

Spatial architecture of immunometabolism: Mitochondrial-organelle interfaces in immune signaling (Review)

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Abstract. Metabolic reprogramming is fundamental to immune cell function, yet the spatial architecture that organizes these metabolic states remains incompletely defined. Rather than functioning as isolated bioenergetic units, mitochondria act as spatial hubs embedded within dynamic organelle networks that coordinate immuno-metabolic signaling. In the present review, the structural and functional basis of mitochondrial organelle interfaces were delineated, including membrane contact sites and vesicular trafficking pathways, with the endoplasmic reticulum, lysosomes, peroxisomes, lipid droplets and the nucleus. It was discussed how these interfaces generate specialized microdomains for the localized exchange of calcium, lipids and redox signals, thereby shaping innate and adaptive effector programs. It was further highlighted how mitochondria-derived vesicles and mitochondria-containing extracellular vesicles extend this regulatory axis, linking intracellular organelle crosstalk directly to systemic tissue homeostasis. Crucially, maladaptive decoupling of these interface circuits emerges as a recurrent feature of infection, sepsis, cancer, autoimmunity and chronic inflammation diseases. Finally, emerging interface-targeted therapeutic strategies were evaluated and the technical methodologies required to validate nanoscale

interactions were critically assessed. By conceptualizing immunometabolism as a spatially coordinated process, the present review provides a comprehensive landscape for decoding immune signaling and identifies tractable avenues for precision immunotherapy.

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1. Introduction

Immune cells continuously adapt to dynamic nutrient availability, tissue oxygenation gradients and inflammatory signals, yet they must simultaneously preserve long-term homeostasis to execute rapid effector programs(1,2). This adaptive capacity is governed by immunometabolism, a process in which innate and adaptive responses are intrinsically coupled to coordinated shifts in glycolysis, oxidative phosphorylation, lipid and amino acid metabolism that shape effector function, memory formation, exhaustion and tolerance in immune cells (3). In this regard, mitochondria are increasingly appreciated as key integrators, transitioning from a canonical role in bioenergetics and metabolism to one that encompasses multifunctional immune signaling. Specifically, mitochondrial effectors modulate pattern recognition receptor signaling, inflammasome activation, type I interferon responses and programmed cell death, thereby linking metabolic reprogramming directly to immune function (4). Accordingly, mitochondrial dysfunction is increasingly viewed as a primary driver of immune dysregulation across infection, cancer, autoimmunity and chronic inflammation rather than merely as a secondary consequence of inflammation (5,6).

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Recent studies have revealed that mitochondrial function is shaped not only by metabolic flux, but also by the spatial organization of mitochondrial organelle interfaces, a mitochondria-centric interaction network composed of two primary regulatory modalities: Membrane contact sites (MCSs) and vesicular trafficking pathways, including mitochondrial-derived vesicles (MDVs) and mitochondrial extracellular vesicles (mitoEVs).

MCSs are defined as regions where two organelle membranes are closely apposed without fusion (7). These contacts facilitate the rapid and spatially restricted exchange of ions, metabolites, lipids and redox signals, serving as platforms for the assembly and coordination of signaling complexes, and thereby regulating the magnitude and duration of downstream responses (8,9). Mitochondria establish such contacts with the endoplasmic reticulum (ER) (10,11), lysosomes (12,13), peroxisomes (14), the Golgi apparatus (15), lipid droplets (LDs) (16,17), the nucleus (18,19) and the plasma membrane (20,21). In parallel with contact-based crosstalk, mitochondria generate MDVs that selectively transport oxidized proteins, lipids and nucleic acids to endolysosomal/lysosomal compartments (22,23), phagosomes (24) and peroxisomal compartments (25,26). Furthermore, mitoEVs distribute mitochondrial cargo to neighboring cells when lysosomal function is impaired (27,28) or when intercellular vesicle exchange remodels the recipient cell organelle network (29-31). Collectively, membrane contact-mediated crosstalk and MDV trafficking establish a mitochondria-centric interaction network that enables rapid, spatially restricted exchange of essential molecular cargo and signaling mediators both within the cell and across intercellular boundaries. This spatial layer of regulation is particularly critical in immune cells, where it coordinates organelle function with immune response-driven metabolic rewiring and signaling, ultimately influencing effector polarization, cytokine secretion, antimicrobial and antiviral responses and cell fate decisions (32-34). Mitochondria-centered organelle crosstalk is dynamically remodeled during immune cell activation and chronic stress, which determines whether stress signals are buffered, resolved, or amplified into sustained inflammation (9-35). When dysregulated, it can skew immune signaling and promote immunopathology across diverse diseases, including infection, sepsis, cancer, autoimmunity and chronic inflammatory disorders (36). Accordingly, coordinated control of mitochondrial organelle communication through MCSs and selective vesicle trafficking is emerging as a key determinant of immune cell fate and a tractable therapeutic target in immunopathology.

In the present review, the spatial organization of immune regulation was explored focusing on mitochondria-centered coordination in immunometabolism. MCSs and vesicle-mediated communication were characterized, elucidating how interfaces between mitochondria and other organelles coordinate signaling and metabolic circuits that tune innate and adaptive immune states, with an emphasis on lipid transfer, Ca^{2+} handling, mitophagy, redox control and epigenetic imprinting. Additionally, shared and context specific patterns of interfacial rewiring were summarized, and it was considered how these structural shifts contribute to immunopathology, including infectious, septic, neoplastic and autoimmune disorders. Crucially, emerging therapeutic strategies that

target interfacial dynamics or vesicle trafficking were highlighted, including small molecule agents, nanobiotechnology and cellular therapies that may advance our understanding of how inter-organelle coordination shapes immune cell states in health and disease, thereby expediting the clinical realization of precision immunotherapies. Finally, the precision and limitations of emerging methodologies were evaluated to ensure objective validation of these nanoscale interactions, bridging the gap between fundamental mechanistic discovery and the development of next-generation interface-targeted diagnostic and therapeutic clinical applications.

2. Structural organization of the mitochondrial interface network

The mitochondrial interface network coordinates cellular processes through structured interactions with multiple organelles, mediated by nanometer-scale MCSs, multi-organelle junctions and vesicle-mediated exchange pathways. By imposing proximity and molecular selectivity, the interface network supports controlled metabolite transfer, lipid trafficking, ion flux and stress sensing, thereby coupling organelle state to immune signaling and effector programs. In the present review, the structural basis of mitochondrial inter-organelle communication was described, its core functional roles discussed, and it was highlighted how alterations in these interfaces shape immune cell behavior in diverse conditions.

ER-mitochondria contacts in immune signaling. ER-mitochondria contact sites (ERMCS) function as highly adaptable immuno-metabolic signaling platforms, integrating calcium flux, lipid exchange, stress sensing and antiviral signaling to fine-tune immune cell fate across developmental, inflammatory and pathological contexts (Fig. 1). ERMCS, often termed mitochondria-associated membranes (MAMs), are specialized microdomains structurally defined by core tethering complexes. This apposition is primarily stabilized by mitofusin 2 (MFN2) and the vesicle-associated membrane protein-associated protein B (VAPB)-protein tyrosine phosphatase-interacting protein 51 (PTPIP51) complex (10,37,38). Beyond physical anchoring, MAMs form dynamic functional hubs dedicated to localized lipid handling and ion flux. For lipid transfer, proteins such as oxysterol-binding protein-related proteins 5 and 8 (ORP5/8) facilitate non-vesicular phosphatidylserine flux by coupling to the mitochondrial MIB/MICOS architecture, which is essential for maintaining cristae organization, mitochondrial morphology and respiratory competence (39,40). Concurrently, calcium transfer is structurally embedded into ERMCS via the inositol 1,4,5-trisphosphate receptor (IP3R)-glucose-regulated protein 75 (GRP75)-voltage-dependent anion channel (VDAC) axis, which funnels ER-released Ca^{2+} across the outer mitochondrial membrane (41,42). Subsequent entry into the mitochondrial matrix is tightly gated by the mitochondrial calcium uniporter complex and its regulators (EMRE and MICU1/2) (43,44). Functionally, this privileged Ca^{2+} transport route directly fuels mitochondrial oxidative metabolism and ATP production, while preventing calcium overload-induced cell death. Ultimately, this localized structural and metabolic network, continuously modulated by molecular chaperones

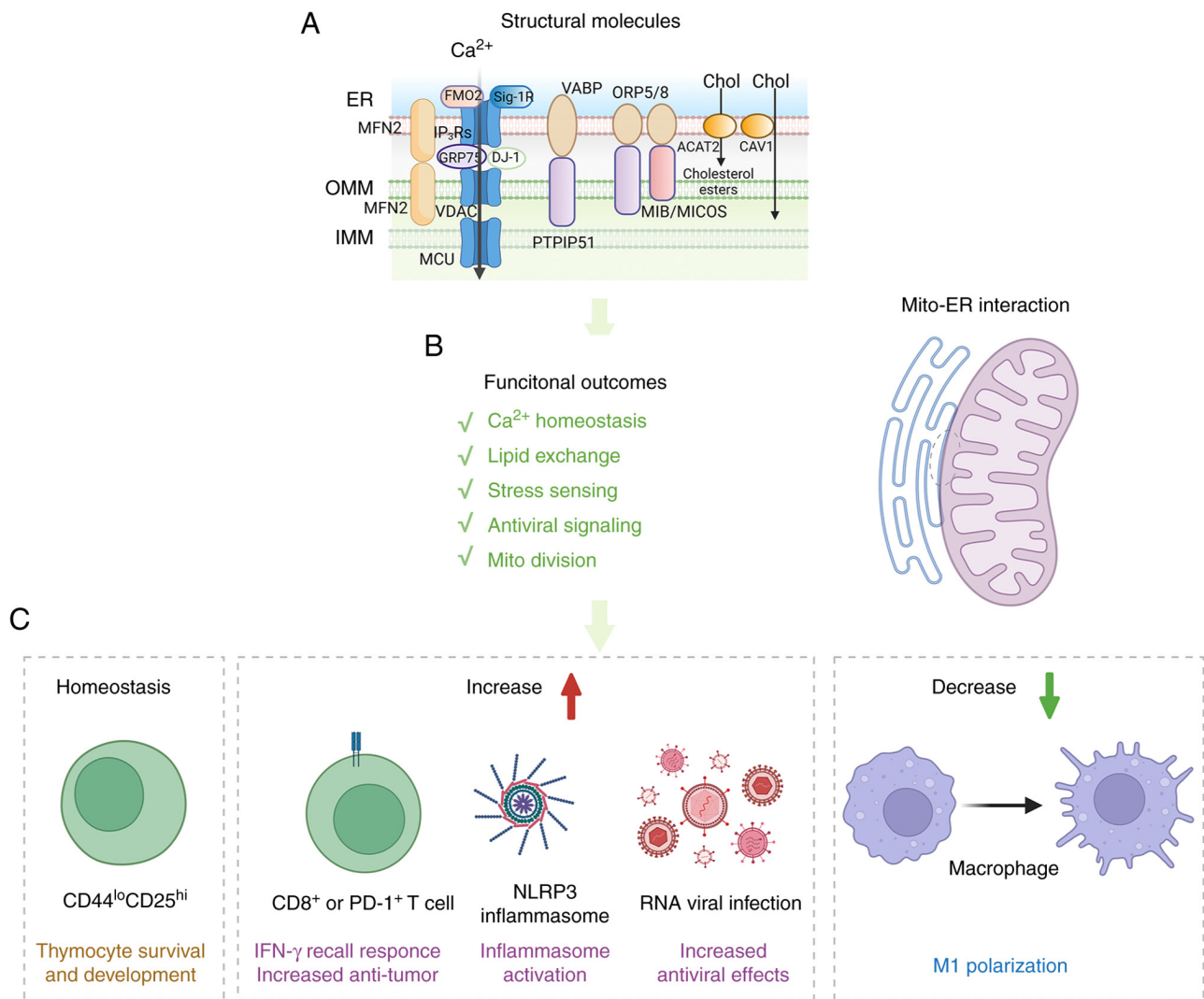


Figure 1. ER-mitochondria contacts as immuno-metabolic signaling hubs in immune cells. (A) Schematic illustration of the structural organization of ER-mitochondria contact sites, highlighting representative tethering and signaling molecules involved in Ca²⁺ transfer, lipid exchange and stress sensing. (B) Major functional outcomes of ER-mitochondria coupling, including Ca²⁺ homeostasis, lipid exchange, stress sensing, antiviral signaling and mitochondrial division. (C) Representative immunological consequences of ER-mitochondria interface remodeling. Under homeostatic conditions, ER-mitochondria contacts support thymocyte survival and development. Increased or reinforced coupling promotes IFN-γ recall responses and antitumor activity in CD8⁺ or PD-1⁺ T cells, enhances NLRP3 inflammasome activation and antiviral signaling, whereas reduced coupling is associated with diminished M1 polarization in macrophages. ER, endoplasmic reticulum; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; MFN2, mitofusin 2; FMO2, flavin-containing monooxygenase 2; Sig-1R, sigma-1 receptor; IP₃R, inositol 1,4,5-trisphosphate receptor; GRP75, glucose-regulated protein 75; VDAC, voltage-dependent anion channel; MCU, mitochondrial calcium uniporter; DJ-1, protein DJ-1; VAPB, vesicle-associated membrane protein-associated protein B; PTP1P51, protein tyrosine phosphatase-interacting protein 51; ORP5/8, oxysterol-binding protein-related proteins 5 and 8; MIB, mitochondrial intermembrane space bridging; MICOS, mitochondrial contact site and cristae organizing system; ACAT2, acetyl-CoA acetyltransferase 2; CAV1, caveolin-1; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3.

