plan. The molecular mechanisms driving NMP self-renewal, and the differential regulation of NMP output as progressively more posterior structures are being laid down, is not well characterised. Here we propose *Nuclear receptor subfamily 6 group A member 1* (*Nr6a1*) as a critical regulator of axial elongation through its control of NMP self-renewal. We show that the expression of *Nr6a1* drops dramatically in the posterior region from embryonic day (E)8.5 to E9.5, a stage at which NMPs relocate from the epiblast to the tailbud and coinciding with the trunk-to-tail transition. Previous genetic ablation of Nr6a1 in mouse caused a severely truncated axis, supporting its role in NMP self-renewal. Furthermore, it has been shown that Nr6a1 plays a key role in controlling the differentiation of the embryonic stem (ES) cells induced by RA signalling by inhibiting the pluripotency factors. To elucidate the roles of Nr6a1 in anterior-posterior axial elongation and NMP proliferation, we have generated a series of novel tools, including Nr6a1-deficient ES cells and stably transduced Dox-inducible Nr6a1 gain-of-function ES cells. Together, these tools will allow us to dissect the role of this orphan nuclear receptor in cell behaviours of mouse *in vitro* derived NMPs, identify the direct downstream targets of Nr6a1 in this context, and reveal novel protein-interacting partners of Nr6a1.

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Comparative analysis of H3K27me3 domains establishes a repressive index for inferring regulatory genes governing cell identity from any chordate cell type

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Identifying the mechanisms governing development and disease remains difficult due to the challenge of enriching for regulatory genes that control cell fate and function. We evaluated chromatin states from 111 NIH epigenome roadmap samples and found that genes having broad H3K27me3 domains with high frequency across diverse cell types, which we termed a repressive tendency (RT), significantly enrich for cell-type specific regulatory genes. We found that a gene's RT value can act as a fixed variable to weight any quantitative gene expression data resulting in enrichment of regulatory genes governing that given cell-type. This analysis approach, which we call TRIAGE (Transcriptional Regulatory Inference Analysis of Gene Expression), is unsupervised and does not depend on external reference data, statistical cutoffs or prior knowledge. We used consortium data from the Human Cell Atlas, FANTOM, and a draft map of the human proteome to show that TRIAGE can enrich for regulatory genes from any cell or tissue type using any quantitative readout of gene expression including RNA-seq (bulk or single cell), CAGE or quantitative proteomics. Given the highly conserved role of regulatory genes, we show that TRIAGE can be applied to quantitative gene expression data from any chordate species, ranging from tunicates to mammals, and identify the regulatory drivers of disease and development. TRIAGE also significantly outperforms prior analysis approaches used to predict regulatory genes using epigenetic data. Lastly, we utilized TRIAGE to analyze scRNA-seq data from cardiac differentiation and identified SIX3, GAD1 and CRLF1 as candidate novel genes governing germ-layer specification. We used CRISPRi hPSCs to show that loss of function for these genes blocks derivation of definitive endoderm and boosts mesoderm induction. Taken together, TRIAGE provides a computational approach for analyzing any quantitative readout of gene expression to identify regulatory genes underlying cell identity and fate in development and disease from any somatic cell-type and chordate species thus opening new opportunities to discover mechanisms underlying organ development, disease and regeneration.

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Discovery of a novel gene required for cardiac rhythm

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Cardiac arrhythmias affect approximately 5% of the population and have a high association with sudden death. Whilst the cause of cardiac arrhythmia is complex, there is a proven genetic component. Genomic sequencing of patients has contributed considerable information towards our understanding of gene mutations in this disease however genetic interpretation relies on our prior knowledge of gene function - typically derived from cell- and animal-based research. From a forward genetic screen in zebrafish, we have identified a completely novel gene required for cardiac rhythm. Mutant phenotypes include slower heart rates, skipped beats and 2:1 (atrium:ventricle) arrhythmias. Optical mapping of action potentials suggest that repolarization of the membrane is prolonged, suggesting an electrical defect in these mutants at the level of the action potential. Whole-genome sequencing mapping identified the causative mutation in a gene encoding a multi-transmembrane domain protein with no ascribed function and its closest homologue also has no known function. Spatial transcriptomics shows enrichment in the sinoatrial node (pacemaker) and atrioventricular node, consistent with the phenotype. We have now established a mouse knockout and find that mice die at birth. Earlier embryonic stages show enlarged hearts and poor blood flow consistent with impaired cardiac function. Importantly, in utero echocardiography imaging of 17.5 dpc embryos confirms cardiac arrhythmia in mutant embryos. Finally, sequencing information from patients with arrhythmogenic disorders reveal heterozygous mutations in the gene, consistent with it acting as a disease susceptibility locus. Together, this work describes a gene discovery project from zebrafish, through to mouse and finally humans, identifying a new regulator in cardiac rhythm.

