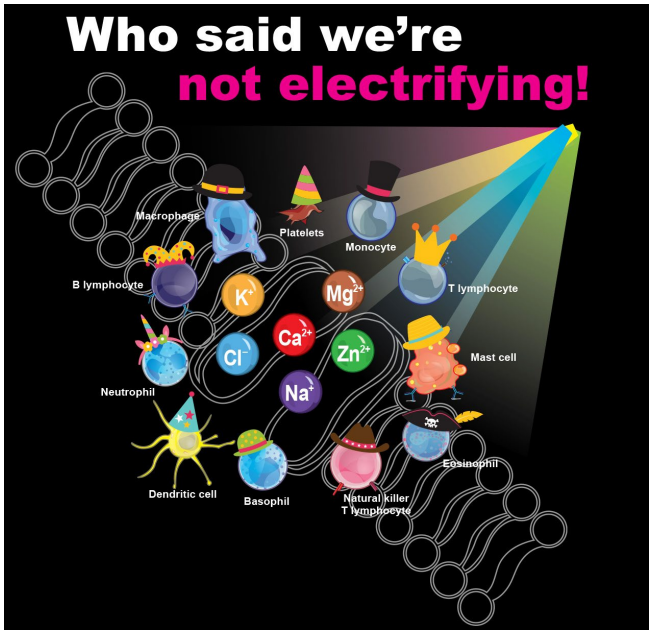


# ABSTRACTS

In-person and Virtual

SGP 2021 SYMPOSIUM

## Ion Channels and Transporters in Immunity, Inflammation, and Antitumor Immunity

22–26 September 2021, Marine Biological Laboratory, Woods Hole, Massachusetts, USA

**Virtual ABSTRACT/TABLE #1 Dalia Alansary**

# Role of purinergic signaling in regulating IL-1α mediated inflammation in chronic kidney disease

MARYAM AMINI[1], PRISKA JOST[1], JANINA FRISCH[2], STEFAN SCHUNK[3], JUTTA ENGEL[2], LETICIA PRATES ROMA[2], THIMOTEUS SPEER[3], BARBARA A. NIEMEYER[1], and DALIA ALANSARY,[11]*Molecular Biophysics, Saarland University, Homburg, Germany; [2]Biophysics, Saarland University, Homburg, Germany; [3]Department of Internal Medicine IV, Nephrology and Hypertension, Saarland University, Homburg, Germany*

Chronic Kidney disease (CKD) is associated with sterile inflammation where endogenous mediators are able to activate innate immune system and trigger pro-inflammatory cytokine production. The cytokine family IL-1 plays a central role in development of CKD. While the contribution of IL-1β to CKD is well characterized, the role of IL-1α is much less understood. We recently identified a novel role of IL-1α as an adhesion molecule mediating immune cells tissue infiltration. The aim of current work is to investigate the role of purinergic signaling in regulation of pro-inflammatory effects of IL-1α. Comparison of monocytes derived from healthy donors and CKD patients showed increased surface expression of IL-1α on CKD-monocytes albeit without alteration of stoichiometry of monocyte subpopulations. Live cell fura-2 based $Ca^{2+}$ imaging showed that the ATP-induced $Ca^{2+}$ signatures were significantly altered in CKD-monocytes. Analysis of expression profile of purinergic receptors revealed upregulation of P2X7 and P2X4 in CKD compared to healthy donor-derived monocytes. Moreover, pharmacological inhibition of P2X4 and P2X7 significantly reduced IL-1α release by human monocytes as measured by ELISA. Furthermore, bone marrow derived macrophages (BMDM) derived from $P2X7^{-/-}$ mice showed significantly reduced IL-1α release. Importantly, $P2X7^{-/-}$ mice were protected against adenine-induced renal injury in a CKD model. Moreover, cardiac myocytes from $P2X7^{-/-}$mice challenged in a model for myocardial infarction showed a significantly reduced IL-1 accumulation within the infarction areas compared to WT cells. Our results indicate a pivotal role of P2X7 regulating IL-1α mediated inflammation and identify P2X7 as a potential therapeutic target to improved outcome of CKD therapies.

**Virtual ABSTRACT/TABLE #2 Ameet A. Chimote**

# Inhibitory effect of Dexamethasone, a COVID-19 therapeutic on Kv1.3 channels in CD8$^+$ T lymphocytes

AMEET A. CHIMOTE[1], ABDULAZIZ ALSHWIMI[1], VAIBHAVKUMAR S. GAWALI[1], MARTINA CHIRRA[1], and LAURA CONFORTI,[1] [1]*Department of Internal Medicine, Division of Nephrology, University of Cincinnati, Cincinnati, Ohio*

An effective adaptive immune response (predominantly mediated by CD8$^+$ T cells) against SARS-CoV-2 infection is critical to prevent inflammation and tissue damage. Ion channels, especially Kv1.3 potassium and Orai1 calcium channels are key regulators of T cell function, but their role in the complications associated with COVID-19 pathogenesis is poorly understood. Furthermore, Dexamethasone (Dex), a corticosteroid administered as a standard of care for severe COVID-19 has been shown to prevent cytokine storm and death, and is known to inhibit Kv1.3 channels in macrophages and Jurkat T cells. We hypothesized that since Kv1.3 regulates T cell cytotoxicity and cytokine release, Kv1.3 blockade may contribute to the mechanism of action of Dex. To test this hypothesis, we isolated peripheral blood mononuclear cells (PBMCs) and CD8$^+$ T cells from healthy donors (HD) and treated them in vitro with 1 uM Dex for 24 and 48 h. Results from electrophysiology and RT-qPCR experiments showed that treatment with Dex significantly decreased Kv1.3 currents and also Kv1.3 gene expression in HD CD8+ T cells. Furthermore, RT-qPCR experiments in HD PBMCs, showed reduced Kv1.3 and Orai1 gene expression after 48h treatment with Dex. These results were validated by measuring Kv1.3 and Orai1 expression in immune cell subsets in PBMCs treated with Dex for 48 h. We observed that Kv1.3 protein expression decreased by ~50% in CD3, CD4 and CD8$^+$ T cells and by ~60% in CD56$^{dim}$CD16$^+$ NK cells. Furthermore, intracellular flow cytometry showed that Dex treatment reduced the ability of CD8$^+$ T cells to secrete IFN$\gamma$ confirming the effect of ion channel downregulation by Dex on T cell function. Thus, our findings suggest that the immunosuppression by Dex is mediated by Kv1.3 channel inhibition. We are currently verifying these findings in PBMC samples from severely ill COVID-19 patients that have been treated with Dex.

**Virtual ABSTRACT/TABLE #3 Björn-Philipp Diercks**

# P2X4 and P2X7 are essential players in basal T cell activity and $Ca^{2+}$ signaling milliseconds after T cell activation

VALERIE J. BROCK[1], INSA M.A. WOLF[1], BJÖRN RISSIEK[2], MARCO ER-LUKOWIAK[2], FRIEDRICH KOCH-NOLTE[3], TOBIAS STÄHLER[3], CHRISTA E. MÜLLER[4], LENA-MARIE WOELK[5], RENÉ WERNER[5], ANDREAS H. GUSE[1], and BJÖRN-PHILIPP DIERCKS,[1* 1]*The $Ca^{2+}$ Signalling Group, Department of Biochemistry and Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany; [2]Department of Neurology, University Medical Centre Hamburg Eppendorf, 20246 Hamburg, Germany; [3]Department of Immunology, University Medical Centre Hamburg Eppendorf, 20246 Hamburg, Germany; [4]Department of Pharmacy, University of Bonn, 53121 Bonn, Germany; [5]Department of Computational Neuroscience, University Medical Centre Hamburg Eppendorf, 20246 Hamburg, Germany*

Initial T cell activation is triggered by channel opening of single $Ca^{2+}$ channels like RYR1, as well as the interaction between ORAI1 with STIM1 proteins leading to the formation of highly dynamic, spatiotemporal $Ca^{2+}$ signals, called $Ca^{2+}$ microdomains (Wolf*, Diercks* et al. 2015. Sci. Signal. 8, 1–13; Diercks et al. 2018. Sci. Signal. 11.). P2X channels, which are ligand-gated cation channels in the plasma membrane of the T cell, are activated by ATP and were shown to influence the cytosolic $Ca^{2+}$ concentration after T cell stimulation. Using a high-resolution $Ca^{2+}$ live cell imaging system, we show that the two purinergic cation channels P2X4 and P2X7 are not only involved in the global $Ca^{2+}$ signals but also promote initial $Ca^{2+}$ microdomains milliseconds after T cell stimulation. Local $Ca^{2+}$ signaling is significantly decreased in T cells from *P2x4*$^{-/-}$ and *P2x7*$^{-/-}$ mice or by pharmacologically inhibiting P2X4 or blocking P2X7 by nanobodies in WT T cells. We demonstrate that the mechanism of $Ca^{2+}$ entry through P2X4 and P2X7 during the formation of $Ca^{2+}$ microdomains is governed by a fast ATP release via pannexin-1. Using an inhibiting peptide called $^{10}$panx1 to inhibit pannexin1 $Ca^{2+}$ microdomains were significant reduced just seconds after T cell stimulation revealing ATP is released through this hemichannel to activate the P2X4 an P2X7 channels. Moreover, we are able to show for the first time that a basal ATP release by pannexin-1 in unstimulated cells activates P2X4 and promotes the formation of spontaneous $Ca^{2+}$ microdomains. Subsequently, T cell stimulation fosters ATP release and autocrine activation of both P2X4 and P2X7, amplifying initial $Ca^{2+}$ microdomains already in the first second of T cell activation.

In summary, basal T cell activity depends on the purinergic axis of PANX1/P2X4 triggering spontaneous $Ca^{2+}$ microdomain formation, which is fostered in frequency and amplitude after T cell activation by P2X4 together with P2X7.

**Virtual ABSTRACT/TABLE #4 Feng Gu**

# NAADP forming enzyme in T cells

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Nicotinic acid adenine dinucleotide phosphate (NAADP) is the most potent endogenous $Ca^{2+}$ mobilizing second messenger known to date and effective in the low nanomolar range in many cells [1]. NAADP functions as a $Ca^{2+}$ trigger in T cells by evoking an initial local $Ca^{2+}$ signal, termed $Ca^{2+}$ microdomain, through type 1 ryanodine receptor mediated $Ca^{2+}$ release [2,3]. However, NAADP's forming enzyme is still controversial. *In vitro*, CD38 can catalyze the formation of NAADP from NADP through base-exchange reaction. But this reaction only proceeds at acidic pH and in the presence of excess of nicotinic acid.

Since both $Ca^{2+}$ microdomains and global $Ca^{2+}$ signals were identical in WT and *Cd38*$^{-/-}$ murine primary T cells, CD38 does not seem to be the NAADP forming enzyme involved in acute stimulation in T cells. We discovered another class of enzymes able to generate NAADP *in vitro* under physiological conditions. We investigated different NADPH oxidases which are expressed in murine and rat T cells. Only knock-out of two of these isozymes, namely DUOX1 and DUOX2, in T cells showed a strong phenotype in global $Ca^{2+}$ signals upon TCR/CD3 activation. Furthermore, the phenotype of *Duox2*$^{-/-}$ T cells was identical with NAADP antagonism, whereas NAADP antagonist BZ194 further reduced global $Ca^{2+}$ signals in *Duox1*$^{-/-}$ T cells. This indicates that DUOX1 altered the global $Ca^{2+}$ signals, at least partially, through other signaling pathway(s). Indeed, less $Ca^{2+}$ microdomains were formed below the plasma membrane in *Duox2*$^{-/-}$ T cells from the first second post-stimulation; DUOX1 did not affect $Ca^{2+}$ microdomain formation. Thus, we identified DUOX2 as NAADP forming enzyme in the early phase of T cell activation.