(for example, Sig-1R and DJ-1) and stress sensors (for example, IRE1α and PERK), directly couples cellular energetic states to downstream immune effector pathways (45-47).

In immune cells, this ER-mitochondrial coupling is not merely a housekeeping function but a tunable platform that translates microenvironmental stress into distinct immuno-metabolic programs. Proximity ligation assays (PLAs) reveal that during early thymocyte development (DN3 stage, defined by CD44^{lo} CD25^{hi}), the assembly of the IP₃R-GRP75-VDAC Ca²⁺ transfer axis at ER-mitochondria contacts is essential. Loss of the chaperone GRP75 disrupts this axis, triggering mitochondrial stress, aberrant mitochondrial DNA (mtDNA) release and type I interferon signaling that ultimately impairs thymocyte survival and development (48).

In mature populations, MAMs serve as spatially restricted signaling hubs, and the functional heterogeneity of ER-mitochondria contacts depends on the specific immune cell subset and its distinct metabolic demands. For instance, transmission electron microscopy (TEM) and PLAs reveal that the abundance of ER-mitochondria contacts is increased in effector memory CD8⁺ T cells (CD62L⁺ CD45RA⁺ populations). These contact sites spatially organize the mTORC2-AKT-GSK3β signaling pathway, which recruits hexokinase I to mitochondrial VDAC, accelerating the substrate flux and respiration required for rapid IFN-γ production during the recall response of memory CD8⁺ T cells. Genetic ablation of the mTORC2 structural component *Rictor*, as well as pharmacological inhibition targeting the mTOR/AKT axis, profoundly abrogates

the recall effector functions (49). Furthermore, in the metabolically hostile tumor microenvironment (TME), TEM and interaction assays reveal that strengthening ER-mitochondria coupling via MFN2-SERCA2 interactions in tumor-infiltrating CD8⁺ T cells (PD-1⁺) prevents mitochondrial Ca²⁺ overload and preserves metabolic fitness. T cell specific *Mfn2* conditional knockout models disrupt these contacts and precipitate metabolic collapse, whereas MFN2 overexpression restored organelle tethering, preserved T cell metabolic fitness, and significantly augments antitumor efficacy, highlighting MAM integrity as a determinant of immunotherapy efficacy (50).

In innate immunity, ER-mitochondria contact dynamics are intricately linked to macrophage polarization and inflammatory thresholds. Confocal microscopy (CM) and TEM demonstrate that classically activated pro-inflammatory macrophage (LPS + IFN- γ -induced) actively orchestrate a distinct spatial uncoupling between the ER and mitochondria, thereby enforcing the glycolytic metabolic switch requisite for robust inflammatory effector functions. By contrast, IL-4⁺ IL-13-induced anti-inflammatory macrophages do not exhibit altered ER-mitochondria interactions (51). Specific MAM tethering components also act as critical inflammatory switches. Subcellular fractionation and CM reveal that the ER-resident tether ORMDL3 physically anchors mitochondrial dynamics regulating protein Fis-1 in human macrophages (THP-1), facilitating targeted calcium flux and concentrated reactive oxygen species (ROS) generation that drive classical NLRP3 inflammasome activation and IL-1 β release. Small interfering RNA (siRNA)-mediated ORMDL3 knockdown dismantles inter-organelle contacts and completely abrogates inflammasome assembly (52). In disease-associated macrophages (CD14⁺ monocytes differentiated), calcium transfer through MAM sites and GSK3 β inactivation drive mitochondrial hyperactivation and amplify macrophage inflammatory responses in diseases such as rheumatoid arthritis and coronary artery disease (53). Beyond metabolic tuning, ER-mitochondria interfaces function as essential signaling scaffolds during host defense. CM identifies that MAMs as the principal platform on which MAVS is spatially anchored to RIG-I during RNA virus infection. This compartmentalization orchestrates downstream antiviral signaling to drive interferon responses, making MAMs a prime membrane-proximal target for viral antagonism (34). Collectively, even subtle shifts in contact abundance, spacing, or composition can markedly alter calcium transfer, metabolic flux and inflammatory signaling, underscoring the sensitivity of immune responses to the precise organization of ER-mitochondria coupling.

Mitochondria-lysosome contacts and immune quality control. Rather than serving primarily as a signaling scaffolds, mitochondria-lysosome interfaces act as adaptive buffering modules that calibrate organelle turnover, degradative capacity and inflammatory stress in response to immune challenge (54) (Fig. 2). Mitochondria-lysosome contacts are governed by a Ras-related protein Rab7A (Rab7) centered tethering cycle, in which GTP-bound lysosomal Rab7 promotes contact formation, whereas mitochondrial fission 1 protein (FIS1) recruits TBC1D15 (TBC1 domain family member 15, a Rab7 GTPase-activating protein) to drive Rab7 GTP hydrolysis and contact release, thereby creating a reversible interface that

spatially coordinates the two organelle networks and biases sites of mitochondrial remodeling (55). During this process, mitochondria, the ER and lysosomes are spatially integrated into a multidimensional crosstalk network. Super-resolution and live-cell imaging further show that ER tubules physically constrict mitochondria to mark the division sites, which subsequently recruits lysosomes in a process driven by RAB7 GTP hydrolysis (56) and spatially regulated by ORPIL-mediated PI(4)P signaling at the ER-lysosome-mitochondrion interface (57). Beyond direct tethering, mitochondria communicate with the endolysosomal system via MDVs. Through PINK1/Parkin-induced and syntaxin 17-mediated pathways, MDVs selectively route oxidized cargo to lysosomes, providing a steady-state quality control route independent of canonical mitophagy (23,58).

On the mitochondrial side, these contacts mark active sites of mitochondrial fission. Super-resolution imaging shows that lysosomes, often alongside the ER, serve as active organizers of mitochondrial division (55). Functionally, these contacts act as fission-competent geometries that can tune broader cell behaviors, such as migration and proliferation (59). Additionally, these interfaces serve as local ion exchange sites. Proximity mapping reveals that lysosomal Ca²⁺ efflux (for example, via TRPML1) is routed to mitochondria, tuning mitochondrial Ca²⁺ dynamics and linking inter-organelle contact remodeling to stress adaptation programs (58). On the lysosomal side, mitochondrial activity constrains lysosomal competence through energetics, spatial organization and transcriptional adaptation. For instance, mitochondrial metabolic dysfunction can elevate lysosomal pH and impair autophagic flux (60). Furthermore, contact tethering dynamics, including mitochondrial Mid51/Fis1 organization, actively shape lysosomal motility, positioning and network remodeling (61). Transcriptionally, mitochondrial damage and redox states are effectively transduced into lysosomal signaling. Elevated mitochondrial ROS and AMPK activation trigger TFEB nuclear translocation, driving a wave of lysosomal biogenesis and increasing autophagic capacity during stress (62,63). Collectively, these pathways establish mitochondria-lysosome interfaces as vital regulatory checkpoints coordinating lysosomal degradative function and positioning with mitochondrial energetic and redox outputs. This tight coupling is particularly consequential in immune cells, which must synchronize endolysosomal processing with rapid metabolic reprogramming to maintain effective immune signaling.

Disruption of mitochondria-lysosome can jointly impair metabolic fitness and signaling integration, reshaping T-cell activation, fate decisions and dysfunction across diverse diseases (64). In CD4⁺ T lymphocytes, Tfam loss or pharmacological inhibition of mitochondrial respiration severely impairs lysosomal degradation capacity and calcium mobilization. This physical and metabolic uncoupling disrupts endolysosomal trafficking and triggers a compensatory but aberrant activation of the transcription factor EB (TFEB) network and synergy causally subverts T cell differentiation towards pro-inflammatory Th1 and Th17 subsets. NAD⁺ restoration rescues lysosomal function, and failure of these mitochondria to lysosome NAD⁺ transfer promotes pro-inflammatory CD4⁺ T cell skewing and worsens inflammation *in vivo* (65). Advanced spatial transcriptomics and dynamic live-cell proximity assays demonstrated

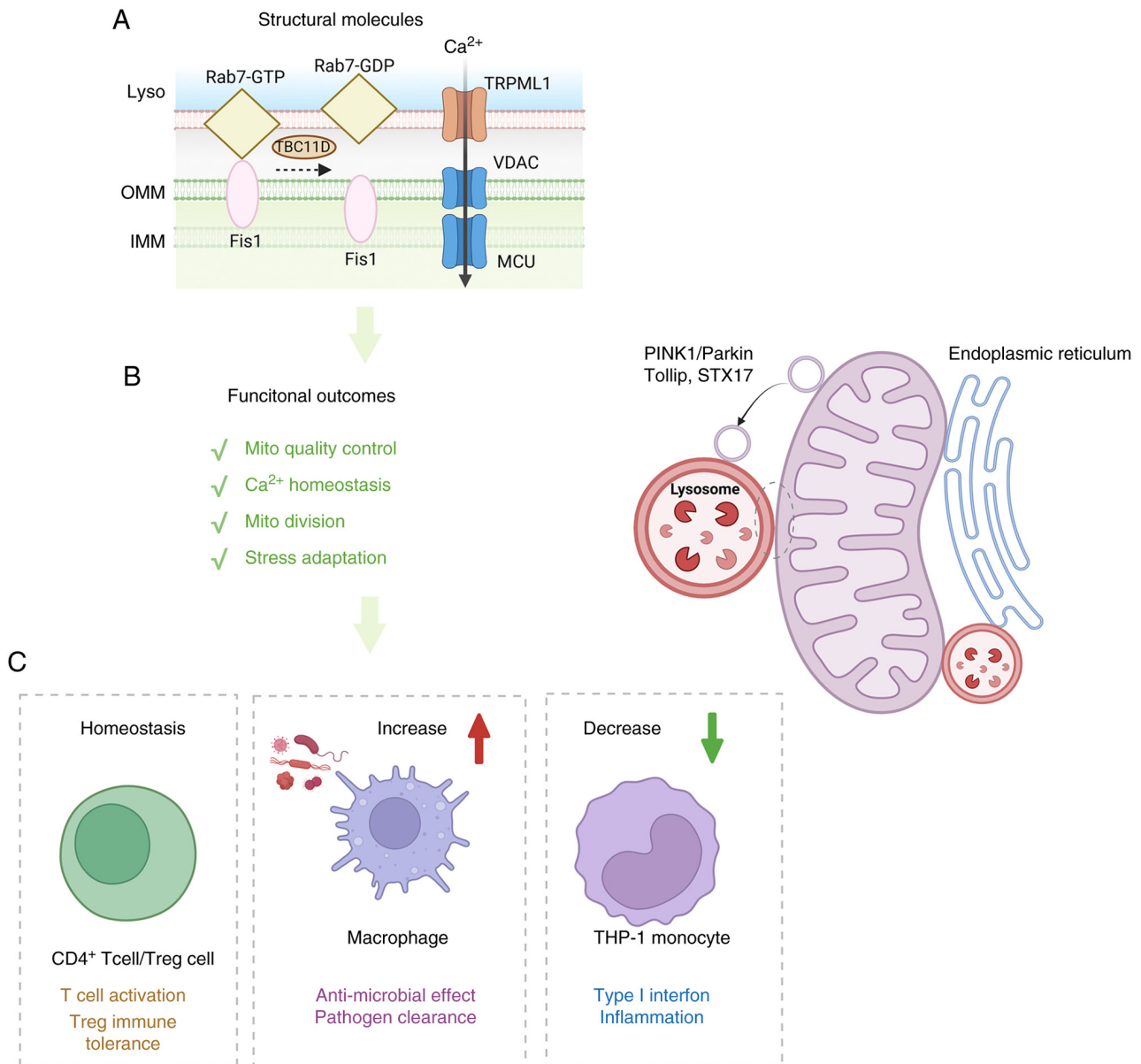


Figure 2. Mitochondria-peroxisome crosstalk coordinates lipid metabolism, redox buffering and antiviral defense. (A) Schematic representation of structural molecules at mitochondria-peroxisome interfaces, which collectively contribute to tethering, peroxisome biogenesis, organelle division and metabolite exchange. (B) Functional outputs of mitochondria-peroxisome communication, including fatty acid oxidation, ROS metabolism, mitochondrial division and peroxisome biogenesis. (C) Representative immunological consequences of mitochondria-peroxisome coupling. Increased coordination between the two organelles enhances antiviral defense and type I/III interferon responses in hMDMs, whereas impaired peroxisomal support in tissue-resident macrophages is associated with defective inflammation resolution. ACBD5, acyl-CoA-binding domain-containing protein 5; PTP51, protein tyrosine phosphatase-interacting protein 51; Fzo1, fuzzy onions 1; PEX, peroxin; Mdm34, mitochondrial distribution and morphology protein 34; Fis1, mitochondrial fission 1 protein; ACBD2, acyl-CoA-binding domain-containing protein 2; EC12, enoyl-CoA delta isomerase 2; TOMM20, translocase of outer mitochondrial membrane 20; MARCH5, membrane-associated ring-CH-type finger 5; ROS, reactive oxygen species; hMDMs, human monocyte-derived macrophages; IFN, interferon.