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A role for Aurka in regulating renal cyst growth downstream of Inpp5e

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Publish consent withheld

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THE MOLECULAR INTERACTION OF SRC FAMILY KINASES WITH THE GROWTH HORMONE RECEPTOR AND ITS ROLE IN LIFESPAN EXTENSION AND LIVER REGENERATION

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Emerging evidence suggests that GHR can signal through pathways additional to JAK2 tyrosine kinase, as has been reported in patients with GH resistance that exhibit normal JAK2-mediated STAT5 but impaired ERK activation. One such pathway involves the Src Family Kinases (SFK). The signalling choice between JAK2 and LYN kinase (a SFK member) by GHR can be modulated by realigning the receptor lower cytokine module in its extracellular domain. Existence of GHR-mediated SFK signalling *in vivo* has been demonstrated in a targeted Box1 motif mutant mouse model allowing complete abrogation of GHR-mediated JAK2/STAT signalling, but the mechanism of activation and its downstream effects remain elusive. In order to determine the structure-function attributes of GHR-mediated LYN activation, we have used co-immunoprecipitation to define the region in the GHR intracellular domain that interacts with LYN and *vice versa* and complemented this with interaction analysis by NMR. Using super resolution microscopy to track single molecule movement on the cell surface, we have identified differences in GHR mobility owing to JAK2 *vs.* LYN binding. Our results indicate that GHR binding to LYN is constitutive, independent of the Box1 motif and unconventional with the interaction site residing in well conserved region between the Box1 and Box2 motif of GHR in the potential lipid interacting domain. We found that cellular levels of JAK2 and LYN compete for binding to GHR and the relative amounts of JAK2 or LYN confers altered signalling. In contrast to JAK2, LYN caused a striking decline in GHR expression with differential ERK1/2 activation kinetics. Hepatic microarray in animal models designed to alter the balance of GHR-mediated signalling, has revealed cohort of genes regulated primarily by GHR-mediated SFK signalling. This study provides insight into the mechanism by which GH-stimulated LYN-ERK signalling is regulated and its physiological relevance in extending lifespan, and clinical relevance to liver regeneration, in Box1 mutant mouse models.

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Design and application of novel chemical probes to detect cathepsin X activation in oral cancer

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Cathepsin X/Z/P is protease in the lysosomal cysteine cathepsin family that exhibits unique monocarboxypeptidase activity. Its expression has been associated with several cancer types and neurodegenerative diseases, although its roles during normal physiology are still poorly understood. Like most proteases, cathepsin X activity is subject to complex post-translational regulation. It is synthesised as an inactive zymogen that must be cleaved by other proteases to permit activation, and its activity can be further controlled by endogenous inhibitors. Thus, measures of protein abundance do not reflect the pool of proteolytically active cathepsin X. Advances in our understanding of the function of cathepsin X have been hindered by a lack of available tools that can specifically measure its activity. We therefore developed a series of new fluorescent activity-based probes that can bind to cathepsin X in an activity-dependent manner. These probes exhibit improved specificity and potency for cathepsin X compared to previously reported methods. We demonstrated the ability of these probes to detect the activity of cathepsin X in cell and tissue lysates, in live cells and in vivo, and to localise active cathepsin X in mouse tissues by confocal microscopy. We have applied our most sensitive and specific probe to study cathepsin X activation in oral squamous cell carcinoma. Biopsies from oral squamous cell carcinomas exhibited a significant increase in cathepsin X activity compared to patient-matched normal tongue tissue. Application of these probes, in combination with specific cathepsin X inhibitors, knockout mice, and oral cancer models, will advance our understanding of cathepsin X in oral cancer pathogenesis.