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**Virtual ABSTRACT/TABLE #5 Lola Hernandez**

# Adhesion-dependent T cell priming via FAK-PLC-γ pathway and SOCE activation demonstrated by advanced optical methods.

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Activation of T-lymphocytes is initiated by the recognition of an antigen via the T cell receptor (TCR). Subsequently, local calcium ($Ca^{2+}$) microdomains near the plasma membrane (PM) are formed, among other signaling pathways by the interaction of stromal interaction molecules (STIM)1 and 2 with ORAI1 channels located in the PM, leading to the opening of these channels and thus to $Ca^{2+}$ influx (1). Recently, we showed that $Ca^{2+}$ microdomains are already formed in T cells before TCR/CD3 stimulation. These $Ca^{2+}$ microdomains are observed on adhesive surface and depend on extracellular $Ca^{2+}$. Further, using our recently published SOCE antagonist, STX564 (2), we demonstrate a major role of ORAI1 contributing to the formation of adhesion-dependent $Ca^{2+}$ microdomains. Furthermore, the proteins focal adhesion kinase (FAK) and phospholipase C-γ (PLC-γ) were identified as signaling molecules upstream of ORAI1 activation, indicating the involvement of the FAK-PLC-γ pathway, as well as the local depletion of endoplasmic reticulum (ER) $Ca^{2+}$-store by D-*myo*-inositol 1,4,5-trisphosphate receptor ($IP_3R$) activation. To investigate the precise contribution and choreography of STIM proteins in translocation to ER-PM junctions and interaction with ORAI1 in adherent and non-adherent conditions, advanced optical methods were used. Therefore, we utilized three different imaging methods: fluorescence resonance energy transfer (FRET), Super Resolution via Optical Re-assignment (SoRa) and Stimulated Emission Depletion (STED) microscopy. With increasing spatial resolution (FRET>SoRa>STED), a lower rate of co-localization was observed between ORAI1 and STIM1, but the difference from non-adherent to adherent condition increased significantly. STIM2 and ORAI1, on the other hand, show an unchanged interaction for each condition. Furthermore, FAK and $IP_3R1$ also showed a significantly higher co-localization with ORAI1 under adherent compared to non-adherent conditions. These results indicate that the FAK-PLC-γ pathway, involving ER depletion by $IP_3Rs$ and subsequent store-operated $Ca^{2+}$ entry (SOCE) activation, is involved in adhesion-dependent T cell activation.

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**Virtual ABSTRACT/TABLE #6 Ronald Immler**

# The voltage-gated potassium channel KV1.3 regulates neutrophil recruitment during inflammation

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Neutrophil functions strongly rely on changes in intracellular calcium concentrations and concomitant calcium signaling. In lymphocytes, sustained $Ca^{2+}$ influx into the cell via $Ca^{2+}$ sensitive ion channels requires a compensatory efflux of potassium via the voltage-gated potassium channel $K_V1.3$ to maintain membrane potential. Although, voltage-gated potassiumcurrents over the plasma membrane have been identified in neutrophils as well, no distinct ionchannel could be attributed to these observations so far. Here, we addressed the question whether $K_V1.3$ is involved in $Ca^{2+}$ signaling of neutrophils thereby affecting neutrophil functionduring acute inflammatory processes.

Using *in vitro* assays and electrophysiological techniques, we show that $K_V1.3$ is functionally expressed in human neutrophils regulating sustained store operated $Ca^{2+}$ entry (SOCE) through membrane potential stabilizing $K^+$ efflux. Inhibition of $K_V1.3$ on neutrophils by the specific small molecule inhibitor 5-(4-Phenoxybutoxy)psoralen (PAP-1) impaired intracellular $Ca^{2+}$ signaling, thereby preventing cytoskeletal rearrangement, adhesion strengthening and appropriate crawling under flow conditions *in vitro*. Notably, interfering with $K_V1.3$ activity did not affect $\beta_2$ integrin inside-out, but outside-in signaling. Intravital microscopy revealed that pharmacological blockade or genetic deletion of $K_V1.3$ in mice decreased intravascularneutrophil adhesion in a shear rate dependent fashion in a mouse model of TNF-α-induced microvascular inflammation. Furthermore, we identified $K_V1.3$ as critical regulator of neutrophilextravasation into TNF-α-inflamed peritoneal cavity. Finally, we show that $K_V1.3$ deficient neutrophils exhibit impaired phagocytosis of *E.coli* particles.

Taken together, our findings do not only provide evidence for a role of $K_V1.3$ in sustained calcium signaling in neutrophils affecting key functions of these cells, they also open up new therapeutic approaches to treat inflammatory disorders characterized by overwhelming neutrophil infiltration.

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**Virtual ABSTRACT/TABLE #7 Qiu-Xing Jiang**

# Chromogranin B anion channel and its potential roles in regulated secretion

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Regulated secretion is an important sensing and feedback pathway for unicellular and multicellular eukaryotes. Regulated release of preformed mediators is important for various types of cells in immune system. In human regulated secretion is a signature function of all exocrine, endocrine and neuronal cells. A long-standing mystery in regulated secretion is the identity of the anion conductance that was found necessary for secretory granule acidification four decades ago. Controversy on CLC-3 acting in such a role has been a deterrent for progress in the past decade. By serendipity, we discovered that recombinant chromogranin B (CHGB) forms a chloride channel in membrane. Here, we provide multiple lines of evidence supporting that native CHGB serves the long-sought anion channel in regulated secretion. High-resolution microscopy and biochemical assays showed that a major fraction of native CHGB in secretory granules is membrane-bound, which keeps a majority of CHGB in close vicinity of granular membranes. Biochemical reconstitution found that native CHGB in the membrane-bound and soluble states both retain the capability of reconstituting anion channels. After granule release, CHGB remains clustered on cell surface and dominates anion flux across cell membranes. Intragranular pH measurements demonstrate that normal granule acidification relies on CHGB anion conduction. Lack of CHGB channel activity impairs insulin maturation and dopamine-loading in INS-1 and PC12 cells, respectively. CHGB-null mice show severe granule deacidification in primary pancreatic beta-cells, accounting for impaired insulin maturation, altered glucose-tolerance and low dopamine concentration in chromaffin granules previously observed in these mice. All experimental observations support coherently that the highly conserved CHGB anion channel licenses normal granule acidification and maturation in regulated secretion.

**Virtual ABSTRACT/TABLE #8 Mariella Kessler**

# Role of adhesion-dependent $Ca^{2+}$ microdomains in T cell priming during migration to inflamed tissue

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Adhesion-dependent T cell priming may play a central role in adaptive immune response. It describes an adhesion-dependent change in sensitivity of T cells to facilitate full T cell receptor (TCR)/CD3 mediated activation[1]. During immune response, T cells migrate from blood vessel walls to inflamed tissue by crossing the endothelial membrane and subsequently elements of the extracellular matrix (ECM). Thereby, integrins, adhesion molecules expressed at the surface of T cells, facilitate binding to endothelial cells and ECM proteins. $Ca^{2+}$ microdomains have been identified as the earliest cytosolic signals that occur upon T cell activation. Previously, our group observed rarely occurring $Ca^{2+}$ microdomains before TCR/CD3 stimulation involving Orai channels and STIM proteins[2].

Here, we hypothesize that infrequent $Ca^{2+}$ microdomains observed before TCR/CD3 stimulation are initial elements of T cell pre-activation and occur in response to adhesion to endothelial cells or to ECM proteins of the basement-membrane or interstitium. Using a high-resolution live cell–imaging technique, we show that blocking with anti-integrin monoclonal antibodies of integrin receptors such as α2/β1 (binds to collagen-IV/VI), α6/β1 (binds to laminin-1) and αL/β2 (binds to the intercellular adhesion molecule, ICAM-1), but not to α5/β1 results in a significant decrease of adhesion-dependent $Ca^{2+}$ microdomains. Next, adhesion of T cells on collagen-IV and laminin-1 resulted in a significant increase of $Ca^{2+}$ microdomains, which was not the case for ICAM-1 or collagen-VI. Moreover, these adhesion-dependent $Ca^{2+}$ microdomains were significantly reduced by blocking the downstream integrin signaling pathway using focal adhesion kinase (FAK) inhibitor PF562,271 and phospholipase C (PLC) inhibitor U73122. Likewise, deletion of all three D-*myo*-inositol 1,4,5-triphosphate receptor ($IP_3R$) genes showed a significant decrease in adhesion-dependent $Ca^{2+}$ microdomains. These results indicate, that the pre-activation state of T cells might be achieved by adhesion to basement-membrane proteins like collagen-IV and laminin-1, involving FAK, PLC and $IP_3Rs$ for $Ca^{2+}$ microdomain formation.

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**Virtual ABSTRACT/TABLE #9 Imrankhan Khansahib**

# HN1L/JPT2: a signaling protein connecting NAADP to $Ca^{2+}$ microdomains

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Nicotinic acid adenine dinucleotide phosphate (NAADP) is the most potent $Ca^{2+}$ mobilizing second messenger that effectively regulate $Ca^{2+}$ signaling in T cells (B.-P. Diercks et al. 2018. Sci. Signal. 11, eaat0358). The target channels of NAADP are controversially discussed. In sea urchin eggs, TPCs were shown to respond to NAADP (P. J. Calcraft et al. 2009. Nature. 459, 596–600). Whereas in T cells, strong experimental evidence for NAADP activating RYR1 was obtained (M. F. Langhorst et al.2004. Cell. Signal. 16, 1283–1289). Surprisingly, neither RYR1 nor TPCs were labeled with NAADP photoaffinity labelling (PAL), but only small cytosolic proteins (T. F. Walseth et al. 2012. Messenger (Los Angel). 1, 86–94). With these findings, we formulated an hypothesis that NAADP bound to one (or more) cytosolic NAADP binding proteins (NAADP BPs) activates different $Ca^{2+}$ channels localized at different organelles, depending on cell type and specific signaling pathway (A. H. Guse. 2012. Sci Signal. 5, pe18). Here, we report identification of an NAADP BP, termed hematological and neurological expressed 1-like protein (HN1L)/ jupiter microtubule associated homolog 2 (JPT2).

HN1L/JPT2 was purified by liquid chromatography and identified by mass spectrometry. PAL results showed that HN1L/JPT2 is a high affinity NAADP BP. Gene knock-out of *Hn1l/Jpt2* in human jurkat and primary rat T cells resulted in decreased numbers of initial $Ca^{2+}$ microdomains, and delayed onset and decreased amplitude of global $Ca^{2+}$ signaling. T cell receptor/CD3 dependent co-precipitation of HN1L/JPT2 with RYR and co-localization data suggest that HN1L/JPT2 connects NAADP formation with RYR1 in first seconds of T cell activation. While we obtained evidence connecting HN1L/JPT2 with NAADP and RYR1 in T cells, interaction of HN1L/JPT2 and TPC1 has also been reported (G. S. Gunaratne et al. 2021. Sci. Signal. 14, eabd5605). Thus, HN1L/JPT2 is an essential component of the NAADP signalosome.

**Virtual ABSTRACT/TABLE #10 Trisha Mahtani**

# The ion channel TRPV5 regulates B cell activation and signalling

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Recognition of antigen by the B cell receptor (BCR) initiates activation and downstream signaling cascades. Calcium is a key secondary messenger and its influx is regulated by ion channels on the plasma membrane, predominantly known to be Calcium Release-Activated Calcium (CRAC) channels. However, B cells deficient in CRAC channel components mount normal antibody responses, suggesting that alternative channels may be important. We identified a member of the calcium permeable transient receptor potential (TRP) ion channel family, TRPV5, as a candidate channel expressed in B cells by a qPCR screen. We found TRPV5 polarized to BCR clusters upon stimulation in a RhoA dependent manner and siRNA knockdown of TRPV5 in a murine B cell line impaired calcium flux upon BCR stimulation. To further investigate B cell responses, we generated a murine TRPV5 knockout by CRISPR-Cas9. B cell development or peripheral B cell compartments were not impacted by TRPV5 deletion. Unexpectedly, calcium influx upon BCR stimulation was not impacted by loss of TRPV5 in these cells; however, BCR signaling was reduced. Immunization with a T-dependent antigen reduced antigen-specific IgG responses in TRPV5-deficient mice in comparison to WT. Thus, our findings demonstrate TRPV5 plays a novel role in BCR signaling and B cell activation.