that the intact spatial signaling between mitochondria and lysosomes is indispensable for orchestrating regulatory T cells (Tregs) metabolic plasticity. Treg cell-specific mitochondria *Opal* deletion or lysosome *Fln* deletion phenocopies and this interplay reprograms Treg cell differentiation and functions with direct consequences for immune tolerance (66). In THP-1 cells, TFAM functions unconventionally as a dedicated autophagy receptor when mtDNA leaks into the cytosol through physically bridges the interaction between the mislocated cytosolic mtDNA and the LC3 protein residing on the autophagosomal membrane. Targeted *Tfam* knockdown and global knockout completely abrogated this receptor-mediated

physical capture. The misplaced mtDNA pathologically hyperactivated the cGAS-STING innate immune sensing pathway, culminating in an explosive downstream cascade of type I interferons and sterile inflammation (67). Furthermore, mitochondria-lysosome coupling also shapes inflammasome biology and antimicrobial defense. For example, in bone marrow-derived macrophage (BMDM), high-resolution confocal fluorescence microscopy with LC-MS/MS-targeted metabolomics reveals that upon stimulation by pathogens such as *Salmonella typhimurium*, the lysosomal biogenesis factor (TFEB) is activated and translocates to the nucleus to upregulate the transcription of aconitate decarboxylase 1 (*Irg1/Acod1*),

thereby driving the massive synthesis of itaconate within mitochondria. The mitochondria-derived itaconate precisely target the *Salmonella*-containing vacuole to suppressing the survival of proliferative *Salmonella* in macrophage. Both Tfeb, Irg1 and Rab32-deficient models confirmed that targeted blockade at any steps of this spatial delivery pathway disrupts the spatial transport of itaconate inhibiting the bactericidal capacity of macrophages (68). Moreover, infection-induced metabolic stress activates AMPK, which subsequently promotes the spatial translocation of TFEB from the cytoplasm or lysosomal surface to the nucleus, synergistically regulating lysosomal biogenesis and mitochondrial function, resulting the accelerated clearance of damaged organelles, ultimate enhancement of the immune clearance capacity against intracellular pathogens, and the fine-tuning of programmed cell death to prevent inflammatory storms (32). Overall, mitochondria-lysosome contacts extend far beyond basic organelle quality control to serve as essential signaling hubs in immunity. By coordinating the clearance of inflammatory triggers (for example, damaged mtDNA) and tuning metabolic flux, these interfaces directly dictate immune cell fate, support robust antimicrobial defenses, and prevent aberrant inflammation, which translating cellular quality control directly into systemic immunological homeostasis.

Mitochondria-peroxisome crosstalk in antiviral immunity. In mammalian cells, *de novo* peroxisome formation can proceed through mitochondria-derived pre-peroxisomal vesicles in which PEX3 and PEX14 first target the mitochondrial outer membrane and are released into vesicular intermediates that later fuse with ER-derived PEX16 carriers (69) (Fig. 3). The outer mitochondrial membrane E3 ubiquitin ligase MARCH5 is essential for this selective biogenic route, specifically controlling the formation of PEX3-positive pre-peroxisomes to maintain organelle abundance and functionality (70,71). Once formed, peroxisomes engage mitochondria through functional contact sites (PerMit) that act as bidirectional hubs coupling lipid flux, redox buffering and organelle homeostasis. These contacts rely on specific tethers, such as the ACBD5-PTPIP51 complex, which facilitates the transfer of mitochondria-produced ROS into the peroxisome for antioxidant buffering under oxidative stress (72), and ACBD2/ECI2, which supports lipid/cholesterol trafficking (73). Additionally, mitochondrial fusion proteins (such as Fzo1 in yeast and MFN1/MFN2 in mammals) promote spatial co-clustering to enhance metabolic coordination, such as transferring peroxisomal citrate to support the mitochondrial TCA cycle (74,75). Productive coupling also depends on spatial distribution and shared remodeling mechanisms. Peroxisome-targeted MIRO1 variants rewire organelle motility and positioning to optimize functional engagement in regions of high metabolic demand (76). Furthermore, mitochondria and peroxisomes utilize a shared, modular division machinery toolkit including DRP1, FIS1, MFF and PEX11 β that enable synchronized tuning of organelle morphology under metabolic stress (77-80), with components such as ERMES Mdm34 further stabilizing these functional contacts (81). Methodological advances, particularly Split-TurboID proximity labeling, now enable precise, high-resolution proteomic mapping of these dynamic PerMit interfaces (82). Peroxisomes perform the initial steps of very

long chain fatty acid (FA) β -oxidation, generating shortened lipid intermediates and acyl-carnitines that are subsequently exported for further oxidation in mitochondria (83). Conversely, disruption of peroxisome biogenesis (for example, PEX3 or PEX5 loss) directly feeds back onto mitochondrial dynamics, inducing DRP1-dependent fragmentation and sensitizing cells to apoptosis, highlighting peroxisomes as active modulators of mitochondrial stress responses (84).

In immune metabolic condition, peroxisomes and mitochondria form an interdependent metabolic redox unit, in which coordinated FA handling and ROS control. In immune microenvironment, this coupling may tune inflammatory signaling thresholds by linking lipid remodeling and oxidative stress management to mitochondrial fitness, thereby shaping how cells respond to infectious or inflammatory stress (85). The Mitochondrial Antiviral Signaling protein (MAVS) localizes not exclusively to mitochondria but also to peroxisomes using high-resolution confocal immunofluorescence microscopy and rigorous subcellular fractionation. This dual localization dictates a bifurcated immunological outcome: Peroxisomal MAVS (pexMAVS) orchestrates a rapid, transient, and Type I interferon-independent induction of interferon-stimulated genes (ISGs, such as Viperin), whereas mitochondrial MAVS (mitoMAVS) coordinates a delayed, stable Type I interferon (IFN- α/β) response. MAVS-deficient (Mavs^{-/-}) genetic background and performed complementation assays by over-expressing MAVS variants engineered to selectively target either the peroxisome (PEX11 β -MAVS) or the mitochondrion (mito-MAVS) indicates that peroxisomes and mitochondria act sequentially and cooperatively as innate immune signaling platforms during the host's antiviral defense (86). In human monocyte-derived macrophages, both peroxisomal and mitochondrial MAVS are capable of activating the classic RLR-dependent Type I and III interferon pathways. However, the hepatitis C virus NS3/4A protease localizes to both organelles but exhibits distinct cleavage efficiencies, effectively dismantling MAVS at these spatial hubs to truncate the innate immune response, and the causality was established through MAVS knockout followed by the lentiviral overexpression of organelle-restricted MAVS constructs (MAVS-pex and MAVS-mito) (87).

More broadly, after severe respiratory viral infection, including SARS-CoV-2 infection, tissue-resident alveolar macrophages undergo marked peroxisomal remodeling. Peroxisomes support macrophage-specific lipid metabolism, including ether lipid synthesis and very-long-chain fatty acid processing, and thereby help preserve mitochondrial metabolic fitness under inflammatory stress. When this peroxisomal support is impaired, alveolar macrophages show reduced mitochondrial respiratory capacity, increased mitochondrial stress and aberrant inflammatory activation. Conditional Pex5 deletion in CD11c⁺ lung myeloid cells, together with complementary macrophage-lineage models, impaired inflammation resolution after viral clearance and disrupted alveolar epithelial repair. Mechanistically, Pex5-deficient alveolar macrophages exhibited reduced ether lipid production, mitochondrial dysfunction, increased inflammasome activation and excessive IL-1 β release, which promoted dysplastic KRT8^{high} transitional epithelial progenitor accumulation, defective alveolar regeneration and chronic post-viral lung pathology (88). Collectively,

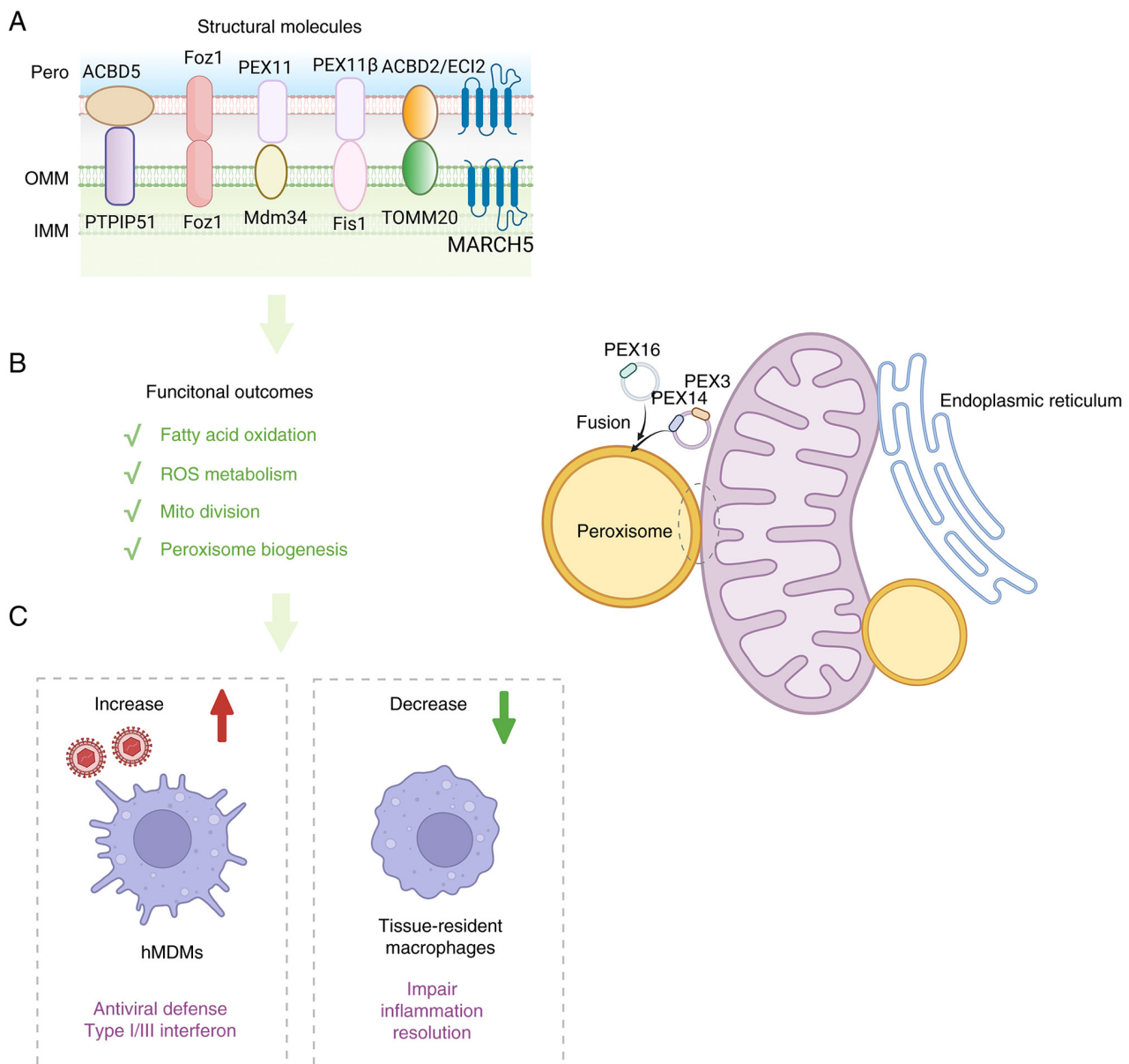


Figure 3. Mitochondria-LD contacts support metabolic flexibility and inflammatory rewiring. (A) Schematic illustration of the structural organization of mitochondria-LD contact sites, showing representative tethering and regulatory molecules. (B) Major functional outcomes of mitochondria-LD coupling, including lipid storage/oxidation, bioenergetics, antioxidant response and metabolic flexibility. (C) Representative immune consequences of remodeling at this interface. Under homeostatic conditions, mitochondria-LD coupling helps maintain energetic supply in BMDM/THP-1 cells. By contrast, reduced coupling in macrophages limits mitochondrial fatty acid oxidation and is associated with a pro-inflammatory phenotype and enhanced antimicrobial defense. LD, lipid droplet; PLIN5, perilipin 5; Hsc70, heat shock cognate 70; FATP4, fatty acid transport protein 4; MIGA2, mitoguardin 2; VSP13D, vacuolar protein sorting-associated protein 13D; TSG101, tumor susceptibility gene 101; ESCRT, endosomal sorting complexes required for transport; Rab8a, Ras-related protein Rab-8A; BMDM, bone marrow-derived macrophage.

peroxisome-mitochondria coordination is viewed through shared lipid metabolism and ROS control, and through organelle-localized signaling modules (for example, MAVS) that together shape antimicrobial and antiviral effects.