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Loss of USP28-mediated BRAF degradation drives resistance to RAF cancer therapies

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RAF kinase inhibitors are clinically active in patients with BRAF (V600E) mutant melanoma. However, rarely do tumors regress completely, with the majority of responses being short-lived. This is partially mediated through the loss of negative feedback loops after MAPK inhibition and reactivation of upstream signaling. Using a genome shRNA screen to target all known deubiquitinating (DUB) enzymes we identify USP28 as a novel regulatory of MAPK activity. Furthermore, we demonstrate that USP28 functions through a feedback loop to destabilize RAF family members. Loss of USP28 stabilizes BRAF enhancing downstream MAPK activation and promotes resistance to RAF inhibitor therapy in culture and in vivo models. Importantly, we demonstrate that USP28 is deleted in a proportion of melanoma patients and may act as a biomarker for response to BRAF inhibitor therapy in patients. To identify novel therapeutic strategies for melanoma patients harbouring USP28 deletions we performed a chemical compound screen and identify rigosertib as being synthetically lethal with USP28 loss. Taken together our results show that loss of USP28 enhances MAPK activity through the stabilization of RAF family members and is a key factor in BRAF inhibitor resistance.

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Identifying causal effectors of platinum resistance in lung adenocarcinoma through multiplexed analysis of signalling dynamics

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Lung adenocarcinoma is an aggressive form of cancer that is frequently resistant to frontline platinum chemotherapy, with a response rate below 30%. Inherent resistance to chemotherapy often involves complex signalling networks, and we have recently performed a genome-wide siRNA screen that identified a number of kinase signalling pathways strongly implicated in platinum resistance (Marini et al, Science Translational Medicine, 2018).

Using magnetic bead-based multiplexing technology, we have now profiled the cisplatin-induced signalling dynamics of the PI3K/mTOR, MAPK, SMAD, STAT and NF-κB pathways alongside key apoptosis mediators and DNA damage response proteins in a panel of lung adenocarcinoma cell lines. A multidimensional analysis of this dataset identified a correlation between platinum resistance and elevated P70S6K activity, which directly opposed a late phase, double-strand break DNA damage response that occurred in platinum sensitive cell lines.

Analysis of patient samples revealed that P70S6K is amplified within ~5% of lung adenocarcinomas, with elevated expression also associated with poor patient survival. Furthermore, inhibition of P70S6K in platinum-resistant cell lines with the dual PI3K/mTOR inhibitor dactolisib promoted an increase in the double-strand DNA damage response and restored sensitivity to platinum chemotherapy both *in vitro* and *in vivo*.

Therefore, this study is a demonstration of how the early phases of a signalling response can be used to predict the eventual cellular outcome, and subsequently applied to a clinical setting. We have also identified a potential therapeutic strategy in targeting P70S6K to overcome platinum resistance in lung adenocarcinoma.

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Subcellular specific targeting of JNK as a novel anti-metastatic therapy in triple negative breast cancer

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The triple negative breast cancer (TNBC) subtype has a much poorer prognosis than hormone receptor or HER2 positive breast cancer, due in part to a higher relapse rate and frequent metastasis to the pleura, lung, liver and brain. The lack of clinically successful targeted therapies for TNBC means that chemotherapy remains the standard-of-care. To date, inhibitors to targets such as EGFR and c-MET have shown promising initial responses, followed by rapid relapse.

Therefore, instead of targeting individual molecules at the top of signalling pathways, we hypothesise that inhibiting the key nodes that integrate signalling from many potential oncogenes will be a more efficient strategy. To this end, we have identified JNK as a critical oncogenic signalling node within TNBC cells, which conveys signals from multiple oncogenic pathways to promote proliferation, invasion and metastasis.

Due to the necessity of JNK for many normal processes, tumour suppression and chemotherapy response in other tissues, systemically targeting JNK is unlikely to yield successful translation to the clinic. Instead we now demonstrate that two distinct JNK network states exist simultaneously within breast cancer cells, with opposing functional and prognostic roles. Through studies of patient cohorts, and the use of genetically encoded localization-specific JNK inhibitors within three-dimensional cell culture models and *in vivo* metastasis assays, we show that these two network states are spatially separated into a tumour suppressing, nuclear JNK pool, and an oncogenic, cytosolic JNK pool.

By adapting our recently published, interaction-based proteomic platform (Croucher et al., Science Signaling, 2016) we have specifically isolated and characterised the oncogenic, cytosolic JNK complex. We also demonstrate that perturbing this signalling complex can specifically inhibit the oncogenic form of JNK, without disturbing the tumour suppressing function of nuclear JNK activity. This finding therefore opens up novel therapeutic options for targeting this critical oncogenic signalling node in TNBC.

