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Review

Noncanonical ion channel signaling in neurovascular barrier regulation and immune cell trafficking

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Ion channels are classically regarded as regulators of electrical excitability, but their role in immune activation and barrier homeostasis extends far beyond global transmembrane ion flux. This functional diversity is, among other things, the result of noncanonical signaling pathways. In this review, we examine the neurovascular endothelium as a paradigm for barrier-associated neuroimmune pathology and discuss three mechanistic principles: (1) permeation-independent scaffolding via cytoplasmic interaction domains, (2) spatio-temporally restricted ion flux within signaling microdomains, and (3) compartmentalized or cargo-mediated signaling. These mechanisms shape endothelial barrier integrity, inflammatory junctional remodeling, and the diapedesis of immune cells across the blood-brain barrier. A deeper understanding of such noncanonical channel functions opens new therapeutic perspectives beyond conventional pore blockade, including the targeted modulation of signaling domains, protein interactions, and subcellular channel localization.

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Introduction

Ion channels were first established as regulators of electrical excitability and later emerged as central determinants of immune activation and tissue barrier homeostasis. While most work has focused on ion permeation, growing evidence supports noncanonical channel functions. We focus on neurovascular endothelium as a tractable paradigm for barrier-associated immune pathology and highlight three distinct forms (Figure 1):

a) *Permeation-independent signaling*. The biological outcome is not proportional to the ion currents and can be attributed to cytosolic interaction domains.
b) *Permeation-dependent signaling with spatial restriction*. Canonical ion flux occurs, but it is confined to microdomains.
c) *Compartment shift or atypical cargo*. Channels enable signal diversification through organelle localization or permeation of organic signaling molecules.

To facilitate conceptual orientation, the main differences between canonical and noncanonical ion channel signaling are summarized in Table 1.

Permeation-independent signaling: ion channels as structural scaffolds and signaling hubs

Permeation-independent signaling describes effects that do not scale with ionic currents, but rather rely on functional cytoplasmic channel domains. These cytoplasmic channel domains orchestrate, for example, assembly, localization, and timing of downstream effector signaling. We contend that in the future, further functional biological outputs will be attributed to the versatile functions of ion channels. This is particularly true given that high-resolution structural analysis also facilitates the elucidation of interactomes. We would like to highlight two notable examples in particular.

Cytosolic interactome assembly

The purinergic P2X7 receptor (P2X7R) provides a clear example of permeation-independent signaling by cytosolic interactome assembly. The exceptionally long C-terminus has no homology to other known proteins and acts as a scaffold for multiple protein–protein interactions (PPIs) independent of canonical ion conduction [1]. Truncation and mutagenesis studies in heterologous expression systems (HEK293 cells) as well as in macrophage-derived cell lines and primary murine macrophages have shown that removal of the distal C-terminus