Mitochondria-LD crosstalk in immuno-metabolic fueling. Unlike other mitochondrial interfaces that primarily organize signaling or degradative buffering, mitochondria-LD contacts are uniquely positioned to govern how immune cells partition lipids between storage, oxidation and inflammatory mediator production (Fig. 4). Mitochondria-LD contacts constitute specialized membrane sites coupling neutral lipid storage with oxidative bioenergetic membranes (89). These interactions

range from transient ‘kiss-and-run’ contacts supporting rapid lipid flux to stable anchors (90), which supports high-demand processes such as thermogenesis in brown adipocytes (91). Mitochondria bound to LDs (peridroplet mitochondria) exhibit distinct proteomes and bioenergetics that preferentially supply ATP for LD expansion while exhibiting reduced FA oxidation (FAO) and fusion/fission dynamics (92). At the molecular level, diverse tethering modules assemble these interfaces. Core scaffolds include LD-localized PLIN5, which couples with FATP4 (93) or mitochondrial Rab8a during energy stress (94) and MFN2-Hsc70 in cardiomyocytes (17). Moreover, systems-level spectral imaging and PLAs reveals the spatial coupling among the ER, mitochondria, and LDs

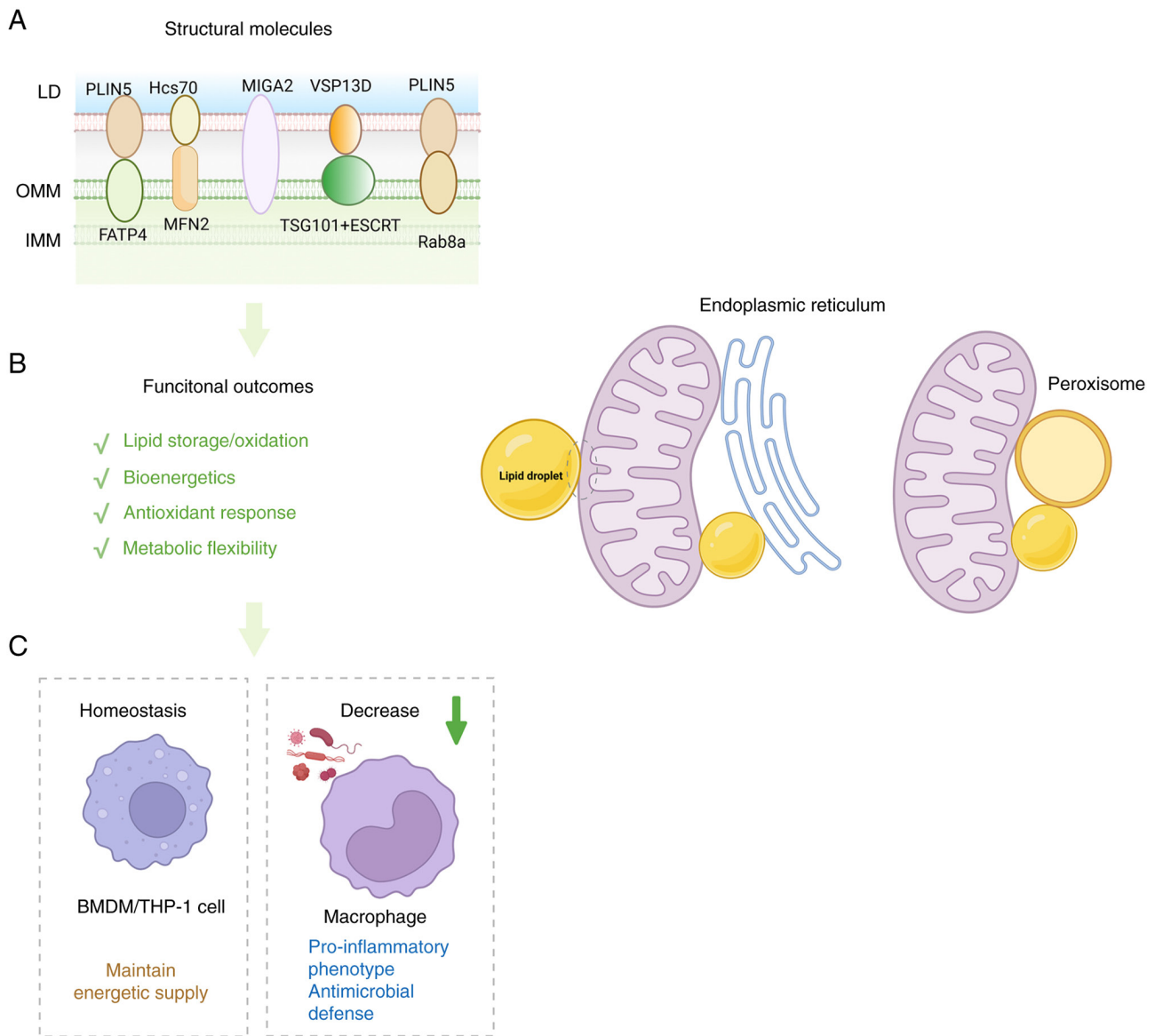


Figure 4. Mitochondria-lysosome interfaces integrate mitochondrial quality control with immune adaptation. (A) Schematic illustration of the molecular architecture of mitochondria-lysosome contacts. (B) Major functional outcomes of mitochondria-lysosome crosstalk, including mitochondrial quality control, Ca^{2+} homeostasis, mitochondrial division and stress adaptation. Mitochondrial cargo is also delivered to lysosomes through vesicular trafficking pathways associated with PINK1/Parkin, Tollip and STX17. (C) Representative immunological consequences of remodeling at this interface. Under homeostatic conditions, mitochondria-lysosome communication supports T-cell activation and Treg immune tolerance. Increased signaling through this axis promotes macrophage antimicrobial activity and pathogen clearance, whereas reduced coordination in THP-1 monocytes is associated with altered type I interferon signaling and inflammatory responses. Rab7, Ras-related protein Rab7; GTP, guanosine triphosphate; GDP, guanosine diphosphate; TBC1D15, TBC1 domain family member 15; TRPML1, transient receptor potential mucolipin 1; PINK1, PTEN-induced kinase 1; Parkin, parkin RBR E3 ubiquitin protein ligase; Tollip, Toll interacting protein; STX17, syntaxin 17; Treg, regulatory T cell.

constitutes a tri-organelle architectures via ESYT-VAPB complexes (16), while factors such as MIGA2 and the VSP13D-ESCRT axis integrate spatial tethering with lipid transfer and membrane remodeling capabilities (95,96) to efficiently execute downstream metabolic programs. However, this tri-organelle crosstalk only reported in non-immune cells (for example, adipocytes and fibroblasts), thus future investigations are required to determine whether the dynamic assembly of the ER-Mito-LD axis play a biophysical switch governing immune cell metabolic fate. Functionally, LD-mitochondria contacts coordinate lipid flux and stress buffering to dictate organelle fate. On the mitochondrial side, peridroplet mitochondria structurally partition activities to preferentially

support processes such as thermogenic fuel delivery in brown adipocytes (92). During nutrient and oxidative stress, these contacts execute critical protective functions. For instance, DGAT1-dependent LD biogenesis buffers excess FAs to prevent acylcarnitine accumulation and mitochondrial lipotoxicity (97), while LD-centered antioxidant responses mitigate lipid peroxidation to preserve mitochondrial fitness (98,99). Efficient FA transfer for β -oxidation and metabolic adaptation is dynamically driven by specific tethers under stress, including PLIN5-FATP4 (93) and AMPK-regulated Rab8a-PLIN5 in starved muscle cells (94), as well as MFN2-Hsc70 and PLIN5-mediated coupling in lipid-loaded oxidative tissues (100). Furthermore, tripartite ER-LD-mitochondria

junctions act as essential hubs for sustained FAO and lipotoxic resistance (16). On the LD side, these interfaces actively regulate lipid retention and mobilization. For instance, PLIN5 restrains basal lipolysis while permitting stimulus-dependent mobilization (101), and the VPS13D-TSG101-ESCRT pathway dynamically remodels the LD surface to enhance transfer efficiency during starvation (96).

In immune cells, the physical coupling between LDs and mitochondria acts as a critical metabolic checkpoint that directly dictates inflammatory signaling and immune cell polarization. Rather than functioning merely as passive lipid storage depots, LDs serve as active innate immune hubs that concentrate lipid metabolic enzymes (such as cyclooxygenases and lipoxygenases) to drive the robust synthesis of potent pro-inflammatory lipid mediators, including eicosanoids and prostaglandins, which intertwined with classic lipid-mediated inflammatory signaling pathways (102). Utilizing high-resolution live-cell confocal microscopy, advanced subcellular fractionation and organelle-targeted proteomics, LDs undergo profound spatial and functional reprogramming to serve as active innate immune hubs in both murine BMDM and THP-1 cells. In resting macrophages, LDs maintain extensive physical contacts with mitochondria to supply FAs for OXPHOS. However, upon pathogen sensing (LPS-induced), this deliberate physical uncoupling restricts mitochondrial FA β -oxidation, thereby forcing a metabolic shift away from oxidative phosphorylation and toward aerobic glycolysis. By preventing the mitochondria from consuming these lipids for ATP production, the immune cell effectively preserves arachidonic acid and other essential FAs within LDs to fuel the massive production of inflammatory mediators. Furthermore, this uncoupling alters mitochondrial ROS production, which further amplifies antimicrobial signaling, increases LD pathogen engagement, and contributes to cellular autonomous defense. Pharmacological inhibitors to ablate LD formation, as well as genetic knockdown models targeting key LD coat proteins (for example, Plin2) rescued intracellular bacterial survival and unequivocally establishing the obligate role of LD-mitochondria spatial uncoupling in host defense (103). During human cytomegalovirus infection, the interferon inducible enzyme viperin is relocalized to mitochondria, where it perturbs mitochondrial FA β -oxidation by engaging mitochondrial metabolic machinery. This mitochondrial rewiring promotes lipogenic flux and LD accumulation, which in turn supports efficient production of infectious virions, indicating that a mitochondria-centered switch can drive LD biogenesis and lipid supply during antiviral stress that is exploited by HCMV for replication (104). Overall, the dynamic remodeling of LD-mitochondria contacts is a fundamental driver of immune cell polarization, dictating the balance between lipid-driven inflammatory mediator synthesis, antimicrobial defense and immune resolution. Although emerging evidence supports a role for LD-mitochondria coupling or uncoupling in shaping inflammatory lipid mediator availability and antiviral effector programs, direct mechanistic studies of the LD-mitochondria axis in immune cells remain relatively limited, making this an important direction for future investigation.

Mitochondria-nucleus signaling and epigenetic imprinting.
Mitochondria and the nucleus maintain continuous,

bidirectional communication driven by defined spatial organization rather than acting as independent compartments. The central structural basis for this coupling is the regulated positioning of nucleus-associated mitochondria (NAM) at the perinuclear region. This extreme spatial proximity enables the direct, highly localized channeling of retrograde signals such as mtROS and TCA cycle intermediates directly into the nucleoplasm without cytosolic dilution, profoundly influencing chromatin remodeling and transcriptomic reprogramming (105) (Fig. 5). During mitochondrial dysfunction, a pro-survival mitochondrial retrograde response is facilitated by direct contact sites between mitochondria and the nucleus that create localized communication microdomains. These promote nuclear stabilization of pro-survival transcriptional effects, and the translocator protein-dependent control of mitochondrial quality as being required for this mitochondria-nucleus coupling, positioning physical proximity as an enabling step for adaptive nuclear reprogramming (19). Upon specific mitochondrial inner membrane stress (such as that induced by mitochondrial uncouplers or misfolded proteins), the resident mitochondrial protease OMA1 is activated and subsequently cleaves the mitochondria-localized protein DELE1. The cleaved form of DELE1 physically translocates from the mitochondria to the cytosol to binds and activates the eIF2 α kinase HRI and attenuation of global cellular translation alongside the selective nuclear expression of cytoprotective factors (such as ATF4) to restore mitochondrial homeostasis. Conversely, upon sensing an elevated AMP/ATP ratio, the cellular energy sensor AMPK directly phosphorylates the transcriptional coactivator PGC-1 α at specific residues and enables the spatial translocation and transactivation of PGC-1 α within the nucleus to induce the mitochondrial biogenesis. The loss-of-function interventions (for example, pharmacological AMPK inhibitor and Pgc-1 α knockdown) completely abrogated the transcription of mitochondrial genes in response to energetic stress (106). Similarly, activated mTOR promotes the interaction between the transcription factor Yin Yang 1 (YY1) and the coactivator PGC-1 α , promoting the transcription of critical mitochondrial genes to match nutrient availability. Pharmacological inhibition using Rapamycin (a specific mTOR inhibitor) and siRNA-mediated specific knockdown of YY1 decoupled mTOR signaling from PGC-1 α transactivation, resulting in severe mitochondrial respiratory defects and diminished oxidative function (107). Collectively, spatial coupling via perinuclear NAM contacts, integrated with these stress-responsive signaling modules, constitutes a vital structural platform for bidirectional mitonuclear coordination.