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A novel feedback loop prevents hyperactivation of Akt

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Cells utilise signalling networks to rapidly respond to environmental cues and initiate appropriate biological responses. These responses arise from a dynamic balance between feedforward signals and feedback regulation, whereby information is relayed to enhance or suppress signal flow as needed. Activation of the protein kinase Akt must be tightly controlled, as it is a key node in a range of signal transduction cascades which regulate fundamental processes such as cell proliferation and metabolism. Disruption of this control can give rise to a plethora of diseases - for example, Akt is often hyperactivated in cancer. It is of upmost importance to understand how Akt activity is regulated in the cell, to inform the design of appropriate therapeutic interventions which can ameliorate abnormal Akt activation in disease. Using live cell total internal reflection fluorescence (TIRF) microscopy, we have discovered a potent feedback mechanism which reduces signal flow across the Akt signalling axis. Our data suggest that following Akt activation in response to growth factors, Akt reduces phosphoinositide 3-kinase (PI3K) activity and subsequently lowers the amount of phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) present at the cell surface. This limits Akt recruitment to the plasma membrane thereby negatively regulating its kinase activity, and consequently the many physiological processes Akt orchestrates. Thus, our findings pinpoint a novel feedback loop which prevents hyperactivation of Akt. This will guide future endeavours to fine-tune Akt activity in disease states which are associated with aberrant Akt signalling.

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Deciphering the functional role of Cep55 in causing chromosomal instability and spontaneous tumorigenesis in vivo

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Introduction: *CEP55*, discovered first by our laboratory, is a key regulator of cytokinesis, with perturbation of its levels causing cytokinesis failure and multi-nucleation. Since its discovery, CEP55 over-expression has been linked to increased aggressiveness of multiple tumor types. This overexpression has been associated with increased AKT activation, caused by the interaction of CEP55 with p110 and promoting stability of the catalytic subunit of *PI3KCA*. Further, it has been shown that wild-type p53 suppresses CEP55 expression by negatively regulating PLK1 creating a p53-PLK1-CEP55 axis to directly modulate CEP55 stability and cytokinesis completion. In addition, *CEP55* has been shown to be part of the CIN70 gene signature that predicts chromosomal instability and poor prognosis of several tumors. However, despite these studies, it is currently unknown whether elevated Cep55 levels alone are sufficient to promote *de novo* tumorigenesis.

M&M: We have generated the first novel transgenic mouse model with overexpression of *Cep55* from the ubiquitously expressed *Rosa26* locus.

Results: We observed that *Cep55* over-expression *in vivo* leads to a wide-spectrum of spontaneous tumor formation with a long latency period. In addition, we have generated a bi-transgenic mouse model ($Cep55^{Tg/Tg}$ $Trp53^{+/-}$) to determine the contribution of p53 loss to Cep55-induced tumorigenesis. Interestingly, we observed a similar latency between $Cep55^{Tg/Tg}$ and $Cep55^{Tg/Tg}$; $Trp53^{+/-}$ mice. However, we detected a higher incidence of tumors in $Cep55^{Tg/Tg}$; $Trp53^{+/-}$ compared to $Cep55^{wt/Tg;}$ $Trp53^{+/-}$ and $Cep55^{wt/wt;}$ $Trp53^{+/-}$ mice with the tumors being significantly larger and more penetrant, proliferative and metastatic in nature. In addition, we demonstrated that *Cep55* overexpression induces chromosomal instability and whole genome doubling. Further, we observed that *Cep55* overexpression is associated with chromosomal segregation errors *in vitro*. We also observed an association of Cep55 with polyploidy in a dose-dependent manner and demonstrated that *Cep55* overexpression protects a polyploid subpopulation upon perturbed mitosis.

Conclusion: Collectively, our data proves the oncogenic potential of Cep55 overexpression *in vivo* and as an important stimulus in the initiation and progression of multiple cancer types.

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Identification of a novel player in insulin-mediated lipolysis inhibition

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Responsible for releasing free fatty acids (FFA) from triglycerides (TG), lipolysis is a catabolic process essential for maintaining whole-body energy homeostasis. It is activated by β-adrenergic stimulation during starvation or prolonged exercise and is inhibited by insulin following nutrient intake. Dysregulated lipolysis, as is the case in insulin resistance, can lead to increased circulating FFA and ectopic TG accumulation, which exacerbates insulin resistance, leading to type 2 diabetes and non-alcoholic fatty liver disease. Despite its importance in such metabolic disorders, the mechanism governing insulin's inhibition of lipolysis remains controversial. Currently, the prevailing model hinges on the enzymatic function of the Akt substrate, phosphodiesterase 3B (PDE3B) in explaining insulin's antilipolytic action. However, several studies have challenged this and suggested that whilst PDE3B is essential, its function alone cannot explain this regulation. Instead, we implicate another Akt substrate, α/β-hydrolase domain-containing protein 15 (ABHD15), as a novel player in insulin's inhibition of lipolysis. Through stable knockdown or knockout of ABHD15 in 3T3-L1 or brown adipocytes, we show an impairment in insulin-mediated inhibition of lipolysis that is rescued upon ABHD15 re-expression. Moreover, through the generation of ABHD15 and PDE3B mutants lacking novel insulin-regulated phosphorylation sites previously identified by our lab, we have explored a putative mechanism by which these proteins facilitate insulin's inhibition of lipolysis. In summary we have identified a novel regulator of lipolysis that will likely shed light on the mechanism of this important process.