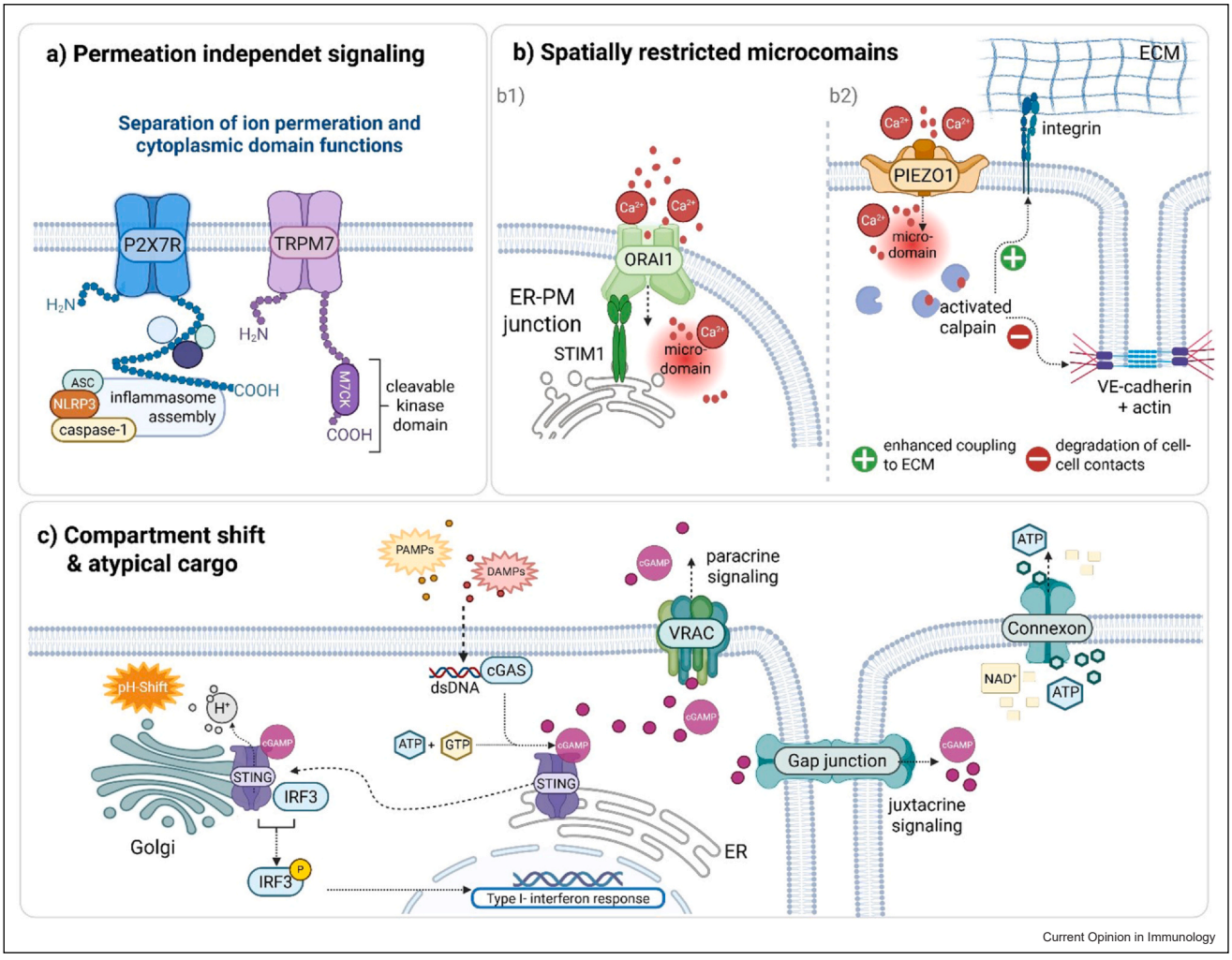
Abbreviations

ADAM A disintegrin and metalloprotease
ADP Adenosine diphosphate
AMP Adenosine monophosphate
ASC Apoptosis-associated speck-like protein containing a CARD
ATP Adenosine triphosphate
BBB Blood-brain barrier
CD Cluster of differentiation
cGAMP cyclic GMP-AMP
cGAS cyclic GMP-AMP synthase
CNS Central nervous system
Cryo-EM Cryo-electron microscopy
Cx Connexin
DNA Deoxyribonucleic acid
dSTORM Direct stochastic optical reconstruction microscopy
dTAG Degron tag system
EAE Experimental autoimmune encephalomyelitis
EB1 End binding protein 1
ECM Extracellular matrix
eEF2 Eukaryotic elongation factor 2
ER Endoplasmic reticulum
FRET Förster resonance energy transfer
GMP Guanosine monophosphate
GPX4 Glutathione peroxidase 4
HEK293 Human embryonic kidney 293 cells
ICAM 1 Intercellular adhesion molecule 1
IFN Interferon
IL Interleukin
IRF3 Interferon regulatory factor 3
K2P Two pore domain potassium channel
KO Knockout
LRRC8 Leucine-rich repeat-containing 8
M7CK TRPM7 kinase fragment
mRNA messenger RNA
MS Multiple sclerosis
MT Microtubule
MyD88 Myeloid differentiation primary response 88
NAADP Nicotinic acid adenine dinucleotide phosphate
NAD^+ Nicotinamide adenine dinucleotideoxidized Form
NFκB Nuclear factor kappa B
NLRP3 NLR family pyrin domain-containing 3
Orai1 Orai calcium release-activated calcium modulator 1
P2X7R P2X7 receptor
PIEZO1 Piezo type mechanosensitive ion channel component 1
PI3K Phosphoinositide 3-kinase
PIP2 Phosphatidylinositol 45 bisphosphate
PKC Protein kinase C
PM Plasma membrane
PPIs protein-protein interactions
PROTAC Proteolysis targeting chimera
RNA Ribonucleic acid
Rorc RAR-related orphan receptor C
SARAF SOCE-associated regulatory factor
Smad2 SMAD family member 2
SNARE Soluble NSF attachment protein receptor
Src Proto-oncogene tyrosine kinase Src
STED Stimulated emission depletion microscopy
STIM1 Stromal interaction molecule 1
STING Stimulator of interferon genes
TGF Transforming growth factor
Th17 T helper 17 cells
TLR Toll-like receptor
TMEM66 Transmembrane protein 66
TRPC6 Transient receptor potential canonical 6
TREK 1 TWIK-related potassium channel 1
TRPM7 Transient receptor potential melastatin 7
TRPML1 Transient receptor potential mucolipin 1
TPCs Two pore channels
Tregs Regulatory T cells
TWIK2 Tandem of P domains in a weak inward rectifying K channel 2
VCAM 1 Vascular cell adhesion molecule 1
VE Vascular endothelial
VRAC Volume-regulated anion channel

preserves small cation currents while selectively abolishing dye uptake, membrane permeabilization, and downstream inflammatory responses [2]. Elevated extracellular ATP levels, which may arise from tissue injury, cellular stress, pathogen-induced danger signaling, or hemichannel-mediated release, activate P2X7R once sufficient local concentrations are reached and thereby promote K^+ efflux and the spatial assembly of the NLRP3-inflammasome core components NLRP3, ASC, and caspase 1 at the plasma membrane (PM) (Figure 1a). This localized assembly facilitates the subsequent cleavage of Gasdermin D, creating conduits for unconventional cytokine secretion like Interleukin (IL)-1β that further amplifies inflammatory signaling cascades [3,4]. This pathway is impaired under C-terminal truncation, illustrating how the P2X7R C-terminal tail fine-tunes a diverse set of downstream responses, thereby coupling inflammatory responses to cytoskeletal remodeling and membrane dynamics. P2X7R localizes preferentially to cholesterol-rich membrane (lipid rafts) and functionally interacts with actin-associated proteins such as α-actinin and filamin [5].

Functional consequences of P2X7R membrane reorganization include the activation of matrix

Figure 1



Three mechanistic principles of noncanonical ion channel signaling. **(a)** Permeation-independent signaling. P2X7R and TRPM7 exemplify functional separation of ion permeation and cytoplasmic domain functions. P2X7R has an extraordinarily long C-terminus that serves as a platform for diverse PPIs, for example, assembly of Inflammasome components ASC, NLRP3, and caspase-1. TRPM7 contains a C-terminal cleavable kinase domain that functions independently of ion flux. **(b)** Spatially restricted microdomains. While the ion-permeation is essential, the functional output is bound to localization and coupling, but not global cytosolic changes or membrane excitability. b1) STIM1 binds to the Ca^{2+} ORAI-1 channel protein, generating localized Ca^{2+} microdomains at these ER-PM junctions. b2) PIEZO1 concentrates Ca^{2+}-signals at low curvature sites like focal adhesions in endothelial cells. Local calpain activation strengthens integrin-mediated cell coupling to the ECM, while destabilizing VE-cadherin-based cell-cell interaction and thereby, for example, modulating BBB stability. **(c)** Compartment shift and atypical cargo. STING can relocate from the ER to the Golgi compartment after sensing raised cGAMP levels. At the Golgi, it facilitates the phosphorylation of IRF3, enabling the type I interferon response. VRAC can export organic molecules, including cGAMP, and supports the paracrine STING activation. Connexin hemichannels and gap junctions can mediate the release or transfer of ATP, NAD^+, and cGAMP, enabling juxtacrine and paracrine signaling. The depicted pathways serve as representative examples for the mechanism, while the functional outcome is cell- and context-specific. *Created in BioRender. Vinnenberg, L. (2026) https://BioRender.com/02t6j6y.*

metalloproteinase-9 that triggers the disruption of tight junction proteins while simultaneously driving the *de novo* expression of adhesion molecules like ICAM-1 and VCAM-1 via the recruitment of proinflammatory signaling scaffolds (e.g. NFκB activation via MyD88) [6,7]. In combination with the ADAM metalloprotease-mediated ectodomain shedding of CD62L on T-lymphocytes, these C-terminal scaffolding functions increase transendothelial diapedesis into the central nervous system (CNS) [1]. Thus, P2X7R non-canonical signaling impacts blood-brain-barrier (BBB) permeability in inflammatory diseases, including multiple sclerosis (MS) [6].

Table 1

Canonical versus non-canonical ion channel signaling: key distinguishing features.

Parameter	Canonical ion channel signaling	Noncanonical ion channel signaling
Signal nature	• Pore-centered ion permeation; biological output follows ion selectivity, gating, and conductance.	• Scaffold-, domain-, microdomain-, compartment-, or cargo-dependent signaling; ion flux may be absent, permissive (locally required but not the primary output), or locally restricted.
Temporal scale	• Typically rapid, ranging from milliseconds to seconds for channel opening and immediate ionic effects.	• Context and readout-dependent; scaffolding and microdomain assembly can be rapid, whereas downstream outputs such as cytoskeletal remodeling, transcriptional activation, junctional reorganization, or barrier changes may evolve over minutes to hours.
Spatial scale	• Often, cell-wide effects on membrane potential, osmotic balance, or bulk cytosolic ion concentrations.	• Spatially restricted to cytoplasmic domains, lipid rafts, ER-PM contact sites, focal adhesions, adherens junctions, organelles, or cell-cell interfaces.
Downstream effects	• Electrical excitability, membrane potential stabilization, global Ca^{2+} elevation, volume regulation, or ion homeostasis.	• Inflammasome assembly, cytoskeletal remodeling, junctional reorganization, immune-cell diapedesis, transcriptional control, organelle stress responses, or paracrine/juxtacrine pathway signaling.
Therapeutic logic	• Mainly pore blockade, channel opening, or modulation of conductance/gating.	• Targeting interaction domains, scaffolding interfaces, localization, trafficking, cargo transport, or microdomain-specific signaling.

A comparative overview of signal nature, temporal and spatial scale, downstream effector mechanisms, and therapeutic targeting logic across both signaling modes.