**Virtual ABSTRACT/TABLE #11 Rakesh Kumar Majhi**

# Thermosensitive TRP channels modulate T cell activation

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Transient Receptor Potential (TRP) channels are present on surface as well as intracellular organelles and act as $Ca^{2+}$-sensing elements. These channels regulate intracellular $Ca^{2+}$ levels and initiate different regulatory pathways, which control immune cell activation and immune response. The TRP channels are regulated by several endogenous and exogenous factors and are often negatively regulated by excess $Ca^{2+}$ ions.

In this work, we have explored the endpgenous expression, specific localization and functional significance of thermosensitive TRP channels TRPV1, TRPV4, TRPA1 and TRPM8 in T cells, especially presenting a comparison of the role of heat and cold sensitive TRP channels in T cell activation.[1-3] Inhibition of TRP channels prevents the increment in intracellular $Ca^{2+}$ levels required for T cell activation by ConA/CD3-CD28. While the heat sensitive TRP channels are essential for TCR mediated T cell activation, cold sensitive TRPM8 appears to be dispensable. The thermosensitive TRP channels regulate T cell proliferation and cytokine response. Subsequently it has been demonstrated that these thermosensitive TRP channels are essential for immune activation during fever, with important functional consequences in both CD4+ve and CD8+ T cells.

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**Virtual ABSTRACT/TABLE #12 Laura Marongiu**

# Inositol 1,4,5-trisphosphate 3-kinase B promotes $Ca^{2+}$ mobilization and the inflammatory activity of dendritic cells

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Innate immune responses to Gram-negative bacteria depend on CD14, TLR4, and MD-2 that form the receptor complex for lipopolysaccharide (LPS). CD14 transfer LPS to TLR4 for NF-kB activation, mediates TLR4 endosome relocation for Type I Interferon production, and induces $Ca^{2+}$ mobilization for NFAT activation. $Ca^{2+}$ mobilization is one of the first events in the activation of the NFATc1-4, the four main members of the NFAT pathway. In electrically non-excitable cells, the main $Ca^{2+}$ entry pathway is regulated by the depletion of intracellular $Ca^{2+}$ stores, coupled with the opening of plasma membrane channels, a mechanism called store-operated $Ca^{2+}$ entry (SOCE). In immune cells, SOCE is the only pathway described for $Ca^{2+}$ mobilization that leads to NFAT activation. SOCE is initiated by antigen binding to the T and B cells receptors, resulting in activation of phospholipase C (PLC) and production of inositol 1,4,5-triphosphate ($IP_3$); this second messenger binds to the $IP_3$ receptors ($IP_3Rs$) located on the membrane of endoplasmic reticulum (ER), and causes a rapid $Ca^{2+}$ release from the ER, followed by the opening of $Ca^{2+}$ release-activated $Ca^{2+}$ (CRAC) channels at the cell surface. This process generates a sustained increase of intracellular $Ca^{2+}$ concentration that is necessary for activation of NFATc.

We have identified the mechanism of $Ca^{2+}$ mobilization in mouse and $CD14^+$ human DCs. In contrast to the T and B cells, following LPS stimulation, $IP_3$ is generated and metabolically converted to $Ins(1,3,4,5)P_4$, by the $IP_3$ kinase (ITPK)B. $Ins(1,3,4,5)P_4$ generation is required to open $IP_3$ receptor 3 ($IP_3R3$) channels co-localized with CD14 at the cell surface; this induces a direct extracellular $Ca^{2+}$ influx, which activates NFAT. ITPKs pharmacological inhibition restrains tissue swelling as well as inflammatory arthritis severity similar to the direct inhibition of NFAT by nanodrugs. ITPKB represents a new target for anti-inflammatory therapies aimed at inhibiting specific DC functions.

**Virtual ABSTRACT/TABLE #13 Doreen Matthies**

# Cryo-EM of channels and transporters: What have we learned in the last decade and what are the current challenges?

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In the last decade the field of Structural Biology has made great advances in using electron microscopy to solve structures of protein complexes including membrane proteins to high resolution. Best practices of how to use Single Particle Cryo-EM and more importantly how to optimize a membrane protein sample such as an ion channel or a transporter will be discussed. Most membrane protein structures are currently resolved in a detergent micelle, but cryo-EM also makes it possible to look at membrane protein complexes in lipid bilayer, such as a lipid nanodisc, liposomes, or even inside cells now. A brief introduction to each of these techniques will be discussed with examples of the conformational landscape of magnesium channel CorA (Matthies et.al. 2016. *Cell.* 164 (4):747-756), voltage-gated potassium channel Kv1.2-2.1 (Matthies et.al. 2018. *Elife*. Aug 15;7), human excitatory amino acid transporter (Qiu et.al. 2021. *Science Advances.* 3:7-10), multi-drug exporter ArcB (Yao et.al. 2020. PNAS. 117:31:18497-18503) and the nuclear pore complex (Allegretti et.al. 2020. Nature. 586:796-800).

**Virtual ABSTRACT/TABLE #14 Ehud Ohana**

# A succinate transport pathway delivers succinate to macrophages thus perpetuating their pro-inflammatory metabolic state

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The gut metabolite composition determined by the microbiota has paramount impact on gastrointestinal physiology. However, the role that transporters which mediate gut metabolite absorption play in communicating with host cells during inflammatory diseases is poorly understood. We have identified the microbiota-determined output of the pro-inflammatory metabolite, succinate, and elucidated the pathways that control transepithelial succinate absorption and subsequent succinate delivery to macrophages. We showed a significant increase of succinate uptake into pro-inflammatory macrophages, which is controlled by $Na^+$-dependent succinate transporters in macrophages and epithelial cells. Furthermore, we found that fecal and serum succinate concentrations were markedly augmented in inflammatory bowel diseases and corresponded to changes in succinate-metabolizing gut bacteria. Together, our results describe a succinate production and transport pathway that controls the absorption of succinate generated by distinct gut-bacteria and its delivery into macrophages. In IBD, this mechanism fails to protect against the succinate surge, which may result in chronic inflammation.

**Virtual ABSTRACT/TABLE #15 Yoshifumi Okochi**

# Method of measuring the membrane potential of phagosomes by optical imaging

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Phagocytes are immune cells that ingest pathogens and dead cells to eliminate them from our bodies. Phagosomes are the specialized vesicles enclosing pathogens to kill and digest them. Since electrogenic proteins, NADPH oxidases and proton pumps, and voltage-gated proton channels function on phagosomes for producing ROS and acidifying within phagosome, the membrane potential of phagosomes have also been believed in regulating phagosomal functions. However, we still do not know the role of membrane potential in phagosome, because we lack the technique to measure membrane potential changes directly. To overcome this problem, we applied a FRET-based voltage sensor protein Merm2[(1)], previously developed in our laboratory, to optically image the phagosomal membrane potential. We utilized RAW264.7, a macrophage cell line, to stably express Merm2 (Merm2-RAW). We verified the robust expression of Merm2 localized in the plasma membrane, but also phagosomes that engulfed IgG-coated beads. Using this tool, we observed that during phagocytosis, phagosomes hyperpolarize, whereas the plasma membrane depolarizes. Occasionally, we found that some of phagosomes depolarize before hyperpolarizing. Pharmacological inhibition of the hyperpolarization observed in phagosomes with paxilline and niflumic acid suggests that $K^+$ and $Cl^-$ contribute to the hyperpolarization. Paxilline blocks the activity of calcium-activated BK (Slo1) channels, thus increased $Ca^{2+}$ in the cytoplasm ($[Ca^{2+}]_{cyto}$) perhaps facilitates the hyperpolarization in phagosomes. To test the idea, we monitored the $[Ca^{2+}]_{cyto}$ together with the membrane potential by imaging Merm2 and RGECO, a $Ca^{2+}$ sensor protein. We observed during phagocytosis, $[Ca^{2+}]_{cyto}$ is increased with timing identical to that of the hyperpolarization in phagosomes in some cases. This result suggests that the elevation of $[Ca^{2+}]_{cyto}$ induces the hyperpolarization in phagosomes, and may be mediated by BK channels.

References:

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**Virtual ABSTRACT/TABLE #16 Camille Rabesahala de Meritens**

# Neutrophil extracellular trap (NET) formation in mice with *Stim1/2 m*yeloid-specific ablation.

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Neutrophils are the most abundant circulating white blood cells protecting the body from infectious agents. Cytosolic $Ca^{2+}$ mobilisation in neutrophil physiology is critical, impacting processes such as phagocytosis, chemotaxis, cytokine transcription, bacterial activity and neutrophil extracellular trap release (NETosis). Store-operated $Ca^{2+}$ entry (SOCE), reliant on STIM-ORAI interactions is the major $Ca^{2+}$-entry pathway, however, how SOCE affects neutrophil activity remains elusive. The formation of NETs, composed of decondensed DNA carrying proteolytic enzymes, neutrophil elastase (NE) and myeloperoxidase (MPO), allows them to immobilize and then degrade target bodies extracellularly. Despite NETosis being known to be involved both in beneficial and detrimental ways in disease, the extent to which neutrophils rely on STIM proteins and $Ca^{2+}$ mobilisation for NETosis is unknown. Thus, we studied NET formation and $Ca^{2+}$-dependent effector functions in bone-marrow neutrophils derived from mice with *cre-lox* dependent ablation of *Stim1* and *Stim2* in myeloid cells (double knockout; dKO). As expected, the cytosolic $Ca^{2+}$ elevations evoked by thapsigargin (Tg) or by the chemoattractant fMIVIL were dramatically lowered in *Stim* dKO neutrophils. Interestingly, reactive oxygen species (ROS) production evoked by fMIVIL was also reduced in *Stim* dKO neutrophils, confirming earlier reports that efficient activation of the phagocytic NADPH oxidase (NOX2) requires STIM proteins. We observed that fMIVIL also induced NETs and these were reduced in dKO animals, suggesting calcium and/or ROS might drive NETosis downstream of fMIVIL. NETosis is also observed with monosodium urate crystals (MSU) treatment, indeed, our data indicate that NET formation evoked by different stimuli is reduced in dKO mice. These results suggest that NETosis may rely on STIM proteins, possibly by downstream $Ca^{2+}$-signalling, exocytosis of NE and MPO, or ROS-dependent signalling pathways leading to DNA decondensation. We are currently using genetic and chemical interventions to define the relative contribution of STIM proteins, $Ca^{2+}$ and ROS signalling for NET production.

**Virtual ABSTRACT/TABLE #17 Yuqing Wang**

# Bioengineered bacteria for the oral delivery of Kv1.3 blockers for the treatment of autoimmune diseases

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CCR7$^-$ effector memory T ($T_{EM}$) lymphocytes are targets for immunomodulation for the treatment of rheumatoid arthritis (RA). Following activation, $T_{EM}$ cells upregulate the expression of the potassium channel Kv1.3 and blocking this channel inhibits $T_{EM}$ cell proliferation and secretion of pro-inflammatory cytokines with minimal effects of CCR7+ naïve and central memory T cells. Analogs of the small peptide ShK, engineered to enhance their selectivity for Kv1.3, are effective in reducing disease severity in animal models of RA and other autoimmune diseases and in patients with active plaque psoriasis. However, like most biologics, these peptides must be injected repeatedly. Here, we propose a novel approach for the delivery of the ShK analog ShK-235 by inducing its production and secretion by a probiotic bacterium, *Lactobacillus reuteri*, for oral delivery into the gastrointestinal tract. We designed LrS235, a bioengineered *L. reuteri* that secretes the ShK-235 peptide, and used single-cell patch-clamp to quantify ShK-235 in the culture supernatant of LrS235 and in the circulation of healthy rats gavaged with LrS235. We used functional assays to define the effects of ShK-235 secreted by LrS235 on the proliferation of $T_{EM}$ cells *in vitro*. We next tested the efficacy of LrS235 in two animal models, delayed-type hypersensitivity (DTH) and collagen-induced arthritis (CIA). Supernatants from LrS235 block Kv1.3 currents and inhibit human $T_{EM}$ cells proliferation and IL-2 and IFN-g production *in vitro*. A single oral gavage of LrS235 in healthy rats results in sufficient levels of ShK-235 in the circulation to block Kv1.3 channels and reduces inflammation in the DTH model. The daily oral gavage of LrS235 is efficacious in reducing clinical signs of disease and joint inflammation in rats with CIA. Our results demonstrate the potential of using the probiotic *L. reuteri* as a novel oral delivery system for ShK-235, and possibly other biologics, to treat RA.