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Histone FLIM-FRET microscopy identifies ATM- and RNF8- dependent rearrangement of chromatin architecture during the DNA damage response

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To investigate how chromatin architecture is spatiotemporally organised at a double strand break (DSB) repair locus, we established a biophysical method to quantify chromatin compaction at the nucleosome level during the DNA damage response (DDR). The method is based on phasor image correlation spectroscopy (ICS) of histone FLIM-FRET microscopy data acquired in live cells co-expressing H2B-eGFP and H2B-mCherry. This multiplexed approach generates spatiotemporal maps of nuclear-wide chromatin compaction that when coupled with laser micro-irradiation, are capable of quantifying the size, stability, and spacing between compact chromatin foci throughout the DDR. Using this technology, we identify that ATM and RNF8 regulate rapid chromatin decompaction at DSBs and formation of a compact chromatin ring surrounding the repair locus. This chromatin architecture serves to demarcate the repair locus from the surrounding nuclear environment and modulate 53BP1 mobility.

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The mouse microbiome is required for sex-specific diurnal rhythms of gene expression and metabolism

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The circadian clock and associated feeding rhythms have a profound impact on metabolism and the gut microbiome. To what extent microbiota reciprocally affect daily rhythms of physiology in the host remains elusive. Here, we analyzed transcriptome and metabolome profiles of male and female germ-free mice. While mRNA expression of circadian clock genes revealed subtle changes in liver, intestine, and white adipose tissue, germ-free mice showed considerably altered expression of genes associated to rhythmic physiology. Strikingly, the absence of the microbiome attenuated liver sexual dimorphism and sex-specific rhythmicity. The resulting feminization of male and masculinization of female germ-free animals is likely caused by altered sexual development and growth hormone secretion, associated to differential activation of xenobiotic receptors. This defines a novel mechanism by which the microbiome regulates host metabolism.

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Classification of the human phox homology (PX) domains based on their phosphoinositide binding specificities

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Phox homology (PX) domains are membrane interacting domains that bind to phosphatidylinositol phospholipids or phosphoinositides, markers of organelle identity in the endocytic system. Although many PX domains many bind the canonical endosome-enriched lipid PtdIns3*P*, others interact with alternative phosphoinositides, and a precise understanding of how these specificities arise has remained elusive. We have systematically screened all human PX domains for their phospholipid preferences using liposome binding assays, biolayer interferometry and isothermal titration calorimetry. These analyses define four distinct classes of human PX domains that either bind specifically to PtdIns3*P*, non-specifically to various di- and tri-phosphorylated phosphoinositides, bind both PtdIns3*P*and other phosphoinositides, or associate with none of the lipids tested. A comprehensive evaluation of PX domain structures reveals two distinct binding sites that explain these specificities, providing a basis for defining and predicting the functional membrane interactions of the entire PX domain protein family.

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Structural insights into the architecture and membrane interactions of the conserved COMMD proteins

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The COMMD proteins are a conserved family of proteins with central roles in intracellular membrane trafficking and transcription. A hallmark feature of the COMMD protein family is a highly conserved C-terminal region, with no known structure, known as the COMM domain. The N-terminal domain of these proteins is highly variable and is proposed to ascribe unique functions to each of the 10 family members. In addition, it is known, that COMMDs form oligomeric complexes with each other and act as components of a larger assembly called the CCC complex, which is localized to endosomal compartments and mediates the transport of several transmembrane cargos. How these complexes are formed however is completely unknown. Here, we have systematically characterised the interactions between several human COMMD proteins and determined structures of COMMD proteins using X-ray crystallography and X-ray scattering to provide insights into the underlying mechanisms of homo- and heteromeric assembly. Our structural analysis revealed that, COMMD proteins possess an a-helical N-terminal domain. While the highly conserved C-terminal COMM domain is composed of two cone shaped chains that are tightly intertwined with each other to form a globular dimeric module. Additional, biochemical analysis demonstrated that this dimeric COMM domains binds directly to CCDC22 and 93, key components of the CCC complex, via a conserved site. Finally, we were able to demonstrate that members of the COMMD family are able to bind nonspecifically to a wide variety of phosphoinositides. Overall, these studies show that COMMD proteins function as obligatory dimers with conserved domain architectures.