Research investigating P2X7R as a noncanonical signaling platform and membrane organizer is a current field of research with ongoing improvement and mechanistic characterizations constantly revealing new players. P2X7R tail interactions are reviewed in detail by Kopp et al. [1].

Integrated proteolytic kinase domain

Transient receptor potential melastatin 7 (TRPM7), consisting of a cation channel and an integrated serine/threonine kinase domain at the C-terminus, is a prototypical example of a 'chanzyme' (Figure 1a). Functional TRPM7 interaction partners and substrates are the subject of intensive research and continuously expanding [8]. We aim to highlight striking pathways potentially relevant in the context of immune and barrier cells.

Characterization of TRPM7 in immune cells revealed modulatory effects on migration, polarization, and differentiation in neutrophils, macrophages, and T-lymphocytes [9]. For example, it is believed that the TRPM7 kinase domain in murine T-lymphocytes modulates the phosphorylation of the C-terminal SXS motif of Smad2 independently of the transforming growth factor β1 (TGF-β1) complex. Downstream, TRPM7 inhibition reduces IL-17 and Rorc expression, resulting in reduced Th17 differentiation [9,10]. This function is abolished in the kinase-dead TRPM7 T-cell mutant with largely preserved channel currents, thereby allowing a kinase-specific interpretation. Since Th17 cells are known to be key proinflammatory effector cells and functional antagonists of regulatory T-lymphocytes (Tregs), these findings highlight basal Smad2 phosphorylation by TRPM7 as a hub of inflammatory T-lymphocyte differentiation.

Beyond immune cell differentiation, published work has connected TRPM7 kinase signaling to metabolic control and cellular stress programs in general. A loss-of-function and rescue design was used to separate TRPM7 kinase activity from other channel-associated functions. Under Mg^{2+}-deprived conditions, enhanced TRPM7 kinase cascade-mediated phosphorylation of eukaryotic elongation factor 2 (eEF2) was detected. eEF2 is an essential protein for translation, as it catalyzes the translocation of the ribosome on the mRNA. eEF2 regulation and reduced protein synthesis are, for instance, associated with cell stress and nutrient deficiency [11].

TRPM7 has furthermore been linked to modulating nuclear functions. The TRPM7 kinase fragment (M7CK) can be cleaved proteolytically and then translocate into the nucleus. The phosphorylation mediated there can regulate epigenetics and gene expression [12]. *In vitro* kinase assays with the purified C-terminal tail of mammalian TRPM7 interestingly showed a multistep autophosphorylation process that precedes substrate phosphorylation and controls access to the catalytic domain [13].

In the context of BBB integrity, TRPM7's control of actomyosin dynamics and the cortical actin ring by phosphorylating myosin II heavy chains and annexin A1 is interesting [8,9]. However, direct separation of kinase versus pore contributions in brain endothelium remains limited so far.

The evaluation regarding the impact of these mechanisms on the (patho)physiology of brain endothelial cells and BBB permeability in neuroinflammatory diseases requires more in-depth investigation.

In addition to these established role models, emerging candidates support permeation-independent signaling in immune and barrier contexts. The noncanonical output is mostly attributed to cytosolic interaction domains in a context- and cell-specific way. For instance, Kv2.1 channels can act as an autonomous scaffold organizing endoplasmic reticulum (ER)-PM junctions and signaling platforms through their C-termini independently of ion conduction. At the same time, the functional impact of these ER-PM junctions is linked to the spatial organization of local Ca^{2+}-signals.

Permeation-dependent signaling with spatial restriction

We define spatially restricted signaling as noncanonical, not because ion flux is missing, but because localization and molecular coupling specify the output, rather than global cytosolic shifts or changes in membrane excitability. It is fascinating to observe how microdomains switch pathway selectivity, for example, by prioritizing local kinases and phosphatases.

Microdomain organizer

Spatially restricted signaling is dependent on organizing proteins such as stromal interaction molecule 1 (STIM1), which interacts with multiple proteins as a scaffold. While the detailed upstream pathways connecting phosphoinositide 3-kinase (PI3K) signaling, ER Ca^{2+} store depletion, and STIM1/ORAI1 activation have been reviewed in depth before, we focus on STIM1 as a scaffolding protein and microdomain organizer that couples store-operated Ca^{2+} entry to spatially restricted effector activation. STIM1 serves as a physical anchor for regulatory proteins such as SARAF (TMEM66) at PM puncta. This interaction stabilizes the junctional architecture and allows spatially limited effector activation without global ion overload (Figure 1b1)[14–16]. Moreover, the STIM1 molecule, which is localized at the ER in its resting state, is a microtubule (MT)-plus-end-tracking protein that directly binds EB1 (End-binding protein 1) through a highly conserved C-terminal domain. The complex formation enables the ER to move through the cytosol to the dynamically growing plus ends of the MTs. This process, known as 'ER hitchhiking', was first described in HeLa and MRC5-SV cells *in vitro* and enables the spatial organization of the ER network.[17–19]. In barrier cells, the STIM1/EB1 axis coordinates the turnover of focal contacts and the directional migration speed in the vascular endothelium by coupling the ER network to cytoskeletal dynamics [20]. In T-cells, STIM1-Orai clusters accumulate at the immunological synapse in a noncanonical manner driven by actomyosin forces. This spatial reorganization is crucial to establish locally restricted Ca^{2+} microdomains required for sustained T-cell activation [21].

Junctional and cytoskeletal coupling in the BBB

The subcellular localization of the mechanosensitive channel Piezo1 is highly dependent on its intrinsic dome shape. The channel accumulates in regions of low curvature. This curvature dependence determines in which membrane domains mechanosensitive signals can arise [22]. One example is the enrichment of Piezo1 at focal adhesions, where the channel is functionally embedded in a bidirectional network of integrin-based adhesion complexes and the cytoskeleton [23]. For BBB endothelial cells, it has been shown that Piezo1 creates locally confined microdomains at focal adhesions, activating calpain in the vicinity of the channel and mediating the cleavage of the adapter protein talin. The cleaved talin efficiently activates integrins and enhances mechanical coupling to the extracellular matrix. Under inflammatory conditions, integrin-mediated forces dominate over vascular endothelium (VE)-cadherin-dependent cell-cell contacts, which weakens the paracellular barrier function and promotes the diapedesis of immune cells across the permeable BBB (Figure 1b2) [24,25]. In immune cells, Piezo1 modulates cell type-specific polarization, adhesion, and migratory/invasive properties, as well as disease burden in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS in the case of Tregs [26,27].

Barrier integrity is controlled at junctions and at the cortical cytoskeleton. Spatially restricted ion channel signaling is critical when ion flux is translated into force transmission, thereby resulting in local remodeling. TRP Canonical 6 (TRPC6) exemplifies this principle in barrier cells. TRPC6 contributes to stabilizing barrier function by binding the actin cross-linker α-actinin 4 via its C-terminus, thereby enabling force transfer between the cytoskeleton and the channel architecture. This interaction anchors TRPC6 in junction-proximal microdomains where local Ca^{2+}-entry can engage PKCα and Src family kinases and couple Ca^{2+}-dependent actin remodeling to contractile force transmission. Through this spatial recruitment of kinases, TRPC6 can regulate the phosphorylation status of the VE-cadherin/β catenin complex at adherens junctions in the endothelium [28–30]. While the basal function supports junctional stability, disruption can occur under pathological overactivation that results in enhanced transendothelial migration during neuroinflammation [31].