Moreover, mitochondrial metabolites orchestrate nuclear gene expression by providing essential substrates for chromatin modifications, competitively inhibiting epigenetic enzymes, and acting as direct signaling intermediates to stabilize transcription factor complexes. For instance, classic mitochondrial metabolic enzymes, specifically the branched-chain ketoacid dehydrogenase (BCKDHA) and pyruvate dehydrogenase (PDC) complexes, physically translocate to the nucleus and anchor to the mediator transcriptional coactivator complex, resulting *de novo* production of acetyl-CoA directly at the chromatin, bypassing the need for mitochondria to nucleus metabolite diffusion. This targeted metabolite production directly fuels localized

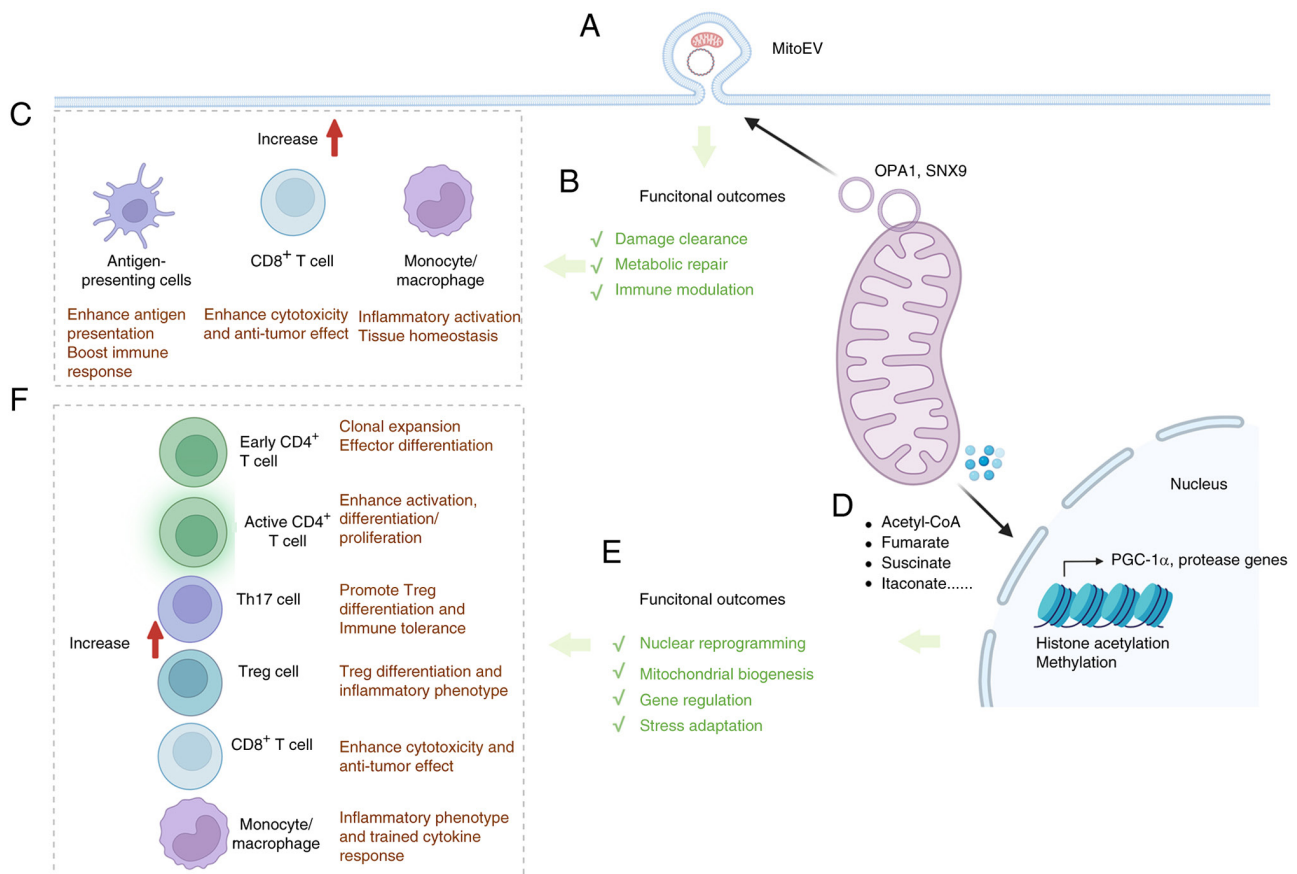


Figure 5. Vesicle-mediated mitochondrial communication and mitochondria-nucleus signaling extend the mitochondrial interface network in immunity. (A) Schematic illustration of the release of mitochondrial-derived vesicles and mitoEVs as an extracellular route of mitochondrial communication and cargo export. (B) Major functional outcomes of mitoEV release, including damage clearance, metabolic repair and immune modulation. OPA1- and SNX9-related pathways are shown as representative regulators of mitochondrial cargo sorting and extracellular vesicle biogenesis. (C) Representative immunological consequences of mitoEV-mediated signaling in recipient cells, including enhanced antigen presentation by antigen-presenting cells, increased cytotoxicity and antitumor effects in CD8⁺ T cells, and inflammatory activation or tissue-homeostatic responses in monocyte/macrophage populations. (D) Schematic illustration of mitochondria-nucleus communication mediated by the transfer of metabolites, which regulate chromatin remodeling and transcriptional programs. (E) Major functional outcomes of mitochondria-nucleus signaling, including nuclear reprogramming, mitochondrial biogenesis, gene regulation and stress adaptation. (F) Representative immune consequences of mitochondria-nucleus communication, including clonal expansion and effector differentiation of early and active CD4⁺ T cells, altered Th17/Treg balance, enhanced CD8⁺ T-cell cytotoxicity and antitumor activity, and inflammatory reprogramming in monocytes/macrophages. OPA1, optic atrophy 1; SNX9, sorting nexin 9; PGC-1 α , peroxisome proliferator-activated receptor gamma coactivator 1-alpha; Th17, T helper 17; Treg, regulatory T cell; mitoEVs, mitochondria-containing extracellular vesicles.

histone acetylation. Targeted knockdown BCKDHA and PDC or using small molecule inhibitors abolished site-specific histone acetylation and disrupted normal cell state transitions (108). Under hypoxic conditions, mitochondrial metabolism is rewired, specifically altering the spatial efflux of citrate and acetyl-CoA from the mitochondria into the cytosolic and nuclear compartments, altering the global landscape of histone acetylation. Exogenous supplementation of acetate or inhibit ATP-citrate lyase (ACLY) reversed the hypoxia-induced epigenetic changes (109). Conversely, the accumulation of specific mitochondrial metabolites exerts regulatory control by competitively inhibiting chromatin-modifying enzymes (110). For example, in cells harboring mutations in the mitochondrial TCA enzymes fumarate hydratase (FH) or succinate dehydrogenase, there is a massive spatial accumulation of fumarate and succinate within the mitochondria, which subsequently spill over into the cytosol and nucleus. These accumulated metabolites act as competitive inhibitors of nuclear α -KG-dependent dioxygenases, including multiple histone demethylases and

TET family DNA demethylases and resulting fundamentally alteration the cellular transcriptomic landscape (111,112). When severe mitochondrial dysfunction triggers the spatial activation of the Integrated Stress Response (ISR) (often via the OMA1-DELE1-HRI axis), it strongly upregulates the transcription factor CHOP. CHOP acts as a highly specific transcriptional tuner to represses the overarching expression of the ISR master regulator ATF4, effectively scaling down the stress response to prevent cytotoxic hyper-activation and allow cellular viability adaptation (113). Thus, mitochondria-nucleus communication couples metabolic state to chromatin and transcriptional regulation while enabling the nucleus to continuously recalibrate mitochondrial function, together supporting sustained cellular adaptation beyond the initiating cue.

In immune cells, mitonuclear signaling can lock in durable activation states, lineage choices and inflammatory thresholds. Upon classic T cell receptor (TCR) and CD28 co-stimulation, early CD4⁺ T cell (CD4⁺ CD62L⁺ CD44⁻) undergo a massive program of mitochondrial biogenesis and proteome remodeling

to specifically upregulate mitochondrial enzymes involved in one-carbon (1C) metabolism (such as SHMT2) to generate formate from serine. This formate then physically effluxes from the mitochondria into the cytosol and nucleus, providing the carbon units necessary for *de novo* purine synthesis and epigenetic methylation, which is key for successful clonal expansion and effector differentiation of T cells. Mitochondrial translation inhibitor and *tfam* genetic deficiency lead to a profound collapse in 1C metabolism and completely arresting T cell activation and proliferation *in vitro* and *in vivo* (114). In pathogenic Th17 cells (CD4⁺ IL-17A⁺ subsets), MTHFD2 restrains inappropriate transcription factor FoxP3 induction, whereas MTHFD2 deficiency favors Treg differentiation (CD4⁺ FoxP3⁺), positioning this axis as a tunable lever for anti-inflammatory immunotherapy target (115). In parallel, upon CD4⁺ T cells activation, the PDH complex, a canonical mitochondrial enzyme, undergoes a notable nuclear translocation. This spatial repositioning allows for the localized, on-site generation of acetyl-CoA directly at the chromatin, which acts as a rate-limiting step to fuel H3K27ac-mediated histone acetylation, promoting T cell activation, differentiation and proliferation, driven by the classic TCR signaling pathway. In PDH-deficiency T cells, acetyl-CoA generation and subsequent histone modification was impaired with limited T cell activation (116). In Treg cells (CD4⁺ Foxp3⁺), elevated levels of the mitochondrial TCA cycle metabolite α -ketoglutarate (α KG) induce a profound metabolic rewiring including oxidative metabolism and altered lipid homeostasis through increasing DNA demethylation, which actively restricting Treg differentiation and shifting the balance toward an inflammatory phenotype. Cell permeable α KG analogs alongside pharmacological inhibitors of mitochondrial complex II (TTFA) and diacylglycerol acyltransferase (DGAT1/2 inhibitors) effectively rescued Treg differentiation (117). In CD8⁺ cytotoxic T lymphocytes (CTLs), mitochondria-derived glutarate exerts its effects by translocating to the nucleocytoplasmic space, where it functions as a competitive inhibitor of nuclear α KG-dependent dioxygenases (such as KDM5 histone demethylases and TET enzymes). This profoundly enhanced the CD8⁺ T cell cytotoxicity, persistence and effector function, significantly boosting antitumor immunity within the classic cytotoxic T cell activation network. Direct *in vivo* dietary supplementation of glutarate, as well as the genetic knockout and pharmacological inhibition of the glutarate-producing enzyme DHTKD1 heighten their antitumor efficacy (118). During inflammatory activation of macrophage (murine BMDMs and human PBMCs), macrophages highly upregulate the mitochondrial enzyme IRG1, catalyzing the production of itaconate to translocation from the mitochondria directly into the nucleus. Within the nucleoplasm, itaconate functions as a direct, competitive inhibitor of Ten-eleven translocation (TET) DNA dioxygenases and inhibit the DNA demethylation at specific loci to dampens the overarching transcription of secondary inflammatory genes, serving as a negative feedback loop for classic NF- κ B inflammatory signaling pathways. Genetic knockout mouse model lacking the itaconate-producing enzyme (*Irg1*^{-/-}), along with treatment using the cell-permeable derivative octyl-itaconate ablation of mitochondrial itaconate production unleashed unbridled nuclear TET activity and exacerbated inflammation (119).

In trained immunity, primary human CD14⁺ monocytes exposed with β -glucan (activating the classic Dectin-1 trained immunity pathway) triggers a sustained upregulation of mitochondrial glutaminolysis, causing the specific accumulation of the TCA cycle intermediate fumarate inside the mitochondria. Fumarate subsequently spatially translocates into the nucleocytoplasmic compartment, where it acts as a competitive inhibitor of nuclear KDM5 family histone demethylases to stable maintaining the activation of epigenetic marks (H3K4me3) at pro-inflammatory gene promoters. Consequently, these cells mount an enhanced, trained cytokine response upon subsequent heterologous infections. Blocking mitochondrial glutamine utilization (treated with glutaminolysis inhibitor BPTES) erased the trained epigenetic phenotype, whereas exogenous fumarate supplementation casually induced trained immunity independently of receptor stimulation (120). Consistently, mevalonate acting as a key mediator via insulin-like growth factor-1 receptor (IGF-1R) -mTOR signaling and downstream histone modifications, establishing a long-lived heightened responsiveness of innate immune cells, directly connecting metabolic state to persistent functional rewiring (121). Under stress condition, herpesviruses induce mtDNA stress is required to fully license interferon stimulated gene expression and robust type I interferon responses (122). TFAM functions as a selective autophagy receptor through an LC3-interacting region, enabling delivery of cytosolic mtDNA-TFAM complexes into the autolysosomal system. By clearing leaked mtDNA, this lysosome-dependent pathway restrains cGAS-STING inflammatory signaling amplification triggered by mitochondrial damage, whereas failure to clear leaked mtDNA can sustain inflammatory signaling (67,123). Together, these studies underscore mitochondria-nucleus communication as a key axis in immune cell regulation, whereby metabolic flux and mitochondrial genome integrity are coupled to nuclear transcriptional and epigenetic programs.