1. Healy, M., et.al. (2018). Structural insights into the architecture and membrane interactions of the conserved COMMD proteins. eLIFE 7. DOI: 10.7554/eLife.35898

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Trafficking and Signalling from Macropinosomes in Microglia

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Macropinosomes are important cellular compartments that allow for the uptake of pathogens, nutrients and cellular debris but also function as key sites of signalling. Many trafficking proteins, including GTPases, are involved in macropinocytosis and also in receptor signalling on these membranes. Our group has previously identified Rab8a and its effector, PI3Kg as regulators of Akt/mTOR signalling downstream of Toll-like receptors (TLRs) in macrophages. Most recently we have identified low dentistry lipoprotein receptor-like protein 1 (LRP1) as a cross talk receptor that recruits Rab8a/PI3Kg in response to activation of TLRs. The LRP1/Rab8a complex, which acts on signalling at the level of macropinosomes, is an important regulator of TLR-induced inflammation. LRP1 has other roles in modulating inflammation and it is heavily implicated in Alzheimer's disease pathogenesis. The role of LRP1 recruited Rab8a/PI3Kg however is poorly characterised in microglia, innate immune cells of the central nervous system tasked with cytokine production of clearance of cellular debris. Here we aim to elucidate where Rab8a and LRP1 intersect on signalling membranes during internalisation from the cell surface and their recycling trajectories in these cells. We have defined the trajectories and machinery involved in Rab8a and LRP1 trafficking from macropinosomes in live microglial cells. These results will allow us to better understand trafficking and activation of LRP1 as a modulator of inflammation and as a clearance receptor in microglia and reveal how the Rab8a/PI3Kg complex is recruited and trafficked for TLR signalling and inflammation.

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Dscam2 suppresses synaptic strength through a synaptic vesicle recycling pathway

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Dscam2 is a cell surface protein expressed on neurons and required for neurodevelopment in *Drosophila*. It mediates boundary formation between different brain regions through homophilic repulsion and promotes contacts between neurons through homophilic adhesion. These functions occur at developmental times when axons and dendrites are actively growing and searching for synaptic partners. Interestingly, we found that a specific isoform of Dscam2 is expressed in larval motor neurons after growth has been completed and synaptic connections have been established.

Aims: To gain insight into post-developmental functions of Dscam2, we analysed Dscam2 mutant phenotypes at the larval neuromuscular junction.

Methods: We used a combination of neurogenetics, electrophysiology, light and electron microscopy (EM) to characterise phenotypes in *Dscam2*mutants.

Results: We found that Dscam2 suppresses the strength of motor neuron synapses. Evoked postsynaptic potentials were increased in *Dscam2^{null}* animals compared to controls. We determined that loss of *Dscam2* increased the number of synaptic vesicles released in response to an action potential. Mutants that suppress synaptic strength are rare so we screened through a number of candidates to identify signalling molecules in this pathway. We found a potential genetic interaction between the PI3K enhancer, *Centauriny1A*, and *Dscam2*. Consistent with this, *Dscam2^{null}* motor neurons exhibited a striking increase in levels of the PI3K substrate, PIP_2. PI3K regulates synaptic vesicle recycling, which in turn regulates synaptic release, so we next investigated whether Dscam2 might be involved in this process. A genetic marker for early endosomes was reduced in *Dscam2* mutants and changes in endosomal structures were observed by EM. EM also revealed an increase in docked synaptic vesicles compared to controls, providing evidence that Dscam2 negatively regulates a necessary step in synaptic vesicle release.

Conclusion: These data argue that Dscam2 contributes to the maintenance of synapses, a strikingly different role than it plays during development. This function appears to be independent of homophilic interactions as *Dscam2*is not expressed in postsynaptic muscle. Our data indicate that Dscam2 interacts with the synaptic vesicle recycling machinery to prevent excess vesicles from tethering to synaptic release sites. This 'brake' on synaptic release may have important implications for neuronal plasticity.