**Virtual ABSTRACT/TABLE #18 Kai Johannes Winterberg**

# NADPH oxidases produce NAADP under physiological conditions

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NAADP is a potent $Ca^{2+}$ releasing second messenger with an important role in T cell activation. After stimulation, NAADP is rapidly formed, contributing to the first $Ca^{2+}$ signals in T cells (Gasser, Bruhn et al. 2006. Journal of Biological Chemistry 281(25): 16906-16913., Wolf, Diercks et al. 2015. Science Signaling 8(398): 1-13). These signals later influence T cell effector functions, making the NAADP signaling pathway an attractive target for pharmacologic interventions (Dammermann, Zhang et al. 2009. Proc Natl Acad Sci U S A 106(26): 10678-10683). The therapeutic potential of modulating NAADP signaling was demonstrated using the NAADP-antagonist BZ194 in a mouse model of multiple sclerosis (Zhang, Watt et al. 2018. Scientific Reports 8(1): 1-17), further highlighting the importance of identifying the key components of this signaling pathway. While recent progress has been made on the nature of NAADP's receptor (Gunaratne, Brailoiu et al. 2021. Sci Signal 14(675), Roggenkamp, Khansahib et al. 2021. Sci Signal 14(675), Zhang, Guan et al. 2021. Nat Commun 12(1): 4739), the mechanism of NAADP formation has remained largely elusive. The previously described candidate enzymes CD38 and SARM1 can form NAADP from NADP in vitro through a base-exchange reaction, but only under specific conditions (acidic pH and millimolar concentration of nicotinic acid) (Aarhus, Graeff et al. 1995. Journal of Biological Chemistry 270(51): 30327-30333, Zhao, Xie et al. 2019. iScience 15: 452-466), thus requiring compartmentalization into lysosomes together with an active transport of NAADP precursors (Nam, Park et al. 2020. FASEB Journal 34(9): 12565-12576), making it unlikely that these enzymes account for the fast rise in NAADP concentration observed during early T cell activation. Therefore, we set out to find other NAADP forming enzymes by establishing an in vitro assay for NAADP synthesis, in which we measured the activity of cell membranes isolated from different cell types. Using a high-performance liquid chromatography (HPLC) system optimized to detect small changes in the concentration of different nucleotides, we discovered that several members of the NADPH-oxidase enzyme family are able to synthesize NAADP from its reduced precursor NAADPH under physiological conditions. Additionally, we identified glucose-6-phosphate-dehydrogenase as the only cellular dehydrogenase able to reduce NAADP to its inactive precursor, pointing towards a potential NAADP/NAADPH redox cycle as a key element in the formation of early T cell calcium signals.

**Virtual ABSTRACT/TABLE #19 Daniel M. Collier**

# Extracellular histone proteins activate P2X7 channels

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Histone proteins are elevated in circulation via neutrophil release during the innate immune response. We previously demonstrated that histone proteins induce $Ca^{2+}$ influx in the vascular endothelium of resistance-sized mesenteric arteries. An increase in endothelial cell $Ca^{2+}$ is canonically expected to promote vasodilation through a variety of pathways including endothelial and smooth muscle cell hyperpolarization. Interestingly, histone induced $Ca^{2+}$ influx does not cause vasodilation, and prevents vasodilation to known endothelium dependent vasodilators (GSK1016790A and NS309; Collier *et al*. 2019. AJP Heart. 316: H1309-H1322). We hypothesized that the mechanism of histone induced vascular dysfunction is due to endothelial cell death or activation of depolarizing currents via unidentified non-selective cation channels. To improve our ability to assay endothelial cell death in native tissue, we used dual-inverted selective plane of illumination microscopy (diSPIM). diSPIM provides higher signal and resolution with lower photobleaching and phototoxicity than confocal microscopy. We found that histone proteins cause low levels of endothelial cell death (assayed by propidium iodide labeling) and less cell death than GSK1016790A - a potent, selective, activator of endothelial cell $Ca^{2+}$ influx and vasodilation via the TRPV4 channel. Thus, we focused on identifying candidate non-selective ion channels with large extracellular domains that could potentially interact with circulating histone proteins. Various members of the ionotropic purinergic receptor (P2X) family have been reported in vascular endothelial and smooth muscle cells. P2X antagonists, Suramin, PPADS, and TNP-ATP, block histone induced EC $Ca^{2+}$ influx, suggesting P2X receptors are necessary for histone induced EC $Ca^{2+}$ influx. We confirmed P2X7 protein expression in resistance-sized mesenteric arteries via immunofluorescence with extracellular, N-terminal, and C-terminal targeted P2X7 antibodies. When expressed in heterologous cells, P2X7 is sufficient for histone-induced depolarizing currents. This discovery provides the framework for understanding the role of endothelial cell resident P2X channels in the innate immune response and cardiovascular physiology.

**Virtual ABSTRACT/TABLE #20 Vivian Gonzalez-Perez**

# Goblet cell LRRC26 regulates BK channel activation and protects against colitis in mice

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Goblet cells (GCs) are specialized cells of the intestinal epithelium contributing critically to mucosal homeostasis. One of the functions of GCs is to produce and secrete MUC2, the mucin that forms the scaffold of the intestinal mucus layer that coats the epithelium and separates the luminal pathogens and commensal microbiota from the host tissues. Although a variety of ion channels and transporters are thought to impact on MUC2 secretion, the specific cellular mechanisms that regulate GC function remain incompletely understood. Previously, we demonstrated that leucine-rich-repeat-containing protein 26 (LRRC26), a known regulatory subunit of the $Ca^{2+}$-and voltage-activated $K^+$ channel (BK channel), localizes specifically to secretory cells within the intestinal tract. Here, utilizing a mouse model in which MUC2 is fluorescently tagged thereby allowing visualization of single GCs in intact colonic crypts, we show that murine colonic GCs have functional LRRC26-associated BK channels. In the absence of LRRC26, BK channels are present in GCs, but are not activated at physiological conditions. In contrast, all tested MUC2-negative cells completely lacked BK channels. Moreover, LRRC26-associated BK channels underlie the BK channel contribution to the resting transepithelial current across mouse distal colonic mucosa. Genetic ablation of either LRRC26 or BK-pore forming □-subunit in mice results in a dramatically enhanced susceptibility to colitis induced by dextran sodium sulfate (DSS). Recently, we have identified the presence of BK channels containing LRRC26 in a subset of cells from human epithelial spheroids derived from colonic biopsies. Overall, our results demonstrate that normal potassium flux through LRRC26-associated BK channels in GCs has protective effects against colitis in mice. Our recent findings in human cells suggest that this type of BK channel might play a similar role in human colonic epithelium.

**Virtual ABSTRACT/TABLE #21 Edries Yousaf Hajam**

# The role of immune cells in dermal white adipose tissue homeostasis

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Homeostasis is a dynamic process as the tissues in our body continuously undergo various changes to maintain their structure and function. For instance, the various compartments of our skin, such as the hair follicle and DWAT (dermal white adipose tissue) undergo continuous cyclic changes of growth and regression. Understanding the mechanism of cyclic changes in DWAT is important as these changes are known to play crucial roles in homeostasis and disease conditions. Various processes that increase or decrease white adipose tissue are known, but the question is which of those processes are involved in the dynamic changes in the DWAT. Here, we report Lipophagy as a main contributor of the decrease in DWAT.

It is well established in the literature that during homeostasis, various immune cells undergo cyclic changes in numbers as well as in the activation status and are in sync with cycling of DWAT. Thus, we asked if inflammatory cells instruct the adipocytes in the skin to undergo cyclic changes. To investigate this hypothetical link, we employed a mouse model with functionally impaired regulatory T cells (T-regs), (which are known to supress both innate and adaptive immune cells). This model is reported to have severe inflammation, Strikingly, we observed a complete loss of DWAT as well. We also found that loss of DWAT was induced in RAG1 null (mouse lacking B and T cells) upon transfer of immune cells from the mouse model with functionally impaired T-regs. Mechanistically we found that CD4+ T and Macrophages create an inflammatory microenvironment prior to the loss of DWAT. Rescue experiments suggest that CD4+ T cells and Macrophages have specific role to play in DWAT homeostasis. Thus, we found a novel non-immune function of immune cells in controlling DWAT homeostasis.

**Virtual ABSTRACT/TABLE #22 Changhao He**

# Mechanism of glucocorticoid-induced TNF receptor activation by an agonistic antibody

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The glucocorticoid-induced tumor necrosis factor receptor (GITR) is a member of the tumor necrosis superfamily of proteins and is expressed on T cells where it promotes and modulates immune responses. Activation of GITR is driven by interaction with its ligand (GITRL) which is proposed to cluster the receptors into a high-order array and thereby trigger a cellular response. Receptor activation drives T cell antitumor activity which has motivated the development of therapeutic agonist IgG antibodies which are believed to mimic the endogenous ligand by clustering receptors. Here we solved the structure of full-length mouse GITR in complex with Fab fragments from the agonistic mouse antibody DTA-1. The receptor is a dimer and each subunit binds one Fab in an orientation that suggests a clustering model of antibody agonism. Binding experiments with purified receptor, ligand, and antibody also support this model, and suggest that antibodies and ligands both cluster receptors as part of a common activation mechanism. Cell signaling functional experiments show that DTA-1, and the anti-human GITR antibody TRX518, drive GITR signaling in their IgG forms but not in their Fab forms. Taken together our results provide a structural and mechanistic framework to understand GITR signaling and activation by therapeutic antibodies.

**Virtual ABSTRACT/TABLE #23 J. Ashot Kozak**

# The function of TRPM7 channel-kinase in T lymphocytes and macrophages: insights from transgenic mouse models

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Transient receptor potential melastatin 7 (TRPM7) is a dual cation channel/protein kinase highly expressed in T lymphocytes, macrophages, mast cells and neutrophils. TRPM7 is one of three mammalian channel-enzymes (*chanzymes*). TRPM7 channels require the membrane phosphoinositide $PI(4,5)P_2$ for activation and are inhibited by cytosolic magnesium, polyamines and protons. The mechanism of channel inhibition by these cations is electrostatic screening/sequestration of $PI(4,5)P_2$ (Zhelay et al. 2018. J. Biol. Chem. 293: 18151-18167). TRPM7 has been linked to cellular and body magnesium homeostasis. Several animal models have been generated to investigate the functions of TRPM7 on cellular and whole-animal levels. The global knockout of TRPM7 or the kinase domain leads to embryonic lethality, whereas the TRPM7 “kinase-dead” (KD) mouse, with an inactivating K1646R mutation in the kinase domain (Kaitsuka et al. 2014. Sci. Rep. 4:5718) is viable. We have used the KD mouse for assessing the contribution of kinase activity to T-cell proliferation, macrophage phagocytosis, calcium signaling, pH and interactions between channel and kinase activities of TRPM7 (Beesetty et al. 2018. Sci. Rep. 8:3023). We are presently exploring a complementary mouse model with a modified channel domain. In combination, these mouse models will allow us to dissect the individual contributions of TRPM7 channel and kinase to specific immune cell functions.