Vesicle-mediated mitochondrial communication in immunity. MDVs (~70-150 nm) and mitoEVs are crucial carriers mediating selective intracellular organelle communication and extracellular cell-to-cell signaling (36,124) (Fig. 5). The biogenesis, cargo selection and routing of these vesicles are orchestrated by a comprehensive network of regulatory molecules. Initial membrane tubulation and scission are driven by MIRO1, MIRO2 and DRP1, coordinated with mitochondrial fission adaptors MID49, MID51 and MFF (125). Following budding, vesicle fate is determined by specific sorting mechanisms. The PINK1-Parkin axis, along with fusion factors such as Syntaxin 17 and the endosomal adaptor Tollip, routes damaged mitochondrial material toward late endosomal and lysosomal pathways (58,123). Alternatively, OPA1 and SNX9 regulate the loading of mitochondrial cargo into extracellular vesicle routes (126), while a distinct pathway involving the E3 ligase MARCH5 and PEX3 generates pre-peroxisomal carriers (70). By packaging functional macromolecular complexes (for example, ATP synthase) rather than random fragments, MDV biogenesis serves as an early, highly selective remodeling step responsive to stress (124). These vesicular pathways exert profound functional impacts on mitochondrial fitness and partner organelles through degradative, metabolic replenishment and signal efflux routes. First, the degradative

MDV-to-lysosome route provides rapid quality control. It selectively clears damaged components independently of bulk mitophagy and acts as an essential compensatory disposal pathway when LC3-dependent autophagy is compromised, thereby preserving mitochondrial homeostasis (127). Second, for metabolic replenishment, specific MDVs support *de novo* peroxisome biogenesis. By routing defined mitochondrial components through an ER-dependent relay, this pathway tightly couples mitochondrial vesiculation to cellular lipid metabolic capacity and peroxisome abundance (25). Beyond intracellular circuits, mitochondrial components can be loaded into multivesicular bodies and released as mitoEVs (27). This signal efflux enables intercellular metabolic repair by transferring functional mtDNA and proteins to support respiration in recipient cells and serves as an alternative degradative clearance route during lysosomal impairment (128). Crucially, the sorting mechanisms governing MDV/mitoEV fate impose strict regulations on what mitochondrial material exits the cell. This selectively limits the release of potentially pro-inflammatory mitochondrial cargo [for example, damage-associated molecular patterns (DAMPs)], directly shaping intercellular communication. In the tissue microenvironment, secreted mitoEVs are captured by professional phagocytes, linking the routing of mitochondrial material directly to phagosomal processing. Ultimately, this dynamic vesicular continuum, balancing internal lysosomal turnover with external vesicle release, determines the final disposal site of mitochondrial antigens and profoundly modulates local inflammatory states and immune responses under stress (27,126).

In immunity, MDVs and mitoEVs constitute an additional mechanism of mitochondrial quality control that intersects with antigen presentation, innate immune sensing, and tissue level immuno-metabolic crosstalk (36,124). In professional antigen presenting immune cells, mitochondrial proteins can be routed for MHC class I presentation, and this pathway is restrained by the Parkinson's-linked PINK1-Parkin quality control axis. Mechanistically, PINK1-Parkin suppresses MDV dependent delivery of mitochondrial cargo into the endolysosomal system for antigen loading, thereby limiting mitochondrial antigen presentation (MitAP) under inflammatory stimuli, thereby coupling organelle surveillance decisions to adaptive immune monitoring (129). In an infection model, loss of PINK1 is associated with rewired early immune responses and altered inflammatory programs, where peripheral myeloid cells emerge as the earliest highly dysregulated subset, subsequently triggering an aberrant CD8⁺ cytotoxic T cell response. This is consistent with mitochondrial quality control acting as an upstream constraint on immunogenic mitochondrial cargoes which shape downstream innate and adaptive immune programs. Although that study's focus was not limited to vesicle ultrastructure, it links a mitochondrial quality control defect to detectable alterations in host immune response dynamics during infection (130). Beyond MitAP, infection can trigger remodeling of MAMs and outer membrane shedding. For example, during infection-associated mitochondrial import stress, mitochondria can generate large outer membrane positive structures (SPOTs) consistent with a vesiculation remodeling response that redistributes outer membrane components. Functionally, this stress-induced remodeling influences infection consequences, suggesting how

mitochondrial membrane remodeling can be hijacked during in host pathogen interactions with downstream consequences for inflammatory damage and cellular defense capacity (131). Under stress condition, EV loading is selectively regulated to avoid exporting oxidized mitochondrial proteins that function as pro-inflammatory DAMPs. Mechanistically, OPA1 and SNX9 promote MDVs' trafficking mitochondrial proteins into EVs, whereas Parkin redirects damaged mitochondrial content toward lysosomal disposal, collectively shaping whether mitochondrial signals are immunologically silent or inflammation enhancing (126). Conversely, when mitochondrial and nuclear DNA are packaged into EVs, transfer of EV-associated dsDNA to macrophages can engage the cGAS-STING pathway and amplify inflammatory programs in Crohn's disease relevant inflammatory condition, providing a direct mechanistic link between mitoEV cargo and innate immune activation (132). Under inflammasome related pyroptotic stress, caspase-1 and gasdermin D promote mtDNA escape into the cytosol, then drive intraluminal vesicle formation that enables mtDNA loading into intraluminal vesicles (ILVs) and release via exosomes. Extracellular mtDNA containing exosomes trigger excessive inflammation that induces Behçet's syndrome such as pathology, directly connecting inflammatory cell death to mitoEV-mediated propagation of sterile inflammation (133). At the tissue level, mitochondrial export pathways can interface with professional phagocytes to support immunologically controlled organelle disposal. In the healthy myocardium, resident macrophages actively take up cardiomyocyte-derived components including mitochondria, supporting tissue homeostasis through phagocytic disposal of expelled mitochondrial cargo (134). Conversely, thermogenically stressed brown adipocytes eject oxidatively damaged mitochondrial cargoes within EVs, and resident macrophages remove these mitoEVs to prevent extracellular accumulation to preserve thermogenic competence. When macrophage clearance is compromised, mitoEV excess accumulation is associated with impaired adaptive thermogenesis, linking immune phagocyte function to mitochondrial quality control at the tissue level (135). In addition, when lysosomal function is compromised, cells can reroute mitochondrial components into EVs generated through late endosome or MVB pathways, creating an extracellular offloading route for mitochondrial components. *In vivo*, these mitochondria containing EVs can be captured by phagocytes and ultimately processed in recipient cell lysosomes, positioning mitoEV release as compensatory organelle quality control with immunological consequences (27). Collectively, these findings support that vesicle-mediated mitochondrial trafficking via MDVs and mitoEVs coordinates cellular intrinsic mitochondrial quality control with immune recognition and intercellular inflammatory signaling and indicate that context-dependent imbalances between immunologically silent disposal and inflammatory mitochondrial export can remodel tissue immune state and influence susceptibility to inflammation-associated disease states.

3. Rewiring mitochondrial interfaces in immune dysregulation-associated diseases

Immune dysregulation across diverse diseases is increasingly recognized that mitochondria function as integrated signaling

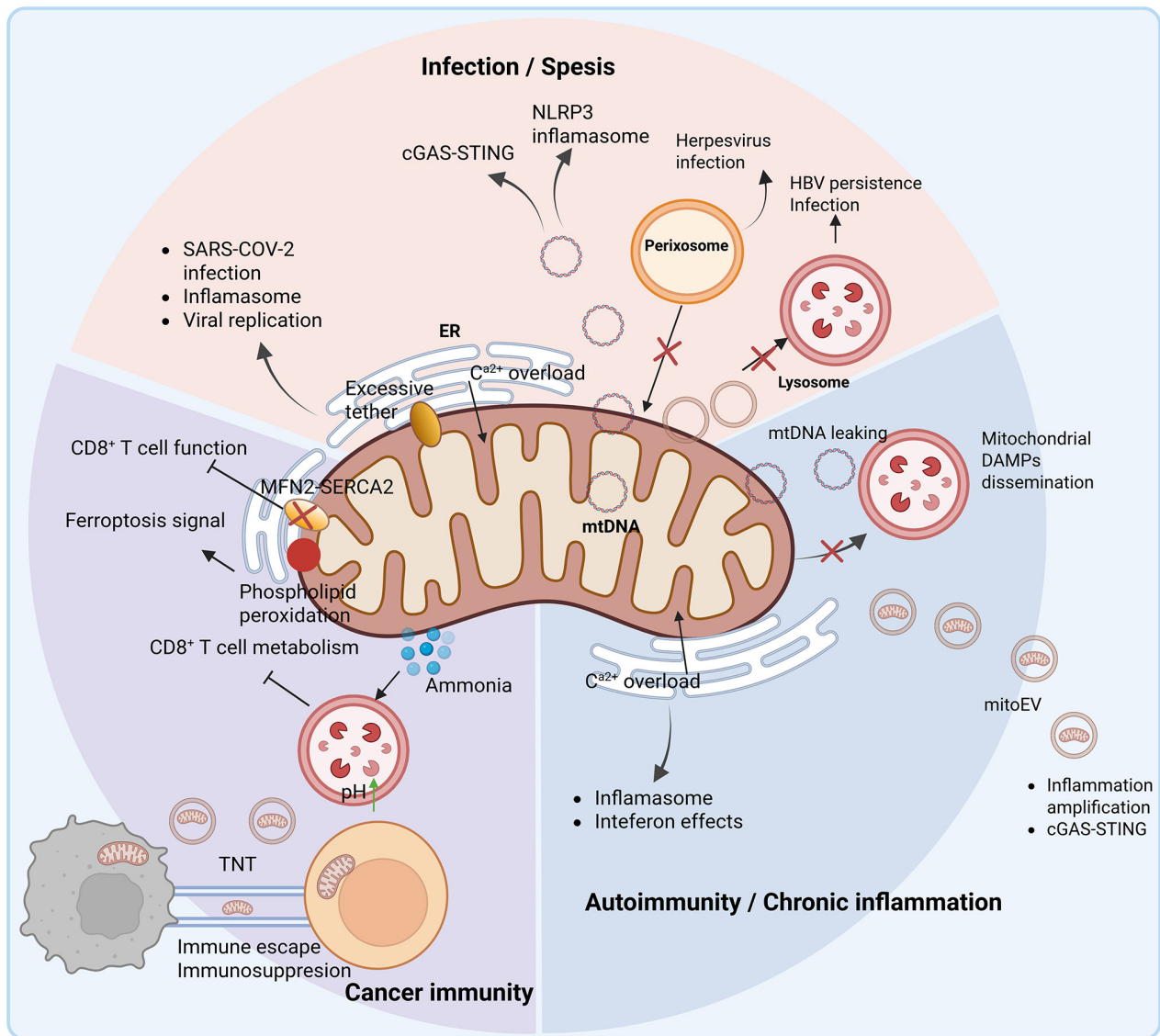


Figure 6. Maladaptive rewiring of mitochondrial interfaces in immunopathology. Mitochondrial organelle interfaces and trafficking routes are remodeled during disease, shifting from homeostatic regulation to pathogenic signaling. In infection and sepsis, pathogens (such as SARS-CoV-2) induce aberrant ER-mitochondria tethering and Ca²⁺ overload, triggering NLRP3 inflammasome activation and viral replication. Mitochondrial stress leads to mtDNA leakage and cGAS-STING activation, while peroxisomes contribute to antiviral signaling. Additionally, inhibition of lysosomal function (for example, during HBV persistence) prevents the clearance of damaged organelles and viral components. In autoimmunity and chronic inflammation, sustained ER stress and Ca²⁺ overload promote the release of mitochondrial DAMPs and oxidized mtDNA. Defective lysosome clearance amplifies sterile inflammation via inflammasome and interferon pathways, while mitoEVs disseminate inflammatory signals systemically. In cancer immunity, the tumor microenvironment disrupts the MFN2-SERCA2 axis at ER-mitochondria contacts, impairing the metabolic fitness of CD8⁺ T cell. Furthermore, interface-associated phospholipid peroxidation promotes ferroptosis signaling, while lysosome accumulation of ammonia inhibits T cell metabolism. Intercellular transfer of mitochondria via TNTs further contributes to immunosuppression and tumor immune escape. NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; mtDNA, mitochondrial DNA; cGAS, cyclic GMP-AMP synthase; STING, stimulator of interferon genes; HBV, hepatitis B virus; mitoEV, mitochondria-containing extracellular vesicle; SERCA2, sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase 2; DAMPS, damage-associated molecular patterns; ER, endoplasmic reticulum; TNTs, tunneling nanotubes.

and metabolic hubs within inter-organelle communication landscapes rather than as autonomous bioenergetic units. Mitochondrial MCSs and vesicle-mediated trafficking routes coordinate metabolite flux, calcium handling, and redox control with innate immune sensing and antigen processing, thereby allowing host defense interface modules to, when chronically activated or aberrantly dysregulated, sustain maladaptive inflammatory responses and tissue injury in infection and sepsis (123,136), cancer (137,138), and autoimmune or chronic inflammatory disorders (139,140). In the present review, the shared and disease specific patterns of mitochondrial interface rewiring were summarized and were

linked to mechanism-targeted immunomodulatory strategies (Fig. 6).

Infection and sepsis. Mitochondria shape host defense in infection and sepsis through interface circuits couple organelle organization to innate signaling and damage handling. During viral infection, pathogens can rewire ER-mitochondria contact topology rather than altering mitochondrial metabolism alone. In human cytomegalovirus infection, stabilized ER encapsulation structures around mitochondria become a dominant contact phenotype that supports viral production, while premature strengthening of ER-mitochondria tethering

can activate STING linked antiviral programs, revealing that contact remodeling can tune the balance between viral replication and immunity (4,141). During SARS-CoV-2 infection, viral structural proteins perturb host calcium homeostasis and remodel ERMCS, linking altered contact architecture to mitochondrial dysfunction and downstream cell stress programs that can influence inflammatory injury in infected tissues (142). SARS-CoV-2 also engages mitochondrial quality control to dampen antiviral signaling. Open reading frame 10 (ORF10) suppresses type I interferon programs by promoting MAVS degradation through mitophagy, thereby connecting a virus driven shift in mitochondrial turnover to impaired innate antiviral response (143). Within infected cells, ERMCS provide spatial control points for inflammasome priming. Enhanced ER-mitochondria coupling can increase localized Ca^{2+} delivery to mitochondria and promote permeability transition-associated release of oxidized mtDNA fragments that support NLRP3 activation, positioning Ca^{2+} hotspots at the interface as a proximal switch for caspase 1-dependent cytokine activation (144). Crucially, genetic knockout or pharmacological blockade of the mitochondrial permeability transition pore and VDAC completely abolishes the cytosolic escape of oxidized mtDNA, confirming the physical opening of these interface channels is the strict molecular trigger that precedes, and directly causes, subsequent NLRP3 inflammasome assembly and pyroptosis. ER stress can further tighten ER-mitochondria communication and augment Ca^{2+} dependent NLRP3 responses together with remodeling of MAM-associated factors and mitochondrial dynamics, reinforcing that inflammatory state is regulated at contact sites (33).