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Heterozygosity for *Nuclear Factor One X* in mice models features of Malan syndrome

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Nuclear Factor One X(*NFIX*)haploinsufficiency in humans results in Malan syndrome, a disorder characterized by overgrowth, macrocephaly and intellectual disability. Although clinical assessments have determined the underlying symptomology of Malan syndrome, the fundamental mechanisms contributing to their enlarged head circumference and intellectual disability in these patients remains undefined. Here, we used*Nfix* heterozygous mice as a model to investigate these aspects of Malan syndrome. We reveal, using magnetic resonance imaging, that brain volume is significantly increased within adult *Nfix$^{+/-}$*mice, most markedly within the cerebral cortex. Moreover, using diffusion magnetic resonance imaging and tractography-based analyses, we reveal microstructural deficits within major forebrain commissures and aberrant connectivity between many crucial brain regions. Finally, we demonstrate that *Nfix$^{+/-}$*mice exhibit cortically-mediated behavioral deficits that model intellectual disability. Collectively, these data provide a significant conceptual advance in our understanding of Malan syndrome by suggesting that megalencephaly underlies the enlarged head size of these patients, and that disrupted cortical connectivity may contribute to the intellectual disability these patients exhibit.

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Breaking constraint of mammalian axial formulae

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Elongation of the main body axis in mouse is driven by self-renewing axial progenitors, initially located within the epiblast and subsequently repositioned to the tailbud. Within axial progenitors and surrounding cells, specific combinations of Hox transcription factors impart the positional information required for regional morphology. While the Hox genes are the ultimate effector molecules, it is the signals and molecules controlling combinatorial Hox expression that orchestrates how the main body axis is laid down. Revealing the identity of these molecules is fundamental to our understanding of developmental and evolutionary mechanisms. We have identified a novel mechanism that controls the timing of a major Hox code transition, the trunk-to-tail transition. The miR-196 family of microRNAs are embedded in Hox clusters and target Hox genes of the trunk region. Genetic deletion of miR-196 in mouse resulted in a predicted upregulation of this trunk Hox code, with a concomitant, yet unexpected, delay in the tail Hox code. Here we use *in vitro* pluripotent stem cell differentiation protocols, coupled with *in vivo* analyses, to demonstrate that miR-196 and Gdf11 signalling act synergistically in the timely activation of the tail Hox code and cessation of axis elongation.

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Molecular Dissection of Box Jellyfish Venom Cytotoxicity Highlights an Effective Venom Antidote

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The box jellyfish *Chironex fleckeri* is probably the most venomous animal in the universe, envenoming leading to tissue necrosis, extreme pain and in the most severe exposures death within minutes. Despite its rapid and potent action, there is a lack of basic molecular insight into how this venom works. Here we perform the first molecular dissection of the jellyfish venom cell death pathway. We used genome-scale lenti-CRISPR mutagenesis to screen for host components required for cell death after venom exposure. Among host factors required for venom cytotoxicity was the peripheral membrane protein *ATP2B1*, a calcium transporting ATPase, and genetic or pharmacological targeting of *ATP2B1*could prevent venom action and confer long lasting protection. Informatics analysis of host genes required for venom cytotoxicity revealed numerous pathways not previously implicated in cell death, and we have validated many of these genes and pathways. Importantly, we identified a venom antidote that can suppress venom action when added up to 15 minutes after exposure, and this compound could also suppress tissue necrosis and pain in mice. These results highlight the power of whole genome CRISPR screening to investigate venom mechanisms of actions and rapidly identify new medicines.

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An improved line scanning confocal technique for enhanced axial resolution and contrast - EDGE™

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Recent developments in super resolution imaging have led to improvement in both axial and lateral resolution for fluorescence imaging. However, the improved axial resolution has come at the cost of imaging depth, with most techniques limited to a few micrometres from the base of the sample. For conventional imaging techniques, improvements in axial resolution are still sought. The advent of lightsheet microscopy.has improved axial contrast in larger specimens such as zebrafish, embryos and worms. However, due to the optical design constraints, increases in throughput are limited to a few dozen samples per hour and have not reached the speeds necessary for routine screening of compound or mutant libraries.

Improvements in axial resolution and contrast would greatly enhance the image quality obtained from conventional techniques allowing greater accuracy of segmentation of structures during image analysis. This would allow more robust quantitative analysis of the acquired images.