**Virtual ABSTRACT/TABLE #24 Yi-Tsang Lee**

# Engineering of photoswitchable single-domain intrabodies for biomedical applications

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As two prominent examples of intracellular single-domain antibodies or antibody mimetics derived from synthetic protein scaffolds, monobodies and nanobodies are gaining wide applications in cell biology, structural biology, synthetic immunology, and theranostics. We introduce herein a generally-applicable method to engineer light-controllable monobodies and nanobodies, designated as moonbody and sunbody, respectively. These engineered antibody-like modular domains enable rapid and reversible antibody-antigen recognition by utilizing light. By paralleled insertion of two LOV2 modules into a single sunbody and the use of bivalent sunbodies, we substantially enhance the range of dynamic changes of photo-switchable sunbodies. Furthermore, we demonstrate the use of moonbodies or sunbodies to precisely control protein degradation, gene transcription, and base editing by harnessing the power of light. Plant-derived photoswitches or genetically-encoded photosensitive modules from plants were used in this study.

**Virtual ABSTRACT/TABLE #25 Guolin Ma**

# Identification of a STIM1 splicing variant that promotes glioblastoma cell growth

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Store-operated Ca2+ entry (SOCE) is regarded as a highly-selective Ca2+ entry mechanism in both non-excitable and excitable tissues in mammals. The existence of two STIM and three ORAI isoforms, as well as the splicing variants of STIM1/2 and ORAI1-3, has greatly contributed to the functional diversity of SOCE in different tissues under varying physiological conditions. The various STIM-ORAI combinations lead to varying magnitudes of Ca2+ signals with distinct spatiotemporal features and different kinetics. Aberrant SOCE signaling has been implicated in a growing number of diseases, such as immunodeficiency, cardiovascular disease, diabetes and cancer. Thus, it is of great importance to explore what STIM-ORAI combinations forming SOC subtypes for each cell type and which ones contribute to Ca2+ entry in health and disease.

Here we report the identification of an alternatively spliced variant of STIM1, designated STIM1β, that harbors an extra exon to encode 31 additional amino acids in the cytoplasmic domain. STIM1β, highly conserved in mammals, is aberrantly upregulated in glioma tissues to induce augmented Ca2+ signaling. At the molecular level, the 31-residue insertion destabilizes STIM1β by perturbing its cytosolic inhibitory domain and accelerating its activation kinetics to more efficiently engage and gate ORAI calcium channels. Functionally, STIM1β depletion reduces SOCE in glioblastoma cells, suppresses cell proliferation and growth both in vitro and in vivo. Collectively, our study establishes a splicing variant-specific tumor-promoting role of STIM1β that could be potentially targeted for cancer intervention.

**Virtual ABSTRACT/TABLE #26 Gary C.H. Mo**

# Inflammatory IL-1β release from Gasdermin D pores is dynamically regulated by calcium-phosphoinositide signaling

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Pyroptosis is an important cell death program in response to caspase activation. It is thought to be a central feature of the inflammatory response in immune and other cells such as the endothelium. During this process, members of the effector gasdermin family, such as gasdermin D, localizes to and inserts into the plasma membrane, creating ~21 nm diameter membrane pores and facilitating the secretion of the matured cytokine IL-1β. These pores are thought to be permanently open, making the resultant osmotic imbalance highly damaging. However, this canonical view is inconsistent with how cells can survive the activation of these pores and suggests a deeper level of control and tuning for these pores. Here, we combine optogenetics, live cell calcium biosensing, and electrophysiology to demonstrate that these pores can behave as dynamic ion channels and show their calcium-phosphoinositide-dependent changes. We quantify repeated and fast opening-closing of these oligomeric pores, visualize their geometry, and identify the signaling components that control pore activity. Through the identification of a regulatory circuit for these pores, we suggest means to control the release of inflammatory cytokines by macrophages.

**Virtual ABSTRACT #27 Hai Nguyen**

# The voltage-gated $K^+$ channel Kv1.3 as a target for modulating neuroinflammation

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Chronic neuroinflammation contributes to the pathogenesis of many neurodegenerative, neuropsychiatric, and neurodevelopmental diseases. However, approaches aiming to relieve neuroinflammation should only target the secondary inflammatory damage without compromising beneficial immune functions. Microglia are the principal macrophages of the brain responsible for the maintenance of homeostasis during normal physiology. Microglia not only can rapidly initiate but can also prolong neuroinflammation in response to pathogenic insults. During microglia activation, many membrane receptors and ion channels undergo significant expression changes. The voltage-gated potassium channel Kv1.3 is an effective regulator of membrane potential and is overexpressed in activated human and rodent microglia associated with numerous inflammatory conditions. We and others have demonstrated that blocking Kv1.3 significantly inhibits microglial activation, proliferation, and production of the inflammatory mediators IL-1β, TNF-α, IL-6, and NO. Blocking microglial Kv1.3 also inhibits components of the inflammatory pathway, including the activation of NFκ-B, p38 MAPK, and the NLRP3 inflammasome. The mechanism underlying this anti-inflammatory action of Kv1.3 inhibition in microglia involves, in parts, the disruption of intracellular calcium signaling. In vivo treatment with Kv1.3-selective inhibitors prevent inflammation in multiple preclinical rodent models of neurodegenerative diseases. Here, we show that the genetic deletion or pharmacological inhibition of Kv1.3 with the selective small-molecule PAP-1 improve neurological deficits, reduce infarct size, and brain levels pro-inflammatory cytokines in both mouse and rat ischemic stroke models. We further show that deleting Kv1.3 is also effective in aged mice and mice of both sexes. Importantly, blocking Kv1.3 does not affect phagocytosis of neuronal debris or levels of IL-10 and brain-derived nerve growth factor. Thus, our findings suggest that selective targeting of Kv1.3-high microglia is an attractive therapeutic approach that fulfills the criteria required of an immunomodulator to not act as a general immunosuppressant for treating neuroinflammation.

**Virtual ABSTRACT/TABLE #28 Gyorgy Panyi**

# Cm28, a peptide from the venom of *Centruroides margaritatus,* obeys unique primary structure and inhibits Kv1.2 and Kv1.3 with high affinity

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Scorpion venoms are rich in ion channel modulator peptides toxins. Proteomic analysis of soluble venom of the Colombian scorpion *Centruroides margaritatus* revealed that it contains mainly peptides with molecular weights ranging between 2.5-8 kDa. Among them, a novel short peptide (named Cm28) consists of 27 amino acids with MW of 2820 Da inhibited voltage-gated potassium channels Kv1.2 and Kv1.3 with $k_d$ value of 0.96 and 1.45nM, respectively. There was no significant shift in the conductance-voltage (G-V) relationship for both channels in the presence of toxin. Toxin binding kinetics showed that the on- and off-rates are consistent with a pseudo-first order reaction, on-rate increases with toxin concentration whereas off-rate remains constant. Based on these we conclude that Cm28 is not a gating modifier, rather a pore blocker. During selectivity profiling against 5 other subtypes of Kv channels (Kv1.1, Kv1.5, Kv11.1, KCa1.1, KCa3.1), 2 subtypes of Nav channels (Nav1.5 and Nav1.4) and the proton channel hHv1, Cm28 did not affect the activity of any channel at a concentration of 150nM (100x of $k_d$ value for Kv1.3) except ~27% blockage of Kv1.1 current. In biological functional assay, we demonstrated that the Cm28 strongly inhibited the expression of the activation markers interleukin-2 receptor a (CD25) and CD40 ligand in anti-CD3-activated $CD4^+$ $T_{EM}$ lymphocytes. Sequence analysis identified Cm28 has less than 40% similarity with other known a-KTx from scorpions and lacks the typical functional dyad (lysine-tyrosine) required to block Kv channels. However, its unique amino acid sequence contains the three disulfide-bond trait of the well-characterized scorpion α-KTx which adopts the cysteine-stabilized a/b scaffold. Results suggest that the novel Cm28 peptide belongs to a new subfamily of a-KTx toxins.

**Virtual ABSTRACT/TABLE #29 Cesare Sale**

# Role of ERG1 $K^+$ channel in lymphocyte development

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It is well established that different types of ion channels, play a relevant role both in the normal process of T- and B- cell development, selection and function[1], as well as during lymphoblast's neoplastic transformation[2]. In our laboratory we have provided evidence that *ERG1b*, an isoform of the ether-a-gò-gò-related gene 1, encoding for a $K^+$ channel, is overexpressed in myeloma and leukemias, where its expression correlates with a worse prognosis both in AML and ALL[3]. The current hypothesis is that these facts can be traced back to a relevant role exerted by ERG1 at specific stages of lymphopoiesis. In this scenario our aim is to characterize the expression of ERG1 during early stages of lymphopoiesis and study how it affects B and T lymphocytes development, and the leukemogenic process. Preliminary studies on mice carrying a selective deletion of the *Erg1b* gene showed an unexpected block in the lymphocyte development both in the B and T lineages respectively at the proB and DN stages. Furthermore, BM cells of *Erg1b KO* mice displayed a reduced capacity to develop colonies in vitro. In the KO transgenic model, the signaling underpinning the lymphocytes differentiative mechanisms was found affected. Particularly we noticed a reduction in the level of AKT phosphorylation in the bone marrow. In WT animals, ERG1 was found expressed in the later stages of B (IgM+) and T (DP) cell development in BM and thymus, when the selection processes occur. Our results indicate an unprecedented physiological role of ERG1 in the processes of differentiation, selection, proliferation, and migration of lymphoid progenitor cells. Furthermore, the developmental block related to *Erg1* dysregulation might represent an initial step in the leukemic transformation. Additional studies are being conducted to elucidate how ERG1 $K^+$ channel dysregulation affects lymphocyte signaling.

[1] (Feske S. et al,. 2012. Nat Rev Immunol. 12(7): 532–547)
[2] (Arcangeli A. et al,. 2012. Curr Med Chem 2012; 19: 683–696)
[3] (Pillozzi S. et al,. 2014. Leukemia (2014) 28, 1352–1355)

**Virtual ABSTRACT/TABLE #30 Mark S. Shapiro**

# Blockers of TRPM7 & TRPM6 cation channels inhibit proliferation of B-lymphocyte lymphoma/leukemia cancers

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Various lymphomas are the seventh most common cancer in men and women in the nation. B lymphocytes (B cells) are physiologically activated by the binding of antigen to receptors on its cell surface, causing the cells to divide, proliferate and aggregate. If the activation process is dysregulated (for genetic, epigenetic or metabolic reasons, etc.) then the mature activated B cells can become neoplastic and with a further insult they can become cancerous. Therapies designed against individual types of cancers often target features that differ between malignant cells and the corresponding normal cells from which they arise. These genetic changes between cancerous and healthy cells can include oncogene activation, gene repression and modified cell surface receptor or ion channel expression. The transient receptor potential (TRP) family of ion channels is involved in a variety of physiological functions, including sensory perception, cellular metamorphosis and regulation of neurons, and are ubiquitously expressed in many different tissues It has recently been shown that activity of $Mg^{2+}$- and $Ca^{2+}$-permeable TRPM7 ion channels are required for activation of B-lymphocytes (lymphopoiesis). We thus *hypothesized* that proper activity of TRPM7, and the related TRPM6, channels are necessary for proliferation and aggregation of B-cell lymphomas. First, lymphopoiesis in LPS & IL4-activated mouse B lymphocytes was assayed via trypan-blue cell counting and optical measurement in terms of aggregation of cells. Immunoblotting showed activated B cells to display higher TRPM7 protein levels than naïve cells. The TRPM7 & 6 blocker, carvacrol (500 μM), greatly reduced proliferation/aggregation, but most cells survived after 48 hours, unlike unstimulated naïve B cells, which die in culture without stimulation. Under whole-cell patch-clamp of naïve and activated B cells, acute application of the TRPM7/6 blockers, carvacrol (500 μM) and NS8593 (50 μM), inhibited ~75-80% of the signature outwardly-rectifying current, indicating that TRPM6/7 channels are the major carriers of the inward cation current in those cells. Similar patch-clamp results were obtained in the lymphoma cell lines, SEM and RS411, which are good models for widely seen and intractable human B-cell lymphomas. In culture, both lymphoma lines proliferated and aggregate in an uncontrolled manner. However, inclusion of either carvacrol or NS8593 in the culture medium, for both cell lines greatly reduced the viability of the cells, with little to any proliferation or aggregation observed within 48 hours, and indeed the great majority of cells were dead within 48 hours. We propose TRPM6/7 channels as novel potential targets for novel anti-cancer therapy for lymphomas and leukemias, especially those with few treatment options.