Complementary effects are reported for the K_{2P} channel TREK-1. A TREK-1 knockout (KO) leads to a worsened EAE phenotype compared to the wild type, which has been linked to an effect on endothelial cells and reduced integrity of the BBB [32,33]. The protective effect of TREK1 can be attributed to its function as a cytoskeletal anchor in endothelial cells. Genetic deletion

as well as inflammatory downregulation triggers the formation of ICAM-1-rich membrane protrusions and the altered regulation of cytoskeletal regulatory proteins such as cofilin-1. Mechanistically, TREK-1 sequesters phosphatidylinositol 4,5-bisphosphate (PIP_2) via its C-terminus, thereby limiting PIP_2 availability for cofilin-1-dependent actin reorganization. This protective mechanism is lost in TREK-1 deficiency, which promotes actin destabilization, impairs BBB integrity, and facilitates immune cell diapedesis [34].

Compartment shift or atypical cargo

Ion channels diversify and specialize signaling beyond inorganic ion conductance at the PM. In our opinion, the concepts of compartment shift and atypical cargo permeation illustrate this through the creation of distinct, luminal environments and specialized paracrine and juxtacrine communication, respectively.

Organelle signaling spaces

Organelle-specific signaling can be discussed as a separate form of noncanonical signaling, as platforms are established independently of the classical PM paradigm and thus establish their own signaling spaces. The stimulator of interferon genes (STING) is an interesting role model for specialized organelle signaling, because it links compartment shift to inflammatory stress programs. Under basal conditions, STING is retained at the ER [35]. In response to activation with cyclic dinucleotides such as cGAMP or exocytic stress, STING translocates to the Golgi, where activated STING has been shown to function as a selective H^+-channel [36–38]. The STING-mediated H^+-efflux causes a pH-shift in neurons and the autophagic degradation of glutathione peroxidase (GPX-4). This triggers specific ferroptotic cell death and subsequent pathological axonal loss in the EAE model [37–39]. The more extensively studied downstream pathway is, however, ion flux independent. STING serves as a scaffold to ensure the specific phosphorylation of the transcription factor IRF3 (interferon regulatory factor 3), which then migrates to the cell nucleus and initiates the type I-interferon response (Figure 1c). The subsequent production of IFNβ has a protective effect in EAE by stabilizing BBB integrity and inducing the differentiation of Tregs [40–42]. Studies with the inhibitor C53 show that the proton channel can be blocked while the subsequent IFN production is completely preserved if the inhibitor is applied after Golgi arrival [39]. Conversely, STING can engage proinflammatory NFκB outputs and stress-associated cascades, highlighting a context-dependent and cell-specific compartment shift platform [43,44].

TRP mucolipin-1 (TRPML1) provides a complementary example for organelle-localized channels within the endolysosomal system. TRPML1-mediated Ca^{2+}-efflux is functionally relevant for α-synuclein degradation, the pathophysiology of lysosomal storage disorders, and the clearance of myelin debris in MS via upregulated autophagy [45–47]. TRPML1 is furthermore functionally relevant for innate and adaptive immune cells. It influences TLR7 responses to single-stranded RNA and directs migration to lymph nodes in dendritic cells. In natural killer cells, TRPML1 maintains mitochondrial fitness via interorganelle crosstalk and lysosome-mitochondria contact sites [48,49].

Two-pore channels (TPCs) are endolysosomal ion channels that conduct either Ca^{2+} or Na^+, depending on the agonist [50]. Under NAADP activation, TPCs generate specific Ca^{2+}-nanodomains independently of global Ca^{2+}-oscillations and activate calcineurin locally. Beyond conductance, TPC function is shaped by PPIs that couple the channel to trafficking and organelle contact logic. TPC channel sensitivity is influenced by N-terminal binding of small GTPases and is functionally coupled to vesicle transport. In addition, TPCs interact with proteins of the SNARE complex, establishing inter-organelle contact sites (e.g. between endosomes and lysosomes) [50].

Metabolite and second messenger transport

Functional signal diversification through ion channels is enhanced by channels that conduct organic cargo molecules in addition to inorganic ions. These channels include volume-regulated anion channels (VRACs) and connexin hemichannels. VRACs are heterohexamers formed from LRRC8A in combination with at least one other LRRC8 homolog (LRRC8B E) and are classically characterized to mediate regulatory volume decrease by conducting Cl^- and organic osmolytes like taurine [51]. This volume regulation can impact BBB integrity, for example, by controlling tight junction stability [52]. Other functions of VRACs are now known, which are mediated via interaction partners or the transport of multiple signaling molecules [51,53]. The composition of the channel-forming LRRC8 subunits significantly determines substrate selectivity and the biological effects in different tissues. One example with direct immunological consequences is the transport of the second messenger cGAMP. In infected or malignant cells, cytosolic DNA acts as a danger signal, which is detected by the enzyme cGAS (cyclic GMP-AMP synthase). In response, cGAS produces the signaling molecule cGAMP, which can leave the cell via LRRC8C- or LRRC8E-containing VRAC-channels. Paracrine signal transduction is particularly important mechanistically. The extracellular cGAMP can be taken up by neighboring cells, where it activates the cGAMP-STING signaling pathways described above. This ensures a coordinated immune response of the cellular environment, which also involves uninfected cells in order to mediate the best possible protective response [54,55]. The functional consequence of reduced cGAMP transport through VRAC-channels is therefore just as broad and cell-specific as with STING [40,41,53,56].

In the context of atypical cargo, connexins (Cx) must be mentioned, which assemble into hexameric PM-spanning connexons or hemichannels and function as essential components of direct cell communication. Their functional diversity results from their ability to form both homo- and heteromeric connexons. In healthy tissue, the hemichannels of two cells dock to form gap junction channels, thereby coupling neighboring cells [57]. Appropriately, gap junction channels transmit, for example, cGAMP, which enables juxtacrine STING signal propagation in cell clusters [55]. Under pathophysiological conditions, however, individual hemichannels can also establish a bidirectional coupling between the cytoplasm and the extracellular space. This enables the flow of inorganic ions such as K^+ and Ca^{2+}, but also organic signaling molecules, including ATP and NAD^+ [58,59]. The hemichannel mediates the efflux of ATP from the cell interior to the extracellular space, where ATP acts as a danger signal and influences adjacent cells. For example, ATP can bind to purinergic receptors such as P2X7R on microglia or macrophages and trigger the NLRP3 inflammasome signaling pathway [6,60,61]. NAD^+ is essential for intracellular energy metabolism and, as an extracellular co-substrate of enzymes such as CD38 and ecto-ADP-ribosyltransferases, regulates other immunomodulatory signaling pathways that, for example, influence the cytokine response and differentiation of T lymphocytes [62,63]. These hemichannel-mediated processes are important, for example, in the neurovascular unit under inflammatory conditions and can undermine BBB integrity. Cx43 is expressed on brain endothelial cells in close proximity to tight junctions, and the probability of Cx43 hemichannels opening increases significantly in an inflammatory environment [64–66]. In the context of neuroinflammatory diseases, Cx43 hemichannel activity correlates with the clinical severity of the disease in a multifactorial manner, making Cx43 hemichannel modulators promising therapeutic approaches [67,68]. The opposite has been described for Cx32, which is essential for oligodendrocyte homeostasis. A loss of oligodendroglial Cx32 and Cx47 has been described in MS lesions, which correlates with impaired glial coupling and may promote demyelination [69–71].