Antiviral signaling is also spatially organized across mitochondria and peroxisomes, as MAVS signals from both membranes and peroxisomes can function as antiviral signaling sites (86). In herpesvirus infection, mitochondria to cytosol communication via mtDNA stress enhance the innate antiviral signaling. Mitochondrial genome instability promotes mtDNA release that engages cGAS-STING and elevates interferon-stimulated gene expression, strengthening antiviral defense, but prolonged activation provides a route to excessive inflammatory signaling that can contribute to immunopathology during infection (122). Consistently, viperin can engage the peroxisome biogenesis factor PEX19 and has been proposed to recruit peroxisomes to MAVS signaling hubs, supporting an inter-organelle view for interferon induction (145). In addition, mitochondria-lysosome coupling and vesicle-mediated trafficking constrain how mitochondrial damage is processed during infection. Mitophagy and MDVs can route selected mitochondrial cargo to multivesicular bodies and lysosomes for degradation, limiting persistent mtROS producing substructures and reducing uncontrolled DAMP release (125,127). When lysosomal function is compromised, mitochondrial cargo can be diverted toward extracellular disposal, including the secretion of mitoEVs under lysosome inhibited conditions, shifting the balance from degradation toward export with potential immunological consequences (27). In HBV infection, mitochondria-lysosome coupling emerges as a decisive antiviral control level. Suppression of mitochondrial respiratory chain activity weakens lysosomal acidification through combined redox

and energetic mechanisms, which reduces autophagic degradation of viral material and thereby favors HBV persistence and disease progression, whereas restoration of respiratory chain support reinstates lysosomal function and promotes viral clearance (146). Specifically, pharmacological inhibition of the mitochondrial respiratory chain using Rotenone or Antimycin A directly triggers defective lysosomal acidification and halts autophagic flux, resulting in delayed HBV clearance; conversely, metabolic restoration of respiratory chain function fully reverses these lysosomal deficits, establishing mitochondria-lysosome energetic crosstalk as the upstream causal determinant of viral clearance. Metabolic stress can also intersect with these routes, because fumarate-driven mitochondrial stress promotes an MDV pathway that enables mtDNA release and cGAS-STING linked inflammation (147). Crucially, while conditional deletion of FH triggers early mtDNA cytosolic release, targeted genetic depletion of Sorting Nexin 9 (SNX9) completely abolishes the generation of these MDVs and prevents the subsequent STING-dependent inflammatory response. This mechanistically proves that SNX9-dependent vesicular trafficking is the indispensable sequence bridging metabolic stress to innate immunity (147). Together, these observations support that infection reshapes mitochondria-ER, mitochondria-lysosome, mitochondria-peroxisome and vesicle mediated mitochondrial routes to balance microbial clearance against host damage.

In sepsis, the same interface circuits are adaptive during early infection can become persistently engaged and augment immunopathology. Circulating mtDNA can activate STING and simultaneously disrupt lysosomal acidification and autophagic clearance, creating a feed forward circuit that sustains inflammatory injury in sepsis associated lung injury models (148). Targeted administration of the STING-specific small molecule inhibitor C-176, or genetic knockdown of STING, explicitly blocks mtDNA-induced microglial polarization, rescues autophagic dysfunction, and significantly alleviates sepsis-associated inflammatory injury, confirming STING as the essential causal trigger in this pathological cascade. Maladaptive interface remodeling also involves the direct dysregulation of mitochondria-lysosome contacts; specifically, reduced TBC1D15-dependent contact control aggravates inflammatory lung injury while restoring TBC1D15 function can improve mitophagy flux and tissue outcomes (149). Knockdown or conditional deletion of the Rab7 TBC1D15 disrupts these contacts, impairs autophagic clearance, and thereby exacerbates sepsis-induced acute lung injury, whereas restoration of TBC1D15 reverses this pathogenic cascade (149). Beyond intracellular contacts, vesicle-mediated mitochondrial transfer can propagate damage signals across immune cells, because pyroptotic macrophage-derived microvesicles can deliver mitochondria to neutrophils and enhance NET formation and coagulopathy, and microvesicle-transferred mitochondria have been linked to cGAS-STING activation and inflammatory reprogramming in sepsis (150). Specifically, genetic knockout of Gasdermin D (GSDMD^{-/-}) or treatment with the GSDMD inhibitor Disulfiram strictly prevents the packaging of GSDMD-N-expressing mitochondria into microvesicles, subsequently abolishing microvesicle-induced NETosis and protecting against sepsis-induced acute injury. This clarifies that GSDMD-mediated pore formation is the

upstream causal event dictating intercellular mitochondrial transfer. Collectively, these studies indicate that mitochondria-centered contact sites and vesicle-based trafficking act as tunable targets during infection but can become maladaptive drivers of sustained inflammation and organ injury in sepsis.

Cancer immunity. The TME enforces chronic bioenergetic and catabolic stress on infiltrating immune cells, and accumulating evidence indicates that mitochondria-centered coupling and trafficking translate this stress into antitumor immune phenotypes. Hypoxic and lactate-enriched niches reprogram solute transport and carbon utilization in tumor infiltrating exhausted T cells, thereby constraining mitochondrial metabolic limits and effector capacity, which provides metabolic conditions in which organelle coordination becomes rate limiting for immune effector function (151). At the interface, ER-mitochondria coupling provides direct evidence that interface architecture can determine intratumoral T cell effector capacity. In TME, ERMCS act as prime hotspots for phospholipid peroxidation that initiates and propagates ferroptotic signaling, indicating that contact site remodeling can tune ferroptosis sensitivity and thereby reshape immune pressure within the tumor ecosystem (152). In CD8⁺ T cells, an MFN2-SERCA2 axis supports ER-mitochondria calcium homeostasis and mitochondrial metabolic competence, and disruption of this module compromises effector function and antitumor immunity, while reinforcement improves therapeutic efficacy, positioning ER-mitochondria interfaces as regulatory hubs that connect chronic stimulation to durable dysfunction (50). Deletion of MFN2 explicitly in T cells (Cd4-Cre mediated deletion *in vivo*) disrupts the SERCA2 interaction, proving that the structural loss of this specific ER-mitochondria tether is the direct upstream trigger for intracellular Ca²⁺ dysregulation. This timeline of events, initial contact loss, followed by calcium and metabolic collapse, definitively shows that interfacial disruption actively drives CD8⁺ T cell exhaustion in the TME, rather than being a secondary byproduct of the exhausted state. Mitochondria-lysosome coupling is also poised to shape immune cell persistence by specifying whether stressed mitochondrial regions are repaired, routed to lysosomal degradation, or rerouted toward inflammatory signals. In effector CD8⁺ T cells, ammonia produced by mitochondrial metabolism is routed into lysosomes, and excessive accumulation elevates lysosomal pH, triggers reflux-associated mitochondrial injury, and blocks effective clearance, thereby weakening antitumor immunity and adoptive T cell therapy responses (153). Mitochondria-lysosome coupling as a core immuno-metabolic module in T cells, where coordinated lysosomes with mitochondrial bioenergetics and redox to shape autophagy, trafficking, and fate decisions, highlighting contact site biology as a still unresolved determinant of exhaustion progression and therapy responsiveness (64).

Vesicle-mediated mitochondrial communication provides an additional aspect that allowing tumors to distribute mitochondrial signals and even mitochondria to reshape immunity at a distance. Senescent or stressed tumor cells can release mtDNA in EVs that are taken up by polymorphonuclear myeloid-derived suppressor cells, thereby activating cGAS-STING together with an ER stress pathway involving

protein kinase R-like ER kinase (PERK) to reinforce an immunosuppressive program in the TME (154). In the TME, cancer cells can transfer dysfunctional mitochondria carrying mutant mtDNA into T cells, and these imported mitochondria persist rather than being efficiently cleared, driving metabolic disruption and functional decline that favors immune escape (138). Consistently, tumor cells can acquire mitochondria from immune cells through physical nanotubes, metabolically empowering cancer cells while depleting mitochondrial capacity in immune cells and weakening their antitumor activity (155). Conversely, bone marrow stromal cells form intercellular nanotubes that deliver mitochondria into CD8⁺ T cells, thereby restoring respiratory capacity, improving resistance to exhaustion, and increasing antitumor efficacy across preclinical models (156). Collectively, these studies indicate that tumors reshape ER-mitochondria contact architecture and lysosome linked quality control within immune cells while deploying mitoEV cargo transfer and direct mitochondrial exchange to impose spatially organized, mitochondria-centered immunosuppression across the TME.

Autoimmunity and chronic inflammation. Mitochondria organelle interfaces provide a core checkpoint that determines whether mitochondrial stress is resolved or converted into persistent sterile inflammation in autoimmunity and chronic inflammatory disease. When nucleoid turnover and endolysosomal disposal are compromised, mtDNA and inner membrane-associated immunostimulatory species accumulate in aberrant location and chronically activate cytosolic DNA sensing and inflammasome pathways, including cGAS-STING and NLRP3 pathway, thereby biasing myeloid and stromal compartments toward type I interferon and IL-1 family responses (144,157,158). Under homeostatic conditions, mtDNA stress is buffered by routing into autophagy and lysosome pathways, and TFAM-dependent sequestration of cytoplasmic mtDNA and nucleoid material restricts inappropriate DNA sensing and inflammatory signaling (67). When Tfam is conditionally ablated (using Cre-loxP conditional knockout models) in specific immune or neural cell lineages, the immediate molecular trigger is the failure of mitochondria to deliver mtDNA to autophagosomes. This localized interface failure rigorously precedes the subsequent pathological accumulation of cytosolic mtDNA and the downstream hyperactivation of the cGAS-STING pathway, proving that the disruption of TFAM-mediated organelle crosstalk is the primary initiator of sterile autoimmune pathology.

Mitochondrial function also feeds back on the degradative pathway of this circuit, because mitochondrial respiratory defects can impair lysosome competence and endolysosomal trafficking, linking primary mitochondrial dysfunction to reduced clearance capacity and exaggerated inflammatory programs (65). In parallel, chronic ER stress and sustained ER-mitochondria calcium coupling at contact sites can increase mitochondrial permeability and facilitate escape of oxidized mitochondrial danger signals, reinforcing inflammasome and interferon circuits (33,144). Vesicle-based routing further determines whether organelle stress remains local or becomes systemically pro-inflammatory, as MDVs execute selective cargo sorting that favors lysosomal disposal of damaged mitochondrial material while limiting extracellular dissemination

of mitochondrial DAMPs, and this selection is reinforced by Parkin dependent MDV trafficking mechanisms that require endosomal adaptors such as Tollip (159). More generally, mitochondrial components can be packaged into EVs in a regulated way that shapes cargo oxidation state and inflammatory potential, supporting the idea that vesicle selection is an active checkpoint that separates protective disposal from DAMP dissemination (126). In inflammatory condition, mitochondria to lysosome signaling controls Treg cell state stability which determine whether tissue inflammation resolves or persists. Mitochondrial stress-driven signaling engages AMPK- and TFEB-dependent lysosomal programs, shifting Tregs away from highly oxidative suppressive states and reducing effective tissue immune suppression, which can permit sustained inflammation and thereby worsen disease-associated immune dysregulation (32). In systemic lupus erythematosus, platelet activation drives release of extracellular mitochondria and mtDNA, providing circulating mitochondrial antigens and nucleic acids that can be taken up by immune cells and linked to interferon-biased programs (160). Collectively, mitochondria-lysosome and mitochondria-ER contact site signaling, together with MDV-based cargo selection, define an integrated inter-organellar network that gates mitochondrial danger signals and provides a mechanistic foundation for sustained autoimmunity and chronic inflammation.