**Virtual ABSTRACT/TABLE #31 Philip Steiner**

# Pharmacologic inhibition of TPC1 diminishes structural basis for endoplasmic reticulum and endo-lysosome interactions

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Rat basophilic leukemia cells (RBL) are often used as a model for primary mast cells, which are innate effector cells of allergic reactions. Previously, we reported that the two-pore channel, TPC1, plays an important role in the $Ca^{2+}$ homeostasis of intracellular organelles, such as endo-lysosomes and the endoplasmic reticulum (ER) of RBL-1 cells and mast cells (Arlt et al. 2020. PNAS. 117:18068-18078). Pharmacologic inhibition or genetic ablation of TPC1 results in enhanced anaphylactic responses in mice. However, there is a lack of ultrastructural knowledge that underlies these processes. We have therefore implemented 2D and 3D transmission electron microscopic (TEM) methods to investigate the ultrastructure of RBL-1 controls and cells treated with the plant alkaloid tetrandrine. It was reported before that tetrandrine acts as an inhibitor of TPCs (Sakurai et al. 2015. Science. 347:995-998). Our TEM investigations with RBL-1 controls depict that ER and endo-lysosomes are in close spatial proximity. Endosome-ER contact sites have been examined for their 2D ultrastructure with regard to a potential $Ca^{2+}$ exchange before (Kilpatrick et al. 2017. Cell Rep. 18:1636-1645). However, only 3D TEM tomography reveals the extent of contact surface between the two organelles. In comparison, these contact sites strongly decrease in cells, treated with tetrandrine. Moreover, it was observed that endolysosomes visibly enlarge after the tetrandrine treatment, which has recently been discussed in an osmotic context (Chen et al. 2021. BBA Mol. Cell Res. 1868:118921). Here, we aim at a better understanding of the role of TPC channels in the regulation of the crosstalk between ER and endo-lysosomes at an ultrastructural level. Correlating our findings with adapted energy dispersive X-ray analysis, as well as with molecular biological and immunological experiments could help clarify whether TPC channels indeed are promising pharmacological targets for the treatment of allergic hypersensitivity.

**In-Person ABSTRACT/TABLE #32 Emerson M. Carmona**

# Understanding the voltage-gated proton channel ($H_v1$) pH dependence to gain insights into its function in the immune system

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The voltage-gated proton channel ($H_v1$) dissipates local proton gradients in cells. $H_v1$ is expressed in a wide variety of cells of the immune system. Here, $H_v1$ is functionally coupled with the NADPH oxidase complex to sustain the production of reactive oxygen species. A significant limitation to characterize $H_v1$ functions in the immune cells is our poor understanding of the molecular basis of its biophysical properties. $H_v1$ has four membrane-spanning segments and lacks a pore domain. It has an intracellular N-terminal domain with unknown function and an intracellular C-terminal domain that forms a coiled-coil structure in the native dimeric channel. $H_v1$ gating is voltage-dependent, pH-dependent, and mechanosensitive. Each subunit of the dimer has a permeation pathway highly selective for protons that open cooperatively. Here, we present our advances in understanding the $H_v1$ pH-dependence using the monomeric form of the *Ciona intestinalis* $H_v1$. $H_v1$ opening probability at steady-state depends on the DpH imposed across the membrane, with a characteristic 40-mV shift per DpH unit of the conductance-voltage (G-V) curves. We showed that the monomeric $H_v1$ G-V curves also shifted according to the DpH value, validating the monomer as a model to study this channel property. Next, we demonstrated that the $H_v1$ voltage sensor is responsible for this DpH dependence by directly measuring the pH dependence of the $H_v1$ gating currents. A 60% of the energy stored in the proton chemical potential is coupled to the electrical work activating the $H_v1$ voltage sensor. Interestingly, gating currents kinetics depended on the internal and external pH values rather than the DpH value, suggesting that the voltage sensor activation involves pH-dependent transitions. We are currently purifying the human $H_v1$ to further understand the channel pH dependence and other functional properties of this interesting channel at the molecular level.

**In-person ABSTRACT/TABLE #33 Axel R. Concepcion**

# The volume regulated anion channel LRRC8C suppresses T cell function by regulating cyclic dinucleotide transport and STING-p53 signaling

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Volume-regulated anion channels (VRACs) are hexameric complexes formed by leucine-rich repeat-containing 8 (LRRC8) proteins. VRACs are responsible for the regulatory volume decrease (RVD) after cell swelling under hypotonic conditions by mediating the efflux of chloride ($Cl^-$), but they can also transport other osmolytes, anticancer drugs, antibiotics and cyclic dinucleotide (CDN) second messengers. LRRC8A is the obligatory subunit of VRACs, and combines with at least one of four other paralogs (LRRC8B-E). Whereas LRRC8A has been extensively studied, the physiological role of other LRRC8 paralogs is much less understood. We here identify LRRC8C as critical component of VRAC in T cells. Expression of LRRC8C was highest in T cells compared to other immune cells and regulated by IL-2/STAT5 signaling. Deletion of LRRC8C in $CD4^+$ T cells abolishes VRAC currents and RVD demonstrating its essential role in T cells. Although *Lrrc8c*$^{-/-}$ mice are phenotypically normal and show no obvious defect in T cell development, T cells of *Lrrc8c*$^{-/-}$ mice have increased cell cycle progression, proliferation, survival and cytokine production after T cell receptor (TCR) stimulation. *Lrrc8c*$^{-/-}$ mice exhibited exacerbated T cell-dependent experimental autoimmune encephalomyelitis (EAE) and enhanced immunity against influenza A virus (IAV) infection. The increased function of LRRC8C-deficient T cells was associated with enhanced $Ca^{2+}$ influx and downmodulation of p53 signaling, whereas inhibition of p53 degradation reversed the phenotype of *Lrrc8c*$^{-/-}$ T cells. We determined that LRRC8C mediates the transport of different immunoreactive CDNs in T cells that activate the STING pathway and p53. Inhibition of STING in wildtype T cells recapitulated the phenotype of *Lrrc8c*$^{-/-}$ T cells including enhanced proliferation and survival. Our results identify LRRC8C as a novel suppressor of T cell function and T cell mediated immunity.

**In-person ABSTRACT/TABLE #34 Miloni S. Dalal**

# Deletion of myeloid Pannexin-1 channels decrease chemokines expression and improves cognitive function after brain trauma

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Brain trauma leads to the activation of microglial and glial cells near the injury site, which consequently release proinflammatory cytokines and chemokines. This, in addition to blood brain barrier (BBB) disruption, promotes infiltration of leukocytes from the peripheral blood stream. One of the key molecules that is released during these events is Adenosine 5' triphosphate (ATP) which acts as a pro-inflammatory and chemotactic molecule through purinergic signaling and correlates with damage severity. A recent study has found that pannexin-1 (panx1) channels are the main conduits of ATP release from the various cell types. Our lab has studied the role of panx1 in the acute phase of TBI and found that pharmacological blockage of Panx1 channels in mice after TBI is neuroprotective and anti-inflammatory. More recently, we found that deletion of Panx1 in myeloid cells improves motor coordination and cognitive function and BBB leakage 6 days post-TBI. In this work, we have extended our study to examine long-term effects and found that myeloid panx1 KO mice still displayed improved memory and locomotor activity at 6 weeks after TBI. This also correlates with a reduction in wound herniation and stagnant BBB leakage compared to injured control mice. Deletion of Panx1 in myeloid cells significantly reduced infiltration of all leukocytes to the injury site 3 days post-TBI. The myeloid panx1 KO mice displayed low expression of various chemokines that were upregulated in control mice after brain trauma. Our findings suggest that Panx1 channels mediate infiltration of leukocytes via activation of chemotaxis signaling. We propose that Panx1 channels could serve as a therapeutic target for neuroinflammation and neuroprotection after brain trauma.

**In-person ABSTRACT #35 Bryce D. Delgado**

# Functional reconstitution of a mitochondrial calcium uniporter

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The mitochondrial calcium uniporter (Uniporter) is the primary mechanism for Ca2+ uptake into mitochondria. Mitochondrial calcium influx enables mitochondrial respiration to respond to cellular signaling events and regulates cells death. Previous work has characterized the Uniporter at a structural level. Difficulties associated with making electrical recordings from mitochondria and with reconstituting Uniporter function using purified protein, however, have limited studies of the channel's function. We present reconstitution of purified Uniporter protein in liposomes, a fluorescence-based assay to study Ca2+ uptake of the purified protein, and macroscopic electrical recordings. We find that the purified protein recapitulates hallmarks of endogenous Uniporters, which include: RuRed sensitivity, rectification, high Ca2+ selectivity, and a Ca2+~Sr2+>>>Mn2+~Ba2+>Mg2+ conductivity sequence. These studies set the groundwork for future investigations with auxiliary subunits and mutagenesis.

**In-person ABSTRACT/TABLE #36 Serap Erdogmus**

# Cavb1 regulates T cell expansion and apoptosis independently of voltage-gated $Ca^{2+}$ channel function

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T cell receptor (TCR) stimulation triggers a rise in intracellular $Ca^{2+}$ that is critical for many T cell functions and immune responses. The best-characterized $Ca^{2+}$ influx pathway in T cells is store-operated $Ca^{2+}$ entry (SOCE) through $Ca^{2+}$ release-activated $Ca^{2+}$ (CRAC) channels encoded by ORAI1. In addition, voltage-gated $Ca^{2+}$ channels (VGCC or $Ca_V$) have been reported to regulate $Ca^{2+}$ signaling and the function of T cells, but their mode of activation and role in electrically non-excitable T cells remains elusive. We here identified the auxiliary $Ca_V$b1 subunit, encoded by the gene *Cacnb1*, as a regulator of T cell function by using an shRNA screen for ion channels and transporters expressed in immune cells. *Cacnb1* was required for clonal T cell expansion after lymphocytic choriomeningitis virus (LCMV) infection. Deletion of *Cacnb1* in T cells increased apoptosis, whereas proliferation, cytokine production and $Ca^{2+}$ signaling were unaffected. Using patch clamp electrophysiology and $Ca^{2+}$ recordings to detect VGCC activity, we were unable to detect voltage-gated $Ca^{2+}$ currents or $Ca^{2+}$ influx in human and mouse T cells upon depolarization with or without prior TCR stimulation. Deletion of stromal interaction molecule 1 (STIM1), previously shown to inhibit the L-type VGCC $Ca_V$1.2 in leukemic T cells, failed to reveal voltage-gated $Ca^{2+}$ influx in primary T cells. Transcripts of several $\alpha_1$ pore subunits of VGCCs can be detected in human ($Ca_V$3.3, $Ca_V$3.2) and mouse ($Ca_V$2.1) T cells, but exon usage analysis showed that the most 5' exons of these genes are not transcribed, likely resulting in N-terminally truncated and non-functional proteins. Our findings demonstrate that although $Ca_V$b1 regulates T cell survival and expansion, these effects are independent of VGCC channel function.