VRAC and connexins thus form the infrastructural backbone for the transport of immune transmitters, coordinating the spread of cellular danger signals and contributing significantly to immunological plasticity at the neurovascular interface.

3D protein structure as the basis for the development of photoactive compounds controlling channel biophysics

High-resolution cryo-electron microscopy (cryo-EM) is an advanced method for the analysis of structural channel components involved in noncanonical ion channel function and the development of high-precision tool compounds for the reversible control of channel function. Active small compounds, including channel blockers, may serve as the basis for the development of photoswitchable chemical probes [72]. Recent advances in cryo-EM enable the identification and characterization of binding sites for photoswitchable molecules and direct visualization of the conformational changes. Elucidating the molecular recognition of photoswitches on the molecular level has, however, still rarely been achieved [73], yet it is crucial in order to facilitate their optimization and to examine the effects on the overall channel structure and function. Azo-compounds, such as azobenzene derivatives, that isomerize between distinct configurations upon irradiation with light, may bind to interacting domains, and their photo-induced change in geometry may allow precise, rapid, and reversible control of the interaction between the protein subunits [72]. Azo-derivatives of channel blockers enable photocontrol of ion channels [74,75]. In combination, small molecule modification and cryo-EM may allow identifying the structural basis of noncanonical ion channel function and its pharmacological modulation.

Therapeutic logic and future directions

The field of non-canonical ion channel signaling is versatile, fast evolving, and opening a new conceptual layer to explain how ion channels shape immune responses and barrier integrity beyond membrane excitability (key points, Table 2).

Despite these advances, mechanistic gaps remain (outstanding questions, Table 2). It is often not clear whether structural effects are fully preserved in permeation-deficient mutants or whether ion conduction plays a permissive role in scaffolding. Ultimately, a clear distinction between noncanonical functions is essential in order to evaluate the therapeutic potential of ion channels. A methodological toolbox for this purpose is summarized in Table 3.

Globally suppressing the channel function through pore block faces several limitations. Among them are a narrow therapeutic window and systemic side effects for ubiquitously expressed ion channels. Pore block can also miss the relevant pathology driving signaling axis if the dominant output is scaffold-based, microdomain- or organelle-restricted. Thus, detailed characterization of non-canonical mechanisms reveals new avenues for precision medicine. Targeted manipulation may address specific intracellular domains or PPI interfaces. Furthermore, allosteric modulators that stabilize distinct signaling-competent conformations, along with strategies to redirect organelle-specific trafficking or subcellular localization, provide unprecedented precision in decoupling pathological outputs from physiological

Table 2

Summary of key points and outstanding questions regarding non-canonical ion channel signaling impacting immune and endothelial barrier cells.

Key points	Outstanding questions
• Ion channels act as scaffolds, organizers, and transporters, not only as pores.	• When is ion flux permissive versus truly dispensable for scaffolding outputs?
• Non-canonical outputs cluster into permeation-independent, microdomain-restricted, and compartment or cargo mechanisms.	• Which non-canonical interactomes are cell-type specific, and which are conserved across tissues?
• Barrier integrity is particularly sensitive to localized channel-cytoskeleton coupling in junction-proximal microdomains.	• Can we pharmacologically target microdomain logic without systemic effects on ubiquitous Ca2+ signaling?
• Neurovascular endothelium offers a tractable paradigm to link non-canonical channel logic to immune cell diapedesis.	• How do organelle channel functions vary across inflammatory states, and what are the best in vivo readouts for compartment-specific signaling?
• Therapeutic strategies should increasingly target domains, interactions, and trafficking rather than pores.	• Which intervention class is most feasible in vivo: domain binders, PPI inhibitors, or trafficking modulators?

Table 3

Methodological toolbox: how to prove non-canonical signaling? To clearly distinguish biophysical ion conduction from structural or coordinative functions, rigorous methodological triangulation is required.

Approach	Details
Pore-dead mutants	Point mutations in the selectivity filter region prevent permeation, while the protein remains intact as a structural scaffold. Patch-clamp or organelle-localized sensors should show that the mutant is indeed current-free. Otherwise, it remains unclear whether residual current has a permissive effect. Caveat: Expression, correct folding, and membrane transport of the mutant must be ensured to avoid artifacts caused by protein misfolding.
Separation-of-function mutants	Targeted deletion or mutation of intracellular binding motifs (e.g., C-terminus or PDZ domains) while maintaining pore permeability. This allows the scaffolding contribution to be considered in isolation.
Acute pharmacology versus genetic deletion	A classic blocker acts on the pore in milliseconds, while a KO removes the entire protein (including the scaffold). If effects persist after pharmacological blockade and only disappear with the KO, this is a primary indication of non-canonical functions.
Compensation & chronic effects	Genetic deletion and long-term blockade can reprogram cells transcriptionally and functionally. This can activate alternative channels, receptors, or signaling axes, which can mask or mimic the primary effect. Therefore, acute approaches and time-controlled depletion should be used. Rescue experiments and short-term readouts help to separate primary mechanisms from secondary adaptations.
Inducible protein degradation (dTAG, PROTACs)	Acute, time-controlled depletion of the channel protein to separate primary non-canonical outputs from chronic adaptation. dTAG systems use genetically introduced degron tags for rapid experimental depletion, whereas PROTACs recruit E3 ligases to endogenous targets and represent a potential translational route toward tissue-, cell- or isoform-selective channel degradation.
Proximity labeling	Fusion of the channel with a biotin ligase to label all proteins in close spatial proximity. This enables the identification of transient interaction partners (interactome) that bridge the cytoskeleton or inflammatory pathways.
Super-resolution microscopy (STED/ dSTORM)	Use of nanoscopy techniques to visualize the organization of channels in spatially confined microdomains below the diffraction limit.
Spatially confined sensors & tension sensors	Use of FRET pairs or localized biosensors. Specialized tension sensors in intracellular loops can also measure whether the channel transmits mechanical force directly to the cytoskeleton.
Structural validation (cryo-EM)	High-resolution imaging of the channel in complex with scaffold partners as a basis for the rational design of PPI inhibitors.
Optogenetic or chemogenetic local activation	Light- or ligand-gated systems can be used to trigger channel activity or channel-associated signaling in defined microdomains or subcellular compartments. This provides temporal and spatial control and enables causal tests of spatial restriction and compartment shift by confining activation to the relevant signaling space.

currents. Comprehensive research into selective small molecules, peptide mimetics, inhibitors of C-terminal interaction surfaces, and tissue-/cell-specific or even isoform-selective proteolysis targeting chimeras (PROTACs) harbors the chance to finally exploit the therapeutic potential of ion channels in neuroimmunology beyond ion conductance.