EDGE™ confocal is an enhanced confocal method that acquires 2D images or 3D image stacks with improved image contrast in all dimensions. This increase in contrast improves visualization and segmentation of structures in thick samples that would otherwise be obscured by high levels of background fluorescence. Inspired by Poher et al., we have implemented this method on the IN Cell 6500 line scanning confocal high content analysis system to allow fast, robust confocal imaging with a theoretical doubling of axial resolution and an order of magnitude attenuation of the out-of-focus signal. With imaging depths beyond 100 micrometers, EDGE confocal allows 3D imaging of tissue and organoids with minimal signal loss and blur in the centre of the objects. The high quality images resulting from this unique hardware combines with the IN Carta image analysis software to improve segmentation and quantitation. We demonstrate that it is the combination of improvements in both hardware and software that lead to improved analysis outcomes.

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Indole-3-propionate, elevated in plasma by high-fibre diets, reduces oxygen free radicals and induces de novo lipogenesis genes in hepatocytes

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Background: Recent research has shown significant health benefits deriving from high dietary-fibre consumption. Compared with native starch, dietary-resistant starch is a highly microbiome-accessible carbohydrate (MAC) that significantly alters the gut microbiome population[1,2]. Many of the benefits of high-MAC diets have been ascribed to increases in short-chain fatty acids, but they alone have not been able to replicate these benefits3.

Aim: To determine the mediators of salutary effects induced by high-fibre diets.

Methods: C57BL/6J mice were fed either native starch (NS) or resistant starch (RS) for 18 weeks (n=20/group). Plasma metabolomics was performed using HILIC HPLC in positive ion mode, and HILIC-AMIDE HPLC in negative ion mode, paired to a 5500 QTRAP QQQ mass spectrometer. The cecal microbial community was profiled using the 16S rRNA V4 region (515F – 806R) and sequenced with Illumina MiSeq. AML12 hepatocytes were stimulated with hydrogen peroxide and the effect of IPA on generation of oxygen free radicals was assessed using a DCFDA/H2DCFDA - Cellular ROS Assay Kit. AML12 hepatocytes were cultured with and without IPA for 48 hours and the expression of ACLY, a gene implicated in de novo lipogenesis (DNL), was determined.

Results: There was 22-fold increase in gut microbiome-derived tryptophan metabolite indole-3-propionate (IPA) (P=7.9E-3), which was positively correlated with several gut microbiota including *Allobaculum*, *Bifidobacterium*, and *Lachnospiraceae*; *Allobaculum* having the most consistently increased abundance of all the IPA-associated taxa across all RS-fed mice. IPA both reduced oxygen free radicals by 2-fold (Figure 1, P-value < 0.0001), and increased ACLY gene expression by 1.5-fold (Figure 2, P-value <0.05) in mouse hepatocytes.

Conclusion: IPA, reported to be a potent scavenger of hydroxyl radical species[4,5], was dramatically elevated by 22-fold in mice on a high-fibre diet. It suppressed free radical generation in hepatocytes exposed to hydrogen peroxide, and increased expression of the DNL gene ACLY in hepatocytes. We propose IPA is an important mediator of the effects of high-fibre diets, and warrants more extensive investigation.

1. Bindels, L. B., Walter, J., and Ramer-Tait, A. E. (2015) Resistant starches for the management of metabolic diseases. Curr Opin Clin Nutr Metab Care 18, 559-565
2. Fuentes-Zaragoza, E., Sánchez-Zapata, E., Sendra, E., Sayas, E., Navarro, C., Fernández-López, J., and Pérez-Alvarez, J. A. (2011) Resistant starch as prebiotic: a review. Starch-Stärke 63, 406-415
3. Keenan, M. J., Zhou, J., Hegsted, M., Pelkman, C., Durham, H. A., Coulon, D. B., and Martin, R. J. (2015) Role of resistant starch in improving gut health, adiposity, and insulin resistance. Adv Nutr 6, 198-205
4. Chyan, Y. J., Poeggeler, B., Omar, R. A., Chain, D. G., Frangione, B., Ghiso, J., and Pappolla, M. A. (1999) Potent neuroprotective properties against the Alzheimer beta-amyloid by an endogenous melatonin-related indole structure, indole-3-propionic acid. J. Biol. Chem. 274, 21937–21942.
5. Karbownik, M., Reiter, R. J., Garcia, J. J., Cabrera, J., Burkhardt, S., Osuna, C., and Lewinski, A. (2001) Indole-3-propionic acid, a melatonin-related molecule, protects hepatic microsomal membranes from iron-induced oxidative damage: relevance to cancer reduction. J. Cell. Biochem. 81, 507–513.