**In-person ABSTRACT #37 Donald Gill**

# Orai Channel C-terminal Peptides – Calcium Signal Suppression and Potential for Immunomodulation

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Store-operated $Ca^{2+}$ signals are critical in controlling cell function. STIM $Ca^{2+}$ sensors in the ER interact with and activate PM Orai channels within ER-PM junctions. The STIM-Orai binding interface leading to channel gating is poorly understood. We proposed that STIM1 remotely gates the Orai1 channel through binding to the C-terminus of the Orai1 protein. The crystal structure of the Drosophila Orai channel suggests adjacent C-terminal helices (M4x) of dOrai subunits in the channel hexamer, form a dimer through hydrophobic Leu-Leu interactions. A key question is whether these M4x helices unfold and directly interact with STIM1 and/or whether they stabilize a dimerized STIM1 binding pocket. We constructed novel PM-tethered, CFP-tagged M4x peptides derived from each of the three mammalian Orai subtypes (Orai1, Orai2, Orai3), and examined FRET interactions with either YFP-tagged concatenated SOAR-SOAR dimers from STIM1, or whole STIM1. The Orai1-M4x peptide showed significant binding to the SOAR-SOAR dimer construct. Mutations in the key leucines (L273D or L276D) that mediate M4x helix-interactions in intact Orai1, reduced this FRET interaction. Intriguingly, the M4x peptides from Orai2 and Orai3 have much higher FRET with SOAR-SOAR, and this FRET was almost completely blocked by the equivalent Orai3 Leu mutations. The high affinity Orai3-M4x peptide is a powerful blocker of Orai1-STIM1 interactions. Moreover, Orai3-M4x peptide provides a valuable tool for defining the STIM-Orai binding interface. Our results reveal that the 19 AA M4x peptide in Orai3 functions as the primary STIM-binding site in mediating channel gating. The peptide may be used to predict new molecular modifiers of STIM/Orai coupling with important immunomodulatory actions.

**In-person ABSTRACT/TABLE #38 Michael E. Green**

# Gating and conduction in the KcsA and $K_v$1.2 channels from quantum calculations

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Potassium channels play a role in a variety of diseases, such as epilepsy, as well as other neurological disorders. Understanding the molecular details of the gating and conduction mechanisms of the channel would be necessary to design molecules for specific interactions with the channel. We are carrying out quantum calculations to determine the molecular configuration of the open and closed states of the $K_v$1.2 voltage gated potassium channel, and the bacterial KcsA potassium channel. The calculations include the local energy minima in the $K^+$ path in the pore of both of these channels, showing the interaction and reorientation of water molecules with respect to both the ion and the protein as the ion moves through the pore. The hydration of the ion before entering the selectivity filter, and its dehydration at the entrance to the selectivity filter, both appear to be critical. The behavior of confined water differs significantly from that of bulk water, and this is apparent in the behavior of water in the pore of the channel. Our calculations also concern a key role for protons in gating. We have already found a path for a proton through the voltage sensing domain (VSD) of Kv1.2 upon application of a voltage of appropriate magnitude (we do not need to use an order of magnitude larger voltage), can see a double proton path from the intracellular end of the VSD to the gate, and, based on results to date, are finding that two protons per VSD, when at the gate, can block $K^+$ passage. We will discuss the relevance of these findings for gating.

**In-person ABSTRACT #39 Ingo Lange**

# Novel compounds target drug-resistant Neuroblastoma through mechanism involving store-operated calcium entry

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Neuroblastoma (NB), an extra-cranial pediatric solid tumor, is the most common cancer in children under 1 year of age. High-risk NB patients have approximately 40% survival rate, a higher likelihood of developing resistance to treatments, and increased incidence of relapse. Despite the plethora of treatment strategies available for patients with NB, current treatment strategies are often ineffective with high-risk and aggressive NB due to widespread metastasis and lack of response to treatment. In addition, NB relapse occurs in more than 50% of high-risk NB patients, and relapsed tumors are resistant to current treatment options. Finding effective treatments for metastatic and drug resistant NB has been a challenge due to the myriad of complex mechanisms that promote drug resistance. Cytoplasmic, endoplasmic reticulum (ER) and mitochondria (MT) calcium homeostasis play a central role in regulating fundamental physiological processes including cell survival and cell death. Interestingly, many of these calcium signaling pathways are deregulated in cancerous cells, resulting in uncontrolled growth, increased metastatic potential, increased survival signaling, resistance to cell death and multi-drug resistance. In order to address this challenge, a library of polyphenolic phytochemicals was screened using a paired patient-derived model of drug-sensitive and resistant Neuroblastoma that would target these altered calcium-signaling pathways. Subsequently, two promising compounds were identified that mobilize calcium thorough mechanisms involving ER calcium release and mitochondrial calcium uptake, as well as store-operated calcium entry (SOCE). A dose-dependent effect was observed. At low concentration the compounds induced cell proliferation that involved components of SOCE, while increase in concentration lead to calcium overload and induction of cell death. Interestingly, the latter effect was more profound in drug-resistant cells that displayed increase in SOCE compared to the drug sensitive counterpart.

The polyphenolic compounds may present a useful tool in understanding of the role of SOCE in cell death and survival and potentially lead to more effective treatment options for high-risk, advanced stage and drug resistant NB.

**In-person ABSTRACT/TABLE #40 Lucile Noyer**

# Distinct roles of ORAI1 in T cell mediated allergic airway inflammation and antiviral immunity in the lung

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T cell activation following antigen recognition by the T cell receptor (TCR) depends on $Ca^{2+}$ signals that regulate many aspects of T cell function. TCR-induced $Ca^{2+}$ influx in T cells is mediated by store-operated $Ca^{2+}$ entry (SOCE) through $Ca^{2+}$ release activated $Ca^{2+}$ (CRAC) channels formed by ORAI1 proteins in the plasma membrane. How $Ca^{2+}$ signals regulate the function of distinct T cell subsets during immune responses *in vivo* is not well understood. We here investigated how SOCE controls Th1 and Th2 cell-mediated immune responses to pulmonary influenza A virus (IAV) infection and allergic airway inflammation after immunization by house dust mite (HDM) allergens. Infection of mice lacking ORAI1 in T cells with the H3N2 strain of IAV did not result in exacerbated pulmonary inflammation and viral burdens despite reduced $Ca^{2+}$ influx in T cells. The same *Orai1*$^{fl/fl}$*Cd4Cre* mice were protected however from airway inflammation after HDM immunization and challenge. A comparative *ex vivo* analysis of antigen-specific $CD4^+$ T cells responding to the HDM challenge or IAV infection showed that ORAI1 controls the expression of genes involved in cell cycle regulation including E2F transcription factors in allergic but not infectious pulmonary immune responses. Genetic deletion of ORAI1 impaired the antigen-specific expansion, differentiation, and function of Th2 cells. Treatment of HDM challenged mice with a CRAC channel inhibitor suppressed allergic airway inflammation but did not compromise immunity to IAV infection. Collectively our data demonstrate that Th2 immune responses are more dependent on CRAC channel function than Th1-mediated antiviral immunity, which may important implications for the treatment of allergic airway disease.

**In-person ABSTRACT/TABLE #41 Trayambak Pathak**

# STIM2 in colorectal cancer metabolism and progression

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Colorectal cancer (CRC) is the third most common type of cancer, accounting for 10% of all cases. Most cancer cells undergo a metabolic transformation to support uncontrolled growth and metastasis. To undergo a metabolic transformation, cancer cells must produce ATP and biomolecules, which requires a healthy mitochondrion. Recent studies indicate that STIM, a stromal interaction molecule present in the endoplasmic reticular membrane, can regulate mitochondrial function by regulating mitochondrial mass, shape, and bioenergetics. TCGA data analysis shows that the reduced STIM2 mRNA level significantly correlates with the reduced survival of CRC patients. To study the role of STIM1 and STIM2 in CRC progression, we have generated multiple clones of STIM1, STIM2, and STIM1/2 double KO HCT116 and DLD1 CRC cells through CRISPR/Cas9.

In contrast to STIM1 KO, loss of STIM2 resulted in increased OCAR, ECAR, ATP generation, and mitochondrial biomass. However, the mitochondria were significantly smaller in STIM2 KO cells. STIM2 KO cells also showed a significantly increased proliferation. Metabolic profiling of STIM2 KO cells showed an increased glucose dependency. Transcriptomic analysis of STIM2 KO cells showed increased expression of glycolysis enzymes, suggesting that STIM2 transcriptionally regulates glycolysis. Xenograft tumor modeling in mice resulted in significantly larger tumor size generation by the STIM2 KO HCT116 cells compared to control and STIM1 KO HCT116 cells. The mice injected with STIM2 KO cells also showed significantly enhanced metastasis to organs such as the liver and colon, resulting in higher lethality in STIM2 KO injected mice than STIM1 KO. Further investigation revealed that the enhanced invasion of STIM2 KO was due to increased expression of MMP14 and 16 in STIM2 KO cells. These results indicate STIM2, not STIM1 is critical for CRC cell viability and function. The specific STIM2-dependent pathways that control these distinct phenotypes are currently under investigation.

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**In-person ABSTRACT/TABLE #42 Jananie Rockwood**

# TRPM7 inactivation and the role of pH in phagocytic activity of murine macrophages

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Transient receptor potential melastatin 7 (TRPM7) is a unique protein that is both an ion channel and a protein kinase. TRPM7 is highly expressed in immune cells such as T cells, macrophages, neutrophils and mast cells. TRPM7 channels are inhibited by cytoplasmic $Mg^{2+}$, polyamines and acidic pH. The TRPM7 kinase-dead (KD mouse model) has been instrumental in understanding the role of this protein in immune cell function. We previously demonstrated that T cells isolated from the spleen of TRPM7 KD mice have a defect in blastogenesis, proliferation and reduced store-operated calcium entry (SOCE) with no change in TRPM7 current magnitudes (Beesetty et al. 2018. Sci. Rep. 8:3023). We have also investigated channel-kinase interaction in macrophages in this mouse model (Beesetty et al., 2021.FEBS J. 288:3585). Splenic macrophages isolated from WT and KD mice expressed F4/80, a marker found on red pulp macrophages, high levels of leukocyte adhesion and migration marker CD11b, C-type lectin receptor CD209b. The marginal metallophilic macrophage marker CD169 was also expressed. We examined phagocytosis using multiple approaches, finding that KD macrophages phagocytize more efficiently compared to WT. Since calcium signaling is required for Fc-receptor-mediated phagocytosis we investigated the intracellular $Ca^{2+}$ levels in these macrophages. KD macrophages had higher baseline calcium levels compared to WT but unlike T-cells, in KD macrophages no obvious differences in SOCE were apparent. Unexpectedly, we found that altered pH regulation was responsible for increased phagocytosis observed in TRPM7 KD macrophages. Increased phagocytosis was accompanied by cytosolic alkalinization and higher basal TRPM7 channel activity. Intracellular $Mg^{2+}$ sensitivity of KD and WT channels was similar. Pharmacological blockade of sodium-hydrogen exchanger 1 (NHE1) reversed the alkalinization and decreased both phagocytosis and channel activity. In conclusion, we have identified a new role for TRPM7 kinase as a regulator of cellular pH and phagocytosis.

**In Person ABSTRACT #43 Jonathan Soboloff**

# Suppression of Ca2+ signals by EGR4 controls Th1 differentiation and anti-cancer immunity in vivo

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While the zinc finger transcription factors EGR1, 2 and 3 are recognized as critical for T cell function, the role of EGR4 remains unstudied. Here we show that EGR4 is rapidly upregulated upon TCR engagement, serving as a critical 'brake' on T cell activation. Hence, TCR engagement of EGR4-/- T cells leads to enhanced $Ca^{2+}$ responses, driving sustained NFAT activation and hyper-proliferation. This causes profound increases in IFNγ production under resting and diverse polarizing conditions that could be reversed by pharmacological attenuation of $Ca^{2+}$ entry. Finally, an *in vivo* melanoma lung colonization assay reveals enhanced anti-tumor immunity in EGR4-/- mice, attributable to Th1 bias, Treg loss and increased CTL generation in the tumor microenvironment. Overall, these observations reveal for the first time, that EGR4 is a key regulator of T cell differentiation and function.