Author contributions

L.V. and S.G.M. conceptualized the manuscript. L.V., B.J.R., and C.G. wrote the original draft. All authors revised and edited the manuscript, approved the final version, and agreed on publishing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used GPT-5.4 and Gemini 3 to check and refine the grammar and structure of the manuscript. After using these tools, the authors reviewed and edited the content and take full responsibility for the content of the published article.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Kopp R, Krautloher A, Ramirez-Fernandez A, Nicke A: **P2X7 interactions and signaling — making head or tail of it**. *Front Mol Neurosci* 2019, **12**:183.
2. Karasawa A, Kawate T: **Structural basis for subtype-specific inhibition of the P2X7 receptor**. *Elife* 2016, **5**:22153.
3. Franceschini A, Capece M, Chiozzi P, Falzoni S, Sanz JM, Sarti AC, Bonora M, Pinton P, Di Virgilio F: **The P2X7 receptor directly interacts with the NLRP3 inflammasome scaffold protein**. *FASEB J* 2015, **29**:2450-2461.
4. Di A, Xiong S, Ye Z, Malireddi RKS, Kometani S, Zhong M, Mittal M, Hong Z, Kanneganti TD, Rehman J, *et al.*: **The TWIK2 potassium efflux channel in macrophages mediates NLRP3 inflammasome-induced inflammation**. *Immunity* 2018, **49**:56-65 e54.
5. Murrell-Lagnado RD: **Regulation of P2X purinergic receptor signaling by cholesterol**. *Curr Top Membr* 2017, **80**:211-232.
6. Oliveira-Giacomelli A, Petiz LL, Andrejew R, Turrini N, Silva JB, Sack U, Ulrich H: **Role of P2X7 receptors in immune responses during neurodegeneration**. *Front Cell Neurosci* 2021, **15**:662935.
7. Liu Y, Xiao Y, Li Z: **P2X7 receptor positively regulates MyD88-dependent NF-kappaB activation**. *Cytokine* 2011, **55**:229-236.
8. • Jonckheere N, Rodat-Despoix L, Dhennin-Duthille I, Girault A, Hague F, Gautier M: **Unlocking TRPM7 interactions: a database-driven quest**. *Comput Struct Biotechnol J* 2025, **27**:5375-5390.

This publication uses a data-driven approach to identify TRPM7 interaction partners in a structured manner. This demonstrates a modern way to identify TRPM7 PPIs, which can then be functionally and therapeutically tested using separation of function strategies.

9. Nadolni W, Zierler S: **The channel-kinase TRPM7 as novel regulator of immune system homeostasis**. *Cells* 2018, **7**:109.
10. Romagnani A, Vettore V, Rezzonico-Jost T, Hampe S, Rottoli E, Nadolni W, Perotti M, Meier MA, Hermanns C, Geiger S, *et al.*: **TRPM7 kinase activity is essential for T cell colonization and alloreactivity in the gut**. *Nat Commun* 2017, **8**:1917.
11. Perraud AL, Zhao X, Ryazanov AG, Schmitz C: **The channel-kinase TRPM7 regulates phosphorylation of the translational factor eEF2 via eEF2-k**. *Cell Signal* 2011, **23**:586-593.
12. Krapivinsky G, Krapivinsky L, Manasian Y, Clapham DE: **The TRPM7 chanzyme is cleaved to release a chromatin-modifying kinase**. *Cell* 2014, **157**:1061-1072.
13. Clark K, Middelbeek J, Morrice NA, Figdor CG, Lasonder E, van Leeuwen FN: **Massive autophosphorylation of the Ser/Thr-rich domain controls protein kinase activity of TRPM6 and TRPM7**. *PLoS One* 2008, **3**:e1876.
14. Jha A, Ahuja M, Maleth J, Moreno CM, Yuan JP, Kim MS, Muallem S: **The STIM1 CTID domain determines access of SARAF to SOAR to regulate Orai1 channel function**. *J Cell Biol* 2013, **202**:71-79.
15. Jardin I, Albarran L, Salido GM, Lopez JJ, Sage SO, Rosado JA: **Fine-tuning of store-operated calcium entry by fast and slow Ca(2+)-dependent inactivation: Involvement of SARAF**. *Biochim Biophys Acta Mol Cell Res* 2018, **1865**:463-469.
16. Albarran L, Lopez JJ, Amor NB, Martin-Cano FE, Berna-Erro A, Smani T, Salido GM, Rosado JA: **Dynamic interaction of SARAF with STIM1 and Orai1 to modulate store-operated calcium entry**. *Sci Rep* 2016, **6**:24452.
17. Grigoriev I, Gouveia SM, van der Vaart B, Demmers J, Smyth JT, Honnappa S, Splinter D, Steinmetz MO, Putney JW Jr., Hoogenraad CC, *et al.*: **STIM1 is a MT-plus-end-tracking protein involved in remodeling of the ER**. *Curr Biol* 2008, **18**:177-182.
18. Sallinger M, Grabmayr H, Humer C, Bonhenry D, Romanin C, Schindl R, Derler I: **Activation mechanisms and structural dynamics of STIM proteins**. *J Physiol* 2024, **602**:1475-1507.
19. Honnappa S, Gouveia SM, Weisbrich A, Damberger FF, Bhavesh NS, Jawhari H, Grigoriev I, van Rijssel FJ, Buey RM, Lawera A, *et al.*: **An EB1-binding motif acts as a microtubule tip localization signal**. *Cell* 2009, **138**:366-376.
20. Tsai FC, Seki A, Yang HW, Hayer A, Carrasco S, Malmersjo S, Meyer T: **A polarized Ca2+, diacylglycerol and STIM1 signalling system regulates directed cell migration**. *Nat Cell Biol* 2014, **16**:133-144.
21. Lioudyno MI, Kozak JA, Penna A, Safrina O, Zhang SL, Sen D, Roos J, Stauderman KA, Cahalan MD: **Orai1 and STIM1 move to the immunological synapse and are up-regulated during T cell activation**. *Proc Natl Acad Sci USA* 2008, **105**:2011-2016.
22. Yang S, Miao X, Arnold S, Li B, Ly AT, Wang H, Wang M, Guo X, Pathak MM, Zhao W, *et al.*: **Membrane curvature governs the distribution of Piezo1 in live cells**. *Nat Commun* 2022, **13**:7467.
23. Yao M, Tijore A, Cheng D, Li JV, Hariharan A, Martinac B, Tran Van Nhieu G, Cox CD, Sheetz M: **Force- and cell state-dependent recruitment of Piezo1 drives focal adhesion dynamics and calcium entry**. *Sci Adv* 2022, **8**:eabo1461.
24. Mui KL, Chen CS, Assoian RK: **The mechanical regulation of integrin-cadherin crosstalk organizes cells, signaling and forces**. *J Cell Sci* 2016, **129**:1093-1100.
25. Albarran-Juarez J, Iring A, Wang S, Joseph S, Grimm M, Strilic B, Wettschureck N, Althoff TF, Offermanns S: **Piezo1 and G(q)/G(11) promote endothelial inflammation depending on flow pattern and integrin activation**. *J Exp Med* 2018, **215**:2655-2672.
26. • Wang J, Jing F, Zhao Y, You Z, Zhang A, Qin S: **Piezo1: structural pharmacology and mechanotransduction mechanisms**. *Trends Pharmacol Sci* 2025, **46**:752-770.

This review combines structural findings on Piezo1 with principles of spatially limited expression and local signal transmission. Importantly, it

links this mechanistic chain from structure to function and to future therapeutic concepts, thereby highlighting the integration of differentiated research.

27. Jairaman A, Othy S, Dynes JL, Yeromin AV, Zavala A, Greenberg ML, Nourse JL, Holt JR, Cahalan SM, Marangoni F, *et al.*: **Piezo1 channels restrain regulatory T cells but are dispensable for effector CD4(+) T cell responses**. *Sci Adv* 2021, **7**:eabg5859.

28. Dryer SE, Reiser J: **TRPC6 channels and their binding partners in podocytes: role in glomerular filtration and pathophysiology**. *Am J Physiol Ren Physiol* 2010, **299**:F689-F701.

29. Shalygin A, Shuyskiy LS, Bohovyk R, Palygin O, Staruschenko A, Kaznacheyeva E: **Cytoskeleton Rearrangements modulate TRPC6 channel activity in podocytes**. *Int J Mol Sci* 2021, **22**:4396.

30. Qadri AH, Prajapati J, Praghna D, Sinha A, Pasupulati AK: **Structural and functional insights of the podocyte slit diaphragm complex**. *Tissue Barriers* 2025, **14**:2575198.

31. Weber EW, Han F, Tauseef M, Birnbaumer L, Mehta D, Muller WA: **TRPC6 is the endothelial calcium channel that regulates leukocyte transendothelial migration during the inflammatory response**. *J Exp Med* 2015, **212**:1883-1899.

32. Ehling P, Cerina M, Budde T, Meuth SG, Bittner S: **The CNS under pathophysiologic attack — examining the role of K(2)p channels**. *Pflug Arch* 2015, **467**:959-972.

33. Bittner S, Ruck T, Schuhmann MK, Herrmann AM, Moha ou Maati H, Bobak N, Gobel K, Langhauser F, Stegner D, Ehling P, *et al.*: **Endothelial TWIK-related potassium channel-1 (TREK1) regulates immune-cell trafficking into the CNS**. *Nat Med* 2013, **19**:1161-1165.

34. •• Lichtenberg S, Vinnenberg L, Steffen F, Plegge I, Hanuscheck N, Dobelmann V, Gruchot J, Schroeter CB, Ramachandran H, Wasser B, *et al.*: **The potassium channel K(2P)2.1 shapes the morphology and function of brain endothelial cells via actin network remodeling**. *Nat Commun* 2025, **16**:6622.
This publication shows that the K2P potassium channel TREK1 influences the morphology and function of brain endothelial cells through the non-canonical remodeling of the actin network. Notably, the functional relevance is confirmed *in vitro* and *in vivo*: the loss of endothelial TREK1 massively increases the diapedesis of immune cells into the CNS. The authors suggest that pharmacological stabilization of the channel would be interesting in order to protect BBB integrity under neuroinflammation.

35. Son A, de Jesus AA, Schwartz DM: **STIM1 holds a STING in its (N-terminal) tail**. *Cell Calcium* 2019, **80**:192-193.

36. •• Liu B, Carlson RJ, Pires IS, Gentili M, Feng E, Hellier Q, Schwartz MA, Blainey PC, Irvine DJ, Hacohen N: **Human STING is a proton channel**. *Science* 2023, **381**:508-514.
This publication highlights how signal retention can be achieved through subcellular organelle localization. It shows that STING is not only a regulator of type I interferon programs. After translocation to the Golgi, STING can act as a proton channel and thereby control autophagy-related pathways and ferroptosis.

37. Lee HG, Quintana FJ: **STING: stay near to STIM(1) neuroprotection**. *Mol Cell* 2024, **84**:2596-2597.

38. Woo MS, Mayer C, Binkle-Ladisch L, Sonner JK, Rosenkranz SC, Shaposhnykov A, Rothammer N, Tsvilovskyy V, Lorenz SM, Raich L, *et al.*: **STING orchestrates the neuronal inflammatory stress response in multiple sclerosis**. *Cell* 2024, **187**:4043-4060 e4030.

39. Xun J, Zhang Z, Lv B, Lu D, Yang H, Shang G, Tan JX: **A conserved ion channel function of STING mediates noncanonical autophagy and cell death**. *EMBO Rep* 2024, **25**:544-569.

40. Morch MT, Reinert LS, Benmamar-Badel A, Dubik M, Burton M, Thomassen M, Kruse T, Asgari N, Paludan SR, Owens T, *et al.*: **STING signaling deficiency exacerbates demyelination and immune infiltration in focal EAE lesions**. *NeuroSci* 2025, **6**:106.

41. Lemos H, Huang L, Chandler PR, Mohamed E, Souza GR, Li L, Pacholczyk G, Barber GN, Hayakawa Y, Munn DH, *et al.*: **Activation of the STING adaptor attenuates experimental autoimmune encephalitis**. *J Immunol* 2014, **192**:5571-5578.

42. Yan Y, Zhang GX, Gran B, Fallarino F, Yu S, Li H, Cullimore ML, Rostami A, Xu H: **IDO upregulates regulatory T cells via tryptophan catabolite and suppresses encephalitogenic T cell responses in experimental autoimmune encephalomyelitis**. *J Immunol* 2010, **185**:5953-5961.

43. Wu J, Dobbs N, Yang K, Yan N: **Interferon-independent activities of mammalian STING mediate antiviral response and tumor immune evasion**. *Immunity* 2020, **53**:115-126 e115.

44. Balka KR, Louis C, Saunders TL, Smith AM, Calleja DJ, D'Silva DB, Moghaddas F, Tailler M, Lawlor KE, Zhan Y, *et al.*: **TBK1 and IKKepsilon act redundantly to mediate STING-induced NF-kappaB responses in myeloid cells**. *Cell Rep* 2020, **31**:107492.

45. Pollmanns MR, Beer J, Rosignol I, Rodriguez-Muela N, Falkenburger BH, Dinter E: **Activated endolysosomal cation channel TRPML1 facilitates maturation of alpha-synuclein-containing autophagosomes**. *Front Cell Neurosci* 2022, **16**:861202.

46. Krogsaeter E, Rosato AS, Grimm C: **TRPMLs and TPCs: targets for lysosomal storage and neurodegenerative disease therapy?** *Cell Calcium* 2022, **103**:102553.

47. Scotto Rosato A, Montefusco S, Soldati C, Di Paola S, Capuozzo A, Monfregola J, Polishchuk E, Amabile A, Grimm C, Lombardo A, *et al.*: **TRPML1 links lysosomal calcium to autophagosome biogenesis through the activation of the CaMKKbeta/VPS34 pathway**. *Nat Commun* 2019, **10**:5630.

48. Spix B, Chao YK, Abrahamian C, Chen CC, Grimm C: **TRPML cation channels in inflammation and immunity**. *Front Immunol* 2020, **11**:225.

49. • Clement D, Szabo EK, Krokeide SZ, Wiiger MT, Vincenti M, Palacios D, Chang YT, Grimm C, Patel S, Stenmark H, *et al.*: **The lysosomal calcium channel TRPML1 maintains mitochondrial fitness in NK cells through interorganelle cross-talk**. *J Immunol* 2023, **211**:1348-1358.
This publication shows that the lysosomal Ca2+ channel TRPML1 is necessary for maintaining mitochondrial fitness in human NK cells. It is thus a prime example of organelle-localized channels and interorganelle signaling impacting functional immune cell outcome.

50. Vassileva K, Marsh M, Patel S: **Two-pore channels as master regulators of membrane trafficking and endocytic well-being**. *Curr Opin Physiol* 2020, **17**:163-168.