**In-person ABSTRACT #44 Sonal Srikanth**

# ORAI1 establishes resistance to SARS-CoV-2 infection by regulating tonic type I interferon signaling

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ORAI1 and STIM1 are the critical mediators of store-operated $Ca^{2+}$ entry by acting as the pore subunit and an endoplasmic reticulum-resident signaling molecule, respectively. In addition to $Ca^{2+}$ signaling, STIM1 is also involved in regulation of a cytosolic nucleic acid sensing pathway. Using *ORAI1* and *STIM1* knockout cells, we examined their contribution to the host response to SARS-CoV-2 infection. *STIM1* knockout cells showed strong resistance to SARS-CoV-2 infection due to enhanced type I interferon response. On the contrary, *ORAI1* knockout cells showed high susceptibility to SARS-CoV-2 infection as judged by increased expression of viral proteins and a high viral load. Mechanistically, *ORAI1* knockout cells showed reduced homeostatic cytoplasmic $Ca^{2+}$ concentration and severe impairment in tonic interferon signaling. Transcriptome analysis showed downregulation of multiple cellular defense mechanisms, including antiviral signaling pathways in *ORAI1* knockout cells, which are likely due to reduced expression of the $Ca^{2+}$-dependent transcription factors of the activator protein 1 (AP-1) family and *MEF2C*. Our results identify a novel role of ORAI1-mediated $Ca^{2+}$ signaling in regulating the baseline type I interferon level, which is a determinant of host resistance to SARS-CoV-2 infection.

**In-person ABSTRACT #45 Marta Stremska**

# Targeting the ion channel TRPM7 promotes the thymic development of regulatory T cells by promoting IL-2 signaling

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Regulatory T cells (Tregs) prevent autoimmunity and excessive inflammation by suppressing the responses of T effector cells (Teff)(Wong and Germain, 2017). Treg identity is largely governed by FOXP3 transcription factor and the loss of function mutations in *Foxp3* gene lead to severe, multiorgan auto-inflammatory syndromes: IPEX (Bennett et al., 2001) in humans and *Scurfy* in mice(Ramsdell and Ziegler, 2014). There is an ongoing quest to harvest Tregs suppressive capabilities, hence the search for pharmaceuticals which drive an increase in Treg numbers are of clinical importance. In our study, we show that TRMP7- deficient T cells in mice conferred their resistance to the T-cell driven concavalin A-induced hepatitis. Lck-Cre TRPM7$^{fl/fl}$ mice displayed lower perivascular infiltration of immune cells and decreased expression of inflammatory cytokines into the liver tissue. Concomitantly, we also found enriched Treg cell population in the livers. Our *in vitro* investigation revealed that TRPM7- deficient T cells activation profile and cell division are comparable to WT Teffs, although their cytokine secretion was somewhat aberrant. Through the assessment of the Lck-Cre TRPM7$^{fl/fl}$ mice thymi and spleens we found both organs to harbor increased Treg populations, which, when isolated, show increased STAT5 phosphorylation status and sensitivity to IL-2. Inhibition of TRPM7 channel with FTY720, an FDA-approved drug, enhanced *Foxp3* gene expression *in vitro* and potentiated the thymic output of functional Tregs into the periphery *in vivo*. Bone marrow transplant experiments revealed that the mechanism leading to increased Tregs in Lck-Cre TRPM7$^{fl/fl}$ mice is achieved through extrinsic factors: TRPM7 deficient cells seem to overproduce IL-2, leading to increased CD25 expression; but also, still unidentified cell autonomous factors, as mixed bone marrow chimeras displayed normal Teff:Treg ratio in their spleens and thymi.

**In-person ABSTRACT/TABLE #46 Anthony Tao**

# Transcriptomic and forward genetic screens to elucidate the role of ion channels and transporters in B cells

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Ion channels and transporters (ICTs) are critical for ion and metabolite homeostasis. Given their expansive repertoire of pharmacological modulators, ICTs represent a promising target for therapeutic manipulation (Alexander, S.P *et al*. 2017. Br J Pharmacol 174: S360-S446). Though understudied, various ICTs have functions in many immune cells (Feske, S *et al*. 2015. Annual Review of Immunology 33: 291-353). However, the role of ICTs in mature B cell function is especially ill-explored (Mahtani, T *et al*. 2019. Immunological Reviews 291: 104-122). B lymphocytes are cells of the adaptive immune system that are important for the antibody-mediated response to infection as well as the pathogenesis of many autoimmune diseases. A better understanding of ICTs in the context of B cells would thus have broad clinical impact. Despite this, a comprehensive investigation of all ICTs in the context of B cells has yet to be undertaken. To address this gap in knowledge, I have performed ICT-focused RNA-seq meta-analyses and CRISPR-based forward genetic screens in primary murine B cells to identify ICTs that potentially contribute to B cell function. Using CRISPR screens, we assayed the role of hundreds of ICTs in B cell activation/proliferation, antibody class-switch recombination, and plasma cell differentiation. Among the candidate ICTs identified by these screens, we found several novel ICTs that affect the function of primary murine B cells. These ICTs include a $Ca^{2+}$ pump, an amino acid transporter, and a bicarbonate transporter. To determine whether these ICTs have physiological roles *in vivo*, I have developed an adoptive transfer model whereby ICT-targeted B cells can be evaluated during an *in vivo* germinal center immune response. Thus far, these findings (1) confirm that many ICTs with various substrate specificities have unique functions in B cells and (2) reveal potential novel drug targets to regulate B cell function and humoral immune responses.

**In-person ABSTRACT/TABLE #47 Liwei Wang**

# Investigation of novel ion channels as potential next-generation therapeutic targets for MS

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Multiple Sclerosis (MS) is a demyelinating disease of the central nervous system (CNS), which affects more than 2.3 million people in the world. Encephalitogenic T cells including T helper (Th)1 and Th17 cells play a crucial role in the pathogenesis of MS. Emerging evidence shows that some ion channels either contribute to the process of autoimmunity or neurodegeneration in MS. However, in general, little is known about the function of ion channels and transporters (ICTs) in encephalitogenic T cells. This represents a major gap in our basic knowledge of immunity and a missed opportunity for the treatment of MS. To fill this gap, we designed and performed a shRNA based screen using the EAE mouse model for MS and complemented the screen with gene expression profiling by RNA-seq, which is aimed to identify ICTs that control the function of encephalitogenic T cells. Our screen identified > 30 ICTs that are depleted in T cells isolated from the CNS. we then prioritized the (i) most depleted and (ii) most highly expressed ICTs from the depletion hits and performed validation experiments by deleting these genes in 2D2 T cells using CRISPR/Cas9 gene editing. As a proof of concept, knockout of 4 depletion hits identified by shRNA almost completely prevent the development of EAE. Protection from EAE was associated with strong reduction of encephalitogenic T cells in the CNS. Of note, deletion of the hits did not affect T cell number in the spleen nor did it decrease the production of encephalitogenic Th1/Th17 cytokines, suggesting that deletion of the hits do not simply kill T cells or affect T cell function globally, but has more specific effects on T cells in the CNS. These findings suggest that depletion hits regulate the migration of $CD4^+$ T cells to the CNS, their proliferation or survival in the CNS. Future studies will i) focus on elucidating the mechanisms of these novel ion channels in encephalitogenic T cell function, and ii) assess if these channels can be the potential drug targets for curing EAE/MS.

**In-person ABSTRACT/TABLE #48 Yin-Hu Wang**

# Characterization of CRAC and other ion channels in inflammatory bowel disease

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Inflammatory Bowel Diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are autoimmune disorders whose prevalence in the US increased from 0.9% to 1.3% between 1999 and 2015. The causes of IBD are complex, and involve the balance between pathogenic, proinflammatory and tolerogenic immune cells including Th1/Th17 and T regulatory (Treg) cells in the intestine, respectively. Previous studies showed that several ion channels and transporters (ICTs), including $Ca^{2+}$ Release-Activated $Ca^{2+}$ (CRAC) channels, regulate T cell differentiation and function in IBD. We here characterize the role of CRAC channels in disease pathology using murine IBD models and patient samples. We found that genetic deletion of several CRAC channel components including STIM1 and ORAI1 prevented or attenuated IBD severity in a T cell transfer model of IBD by suppressing Th1 and Th17 cell function. Likewise, pharmacologic blockade using a selective CRAC channel blocker ameliorated disease activity and proinflammatory cytokine production. To analyze the effects of CRAC channel inhibition on immune cells in the colon lamina propria (CLP) of therapy refractory IBD patients, we treated lamina propria mononuclear cells (LPMCs) with a CRAC inhibitor followed by multiparametric analysis of the immune cell composition and function using mass cytometry (CyTOF). The LPMC composition of IBD patients was characterized by an enrichment of $CD4^+$ and $CD8^+$ effector T cells, Th17 and Treg cells as well as innate lymphoid cells (ILCs). CRAC channel inhibition attenuated B cell activation, the production of IL-2, IL-4, IL-6, IL-17, TNF-α and IFN-γ by human colonic T cells and ILCs, and that of IFN-γ by myeloid cells, without affecting the viability, differentiation and function of human intestinal epithelial cells. To investigate if other ICTs besides the CRAC channel regulate the differentiation and function of proinflammatory and regulatory T cells in IBD, we conducted two unbiased screens using a murine IBD model driven by the pathobiont *Helicobacter Hepaticus.* The first screen involved a transcriptomic analysis of ICT expression in lamina propria Treg and Th1/Th17 cells and the second a CRISPR based functional ICT screen *in vivo.* Both screens identified several ICTs that are likely to regulate T cell mediated inflammation in IBD and that may be novel targets for immunotherapy in IBD.

**In-person ABSTRACT #49 Wenlei Ye**

# A tale of yin and yang: TMEM16F gating, ion selectivity, and functional implications

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TMEM16F manifests dual functions of a $Ca^{2+}$-activated small-conductance ion channel and phospholipid scramblase, playing critical roles in T cell activation, syncytia formation and blood coagulation. In contrast to its paralogs, the TMEM16A/B calcium-activated chloride channels, the biophysical properties of TMEM16F were controversial in both gating kinetics and ion selectivity. Over the past few years, we have shown that TMEM16F $Ca^{2+}$-activation is synergistically regulated by membrane potential and membrane-tethered $PIP_2$ (Ye, et al., 2018, *PNAS*, 115: E1667). In addition, with the Q559K mutant that shows no current rundown and less outward rectification in excised patch, we found that the channel shifted its ion selectivity in response to the change of intracellular $Ca^{2+}$ concentration, with an increased permeability ratio of $Cl^-$ to $Na^+$ ($P_{Cl-}/P_{Na+}$) at a higher $Ca^{2+}$ level (Ye, et al., 2019, *eLife*, 8). These dynamic features in gating and ion selectivity suggest a mechanism that might unify the functional distinctions among the membrane proteins in the TMEM16 family, helping us better reveal their physiological roles.

**In-person ABSTRACT/TABLE #50 Wandi Zhu**

# PIEZO1 mediates a novel mechano-thrombotic pathway in diabetes

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Thrombosis, as the leading complication of common human disorders including diabetes, coronary heart disease, and infection, remains a global health burden. Current anticoagulant therapies which target the general clotting cascade are associated with unpredictable adverse bleeding effects, as the understanding of hemostasis remains incomplete. Here, using perturbational screening of patient peripheral blood samples for latent phenotypes, we identified dysregulation of the major mechanosensory ion channel PIEZO1 in multiple blood lineages in Type 2 diabetes mellitus (T2DM) patients. Hyperglycemia activates PIEZO1 transcription in mature blood cells and selects high PIEZO-expressing hematopoietic stem cell (HSC) clones. Activation of Piezo1 in HSCs under hyperglycemia upregulated genes that promote differentiation towards monocyte and dendritic cell lineages and those involved in the inflammatory responses. Elevated Piezo1 activity in platelets, red blood cells, and neutrophils in T2DM triggered discrete prothrombotic cellular responses, including an increase in thrombin generation and the formation of neutrophil extracellular traps. Inhibition of Piezo1 protected against thrombosis in both human blood and in zebrafish genetic models, particularly in hyperglycemia. Our findings identify a novel candidate target to precisely modulate mechanically induced thrombosis in T2DM and a screening method to predict patient-specific risk. Ongoing remodeling of cell lineages in hematopoiesis is an integral component of thrombotic risk in T2DM and related mechanisms may have a broader role in chronic disease.