51. • Thone FMB, Polovitskaya MM, Jentsch TJ: **LRRC8/VRAC chloride and metabolite channels in signaling and volume regulation**. *Trends Biochem Sci* 2025, **50**:873-891.
This review integrates work on the versatile VRAC signaling in physiology and disease. It is particularly relevant because VRACs' diverse functions continue to be identified. The authors emphasize that cargo selectivity depends on channel composition and discuss that specific LRRC8 subunits can gate distinct cargos. For instance, LRRC8E is essential for permissive cGAMP transport.

52. Tian Y, Wang Y, Zhao Y, Liu C, Zhang X, Zhang Y, Wu Z, Kong Y, Wang B, Zhang H, *et al.*: **LRRC8A in endothelial cells contributes to the aberrant blood-brain barrier integrity in ischaemic stroke**. *Stroke Vasc Neurol* 2025, **10**:625-636.

53. Okada Y, Sabirov RZ, Sato-Numata K, Numata T: **Cell death induction and protection by activation of ubiquitously expressed anion/cation channels. Part 1: Roles of VSOR/VRAC in cell volume regulation, release of double-edged signals and apoptotic/necrotic cell death**. *Front Cell Dev Biol* 2020, **8**:614040.

54. Zhou C, Chen X, Planells-Cases R, Chu J, Wang L, Cao L, Li Z, Lopez-Cayuqueo KI, Xie Y, Ye S, *et al.*: **Transfer of cGAMP into bystander cells via LRRC8 volume-regulated anion channels augments STING-mediated interferon responses and anti-viral immunity**. *Immunity* 2020, **52**:767-781 e766.

55. Ablasser A, Schmid-Burgk JL, Hemmerling I, Horvath GL, Schmidt T, Latz E, Hornung V: **Cell intrinsic immunity spreads to bystander cells via the intercellular transfer of cGAMP**. *Nature* 2013, **503**:530-534.

56. Concepcion AR, Wagner LE, 2nd, Zhu J, Tao AY, Yang J, Khodadadi-Jamayran A, Wang YH, Liu M, Rose RE, Jones DR, *et al.*: **The volume-regulated anion channel LRRC8C suppresses T cell function by regulating cyclic dinucleotide transport and STING-p53 signaling**. *Nat Immunol* 2022, **23**:287-302.

57. Peng B, Xu C, Wang S, Zhang Y, Li W: **The role of connexin hemichannels in inflammatory diseases**. *Biology* 2022, **11**:237.

58. Takeuchi H, Suzumura A: **Gap junctions and hemichannels composed of connexins: potential therapeutic targets for neurodegenerative diseases**. *Front Cell Neurosci* 2014, **8**:189.

59. Goodenough DA, Paul DL: **Beyond the gap: functions of unpaired connexon channels**. *Nat Rev Mol Cell Biol* 2003, **4**:285-294.

60. North RA: **Molecular physiology of P2X receptors**. *Physiol Rev* 2002, **82**:1013-1067.

61. Yang F, Zhao K, Zhang X, Zhang J, Xu B: **ATP induces disruption of tight junction proteins via IL-1 beta-dependent MMP-9 activation of human blood-brain barrier in vitro**. *Neural Plast* 2016, **2016**:8928530.

62. Fang J, Chen W, Hou P, Liu Z, Zuo M, Liu S, Feng C, Han Y, Li P, Shi Y, *et al*.: **NAD(+) metabolism-based immunoregulation and therapeutic potential**. *Cell Biosci* 2023, **13**:81.

63. Wu J, Han K, Sack MN: **Targeting NAD+ metabolism to modulate autoimmunity and inflammation**. *J Immunol* 2024, **212**:1043-1050.

64. Yamasaki R: **Connexins control glial inflammation in various neurological diseases**. *Int J Mol Sci* 2023, **24**:16879.

65. Johnson AM, Roach JP, Hu A, Stamatovic SM, Zochowski MR, Keep RF, Andjelkovic AV: **Connexin 43 gap junctions contribute to brain endothelial barrier hyperpermeability in familial cerebral cavernous malformations type III by modulating tight junction structure**. *FASEB J* 2018, **32**:2615-2629.

66. Saez JC, Contreras-Duarte S, Gomez GI, Labra VC, Santibanez CA, Gajardo-Gomez R, Avendano BC, Diaz EF, Montero TD, Velarde V, *et al*.: **Connexin 43 hemichannel activity promoted by pro-inflammatory cytokines and high glucose alters endothelial cell function**. *Front Immunol* 2018, **9**:1899.

67. • Takase EO, Yamasaki R, Nagata S, Watanabe M, Masaki K, Yamaguchi H, Kira JI, Takeuchi H, Isobe N: **Astroglial connexin 43 is a novel therapeutic target for chronic multiple sclerosis model**. *Sci Rep* 2024, **14**:10877.
This manuscript underscores the pathological relevance of hemichannel-mediated ATP release (representing atypical cargo) in MS, demonstrating that targeting this specific non-canonical modality offers therapeutic efficacy.

68. De Bock M, De Smet M, Verwaerde S, Tahiri H, Schumacher S, Van Haver V, Witschas K, Steinhauser C, Rouach N, Vandenbroucke RE, *et al*.: **Targeting gliovascular connexins prevents inflammatory blood-brain barrier leakage and astrogliosis**. *JCI Insight* 2022, **7**:e135263.

69. Cotrina ML, Nedergaard M: **Brain connexins in demyelinating diseases: therapeutic potential of glial targets**. *Brain Res* 2012, **1487**:61-68.

70. Zhao Y, Yamasaki R, Yamaguchi H, Nagata S, Une H, Cui Y, Masaki K, Nakamuta Y, Iinuma K, Watanabe M, *et al*.: **Oligodendroglial connexin 47 regulates neuroinflammation upon autoimmune demyelination in a novel mouse model of multiple sclerosis**. *Proc Natl Acad Sci USA* 2020, **117**:2160-2169.

71. Markoullis K, Sargiannidou I, Schiza N, Roncaroli F, Reynolds R, Kleopa KA: **Oligodendrocyte gap junction loss and disconnection from reactive astrocytes in multiple sclerosis gray matter**. *J Neuropathol Exp Neurol* 2014, **73**:865-879.

72. Kobauri P, Dekker FJ, Szymanski W, Feringa BL: **Rational design in photopharmacology with molecular photoswitches**. *Angew Chem Int Ed Engl* 2023, **62**:e202300681.

73. • Muller M, Niemeyer K, Ojha NK, Porav SA, Vinayagam D, Urban N, Buchau F, Oleinikov K, Makke M, Bauer CC, *et al*.: **Ideal efficacy photoswitching for chromocontrol of TRPC4/5 channel functions in live tissues**. *Nat Chem Biol* 2026, **22**:180-191.
This study shows that endogenous TRPC4/5 channels can be controlled photopharmacologically in live tissues. This is particularly relevant because it demonstrates that rational photoswitch design enables precise and robust modulation of ion channel function in complex biological systems, thereby underscoring the translational potential of this approach.

74. Schonberger M, Althaus M, Fronius M, Clauss W, Trauner D: **Controlling epithelial sodium channels with light using photoswitchable amilorides**. *Nat Chem* 2014, **6**:712-719.

75. Fehrentz T, Huber FME, Hartrampf N, Bruegmann T, Frank JA, Fine NHF, Malan D, Danzl JG, Tikhonov DB, Sumser M, *et al*.: **Optical control of L-type Ca(2+) channels using a diltiazem photoswitch**. *Nat Chem Biol* 2018, **14**:764-767.