4. Therapeutic targeting of mitochondrial interfaces

Small molecule and biologic targeting contact sites and mitochondrial traffic. Therapeutic manipulation of mitochondria-organellar interfaces aims to reroute inflammatory signals flow at nanoscale contact sites and vesicular trafficking checkpoints rather than globally suppressing mitochondrial metabolism. In this context, small molecules currently represent the most tractable therapeutic modality, whereas interface-selective biologic strategies remain largely experimental (161). A pharmacologically accessible target is the sigma 1 receptor enriched at ER-mitochondria contacts, which constrains IRE1 α RNase activity during stress. In murine models of systemic inflammation and sepsis, pharmacologic sigma-1 receptor agonism with fluvoxamine suppressed IRE1-dependent cytokine programs and improved survival, supporting an interface-enriched molecular pathway as a druggable immunomodulatory target (162). In LPS or fecal-induced inflammatory murine models, fluvoxamine dampened cytokine productions in *ex vivo* LPS-stimulated human whole-blood leukocytes (162). More direct MERC-directed screening has also yielded compound 24, first identified in HCT116 cells as a MERC enhancer, and subsequently shown to restore defective cholesterol trafficking, increased MERCs, enhanced LD formation and restoration of mitochondrial cristae architecture in an amyotrophic lateral sclerosis patient-derived cell model (163). In parallel, several clinically used or bioactive compounds appear to remodel MERC function in a context-dependent manner rather than by directly targeting tether proteins. For example, metformin normalized excessive sarcoplasmic reticulum (SR)/ER-mitochondria coupling in ventricular cardiomyocytes. This drug decreased myocardial MICU1 expression, mitochondrial Ca²⁺ content and improved complex I-driven respiration (164). Sulforaphane

was reported to counteract MAM disruption and exaggerated glucose production in palmitate-treated mouse hepatocytes and diabetic animal models (165). Luteolin, identified in a neuronal cell-based high-throughput screen and validated in primary neurons, increased mitochondria-ER contacts, mitochondrial Ca²⁺ and Ca²⁺-dependent PDC activity without changing mitochondrial mass or structure (166). In neuronal cells, urolithin A treatment reduced mitochondrial Ca²⁺ influx, mtROS accumulation, and cognitive impairment in high glucose and in streptozotocin-induced diabetic mice (167). Oxidative stress can promote efflux of mtDNA fragments through pores associated with VDAC oligomerization at the mitochondrial outer membrane, thereby potentiating cytosolic DNA sensing and downstream inflammatory responses. Pharmacologic inhibition of VDAC oligomerization with VBIT-4 reduced mtDNA release and attenuated interferon linked pathology in a lupus model, providing proof of principle that restricting mitochondrial danger signals escape can indirectly dampen downstream organelle coupled inflammatory signaling, including cGAS-dependent STING signaling (168). The translational attractiveness of this strategy lies in its action at a proximal checkpoint upstream of several inflammatory sensing pathways, although this advantage is tempered by a relatively narrow safety window, because higher concentrations of VBIT-4 can suppress mitochondrial respiration, perturb membrane potential and reduce cell viability (169). Pharmacologic manipulation of MDV routing remains at an early stage, yet recent mechanistic work has begun to identify druggable intervention targets. Parkin, acting in concert with the endosomal adaptor Tollip, facilitates the incorporation of MDVs into the endosomal system for subsequent lysosomal delivery independently of canonical mitophagy, supporting the concept that vesicle-mediated mitochondrial quality control can be selectively reinforced to constrain inflammatory mitochondrial cargo (159). In this context, inhibition of the mitochondrial deubiquitylase USP30 represents a practical chemical lever because it amplifies PINK1-Parkin-dependent ubiquitin signaling positioned upstream of multiple mitochondrial disposal routes, including vesicle mediated trafficking and lysosome directed clearance, thereby providing a plausible way to shift mitochondrial traffic toward protective containment when the pathway is intact or only partially compromised (170). Compared with VDAC blockade, USP30 inhibition benefits from enzymatic tractability and measurable pharmacodynamic readouts, although its efficacy is still likely to depend on residual integrity of PINK1/Parkin-associated quality control pathways in the target cells. Translational development will require precise characterization of therapeutic windows, given that interface components regulate essential calcium and lipid exchange. Excessive enhancement of ER-mitochondria calcium coupling may increase susceptibility to permeability transition, whereas excessive attenuation can impair antimicrobial signaling and lymphocyte activation, indicating that efficacy and clinical acceptability are likely to depend on cell type, disease stage and dosing regimen (162). These drugs are translationally attractive because it combines repurposable agents with established human pharmacology, yet their further clinical translation remains limited by several issues. The field lacks robust biomarkers, target engagement assays, and delivery strategies capable of demonstrating selective

MERC remodeling in immune disease relevant conditions. Moreover, the relevance of these agents to immune-related disease remains incompletely defined, as most have yet to be validated in bona fide immune cells or in established models of immune-mediated pathology. Future translational studies should therefore focus on demonstrating target engagement in relevant immune cell subsets.

For mitochondria-lysosome communication, the current small-molecule landscape is somewhat more mechanistically focused, although it remains at an early stage. In palmitate-injured human endothelial cells and renal tubular epithelial cells, TRPML1 agonists ML-SA5 and ML-SA8 reduced mitochondrial ROS, indicating that pharmacologic activation of lysosomal Ca^{2+} signaling can stabilize mitochondrial homeostasis through the lysosome-mitochondria axis (171). Importantly, this pathway also has emerging relevance to immune biology, because ML-SA5 suppresses IL-1 β secretion in bone marrow-derived macrophages and RAW264.7 cells exposed to LPS, zymosan A, or resiquimod, thereby linking lysosomal signaling directly to inflammatory control in macrophages (172). Currently available pharmacology for mitochondria-LD and mitochondria-peroxisome interfaces is rare and is dominated mainly by indirect metabolic remodeling rather than direct tether targeting. At the mitochondria-LD interface, atorvastatin promoted PKA-dependent phosphorylation of PLIN5 in livers of high-fat diet-fed mice and in isolated hepatocytes, thereby enhancing lipolysis and reducing hepatic triglyceride accumulation (173), whereas glycycomarin induced the PLIN5-SIRT1 axis *in vitro* and *in vivo* and attenuated palmitate-induced hepatocyte lipo-apoptosis and inflammatory responses (174). Because PLIN5 is a key organizer of LD-mitochondria coupling, these observations are therapeutically relevant, but they also indicate that existing compounds mainly act through metabolic regulators of the interface rather than through dedicated contact site ligands. At the mitochondria-peroxisome interface, the best developed small molecules are PPAR agonists. Fenofibrate enhanced the expression of peroxisomal genes and proteins involved in peroxisomal biogenesis and function in livers of high-fat diet-fed mice, while also improving FAO programs linked to mitochondrial metabolism (175). Bezafibrate was explored in VLCAD-deficient patient fibroblasts as a metabolic reprogrammer for mitochondrial FAO disorders (176), although subsequent work in stressed VLCAD-deficient cells showed that bezafibrate-driven PPAR activation could also disturb mitochondrial redox bioenergetics and reduce cell viability (177). Several drugs (for example, fenofibrate and bezafibrate) already have systemic pharmacology, oral dosing paradigms, or early clinical safety information, which lowers the barrier to repurposing; however, their limitations are especially relevant for immune-oriented interpretation. Accordingly, for immune-related diseases these compounds are considered enabling metabolic modulators whose relevance to organelle interface immunobiology remains to be established directly.

Interface-targeted delivery and nanotechnologies. Following from pharmacologic manipulation of interface proteins, targeted delivery and nanotechnology provide a targeting strategy that localizes bioactivity at nanoscale interfaces and

MDV pathways, thereby improving efficacy while limiting system wide perturbation of organelle homeostasis. A practical design principle is dual or sequential subcellular targeting, in which a mitochondria-enriched motif is combined with a second motif that biases accumulation at a partner compartment such as the ER, increasing the probability that payloads reside near contact zones where calcium, redox and lipid exchange are integrated. In ER-mitochondria co-targeting strategies, the molecular delivery platform DDY enables simultaneous mitochondrial and ER localization with glutathione (GSH) responsive intracellular parent drug release. Cancer cells exhibit significantly higher GSH concentrations than normal cells; DDY safely and selectively releases the repurposed antihistamine desloratadine directly at mitochondria-ER interfaces. This precision delivery induces profound ferroptosis in xenograft and metastasis tumor models while avoiding systemic toxicity, highlighting a translational pathway to reactivate approved drugs lacking innate anticancer activity. An additional interface-directed strategy is mitochondria-ER click systems built on the benzothiophene-fused azacyclononyne BT9N. By conjugating an ER-directing sulfonamide moiety with mitochondria maintaining thiophene and thiourea groups, BT9N relies on mitochondrial membrane potential independent targeting to undergo direct intra-organelle click reactions with azides. This modularity enables organelle resolved functionalization proximal to mitochondria-ER interfaces, providing a contact proximate perturbation strategy that avoids bulk organelle disruption (178). Interface-resolved profiling further enables rational anchor selection, specifically, proximity-dependent biotinylation and split proximity labeling at LD-mitochondria contact sites identified a multimeric ESYT1-ESYT2-VAPB/VAPC architecture localized at LD-mitochondria-ER junctions that supports FA transfer and links tether composition to lipotoxic stress control with implications for metabolic disease biology (16). Photoactivatable THTTPy-PTSA enables sequential ER-mitochondria targeting to rewire ER-mitochondria coupling and enhance immunogenic cell death coupled antitumor immunity *in vivo* (179). Mechanistically, THTTPy-PTSA employs a p-toluene-sulfonamide (PTSA) group for initial ER localization. Upon light irradiation, its tetrahydropyridine group undergoes photo-oxidative dehydrogenation into a mitochondria-targeting pyridinium moiety, driving a spatial translocation that triggers cascade-amplified ER stress. This targeted perturbation overcomes ER-mitochondria mediated immune escape by releasing abundant DAMPs to induce widespread ICD (179). Similarly, the nanocomposite ($\text{Cu}_2\text{Se}-\text{CaO}_2$)@LA evokes mitochondria-associated ER stress and engages PERK-mediated eIF2 α phosphorylation to drive ICD with dendritic cell maturation and cytotoxic T cell recruitment (180). Triggered by second near-infrared (NIR-II) irradiation, this H_2O_2 -self-supplying platform integrates photothermal and chemodynamic therapies (PTT/CDT) to induce severe Ca^{2+} overload and ROS formation. This sequence selectively damages the mitochondria and ER networks, exerting potent *in vivo* tumor suppression with high biosafety (180). Additional ER-mitochondria dual targeting phototherapeutic implementations include the near infrared photosensitizer TCy5-I-3F that synchronizes ER stress with mitochondrial dysfunction to inhibit tumor growth and enhance immune activation under irradiation (ER and mitochondrial

double-targeted NIR photosensitizer synergistically promote tumor cell death). Additionally, the colorectal cancer nano-platform (NP) HA/pCe6-A to NPs that suppresses cancer stemness-linked resistance while enhancing immunotherapy, consistent with the principle that interface-synchronized ER stress and mitochondrial damage can be therapeutically beneficial (181). By utilizing a hyaluronic acid coating to actively target CD44 receptors on cancer stem-like cells via endocytosis, this platform co-delivers the ER-targeting photosensitizer pCe6 and the mitochondrial inhibitor atovaquone. This dual organelle disruption effectively reprograms the immunosuppressive TME by promoting apoptosis and ROS production, achieving superior synergistic tumor inhibition compared with conventional monotherapies (181).

Beyond ER coupling, mitochondria-lysosome co-targeting nanostrategies are emerging through dual localization photosensitizer or nanoparticle designs to modulate autophagy flux and mitochondria-lysosome crosstalk. For example, a biomimetic nanoplateform utilizing a hollow MnO₂ core loaded with tetrandrine and a mitochondria-targeting photosensitizer (Ce6-Apt) initiates a triple-killing mechanism. Under near-infrared irradiation, Ce6-Apt inflicts mitochondrial photodynamic damage to activate mitophagy, while tetrandrine induces lysosomal alkalization to block the mitophagic flux and simultaneously triggers excessive macropinocytosis (methuosis). This synergistic spatiotemporal control completely severs the compensatory energy supply of tumor cells, offering a precision strategy to overcome metabolic drug resistance (182). Vesicle focused engineering provides an interface informed delivery level because mitochondria-lysosome quality control decisions can be switched toward extracellular secretion when lysosomal function is impaired, as lysosomal inhibition increases the secretion of mitochondria within large EVs generated in multivesicular bodies and released independently of autophagy, establishing a degradation vs. export switch that is directly relevant to MDVs or mitoEVs (27), while regulated cargo selection limits dissemination of oxidized mitochondrial DAMP proteins into EVs, indicating that vesicle loading is an active checkpoint that separates protective disposal from pro-inflammatory release (126). Enabling EV platforms that enhance active cargo loading and endosomal escape can be adapted to interface-targeted delivery, when coupled to validated dual organelle targeting signals. Examples include TOP-EVs for intracellular protein delivery (183) and multimodal engineered EVs that combine mini-intein mediated cargo loading with VSV-G driven endosomal escape (184). By contrast, strategies such as IDEA-mediated cGAS delivery mainly demonstrate intracellular pathway engagement, with limited evidence for preferential enrichment at organelle contact microdomains (185). Overall, current interface-directed delivery is best developed for ER-mitochondria coupling and for mitochondria linked endolysosomal quality control and offloading routing, whereas other mitochondria organelle interfaces remain comparatively limited, underscoring the need for future interface resolved profiling guided ligand design and validation.

Despite these innovative designs, the clinical translation of nanoparticle-based delivery systems is severely hindered by *in vivo* bottlenecks, including poor solid tissue penetration, rapid clearance by the mononuclear phagocyte system,

and variable immunological responses and translational uncertainty in humans (186). The leap from murine xenograft models to the complex human immune ecosystem remains a formidable challenge. Alternatively, vesicle-focused engineering (such as EVs) provides an interface-informed delivery platform with superior biocompatibility (126). Strategies such as TOP-EVs (183) and mini-intein-mediated EVs (184) enhance precise cargo loading. However, scaling up engineered EVs for clinical grade manufacturing with consistent batch-to-batch uniformity remains a core industrial obstacle. Future translational efforts must prioritize simplifying these complex delivery platforms into scalable, immune-cell-directed formulations that maintain high interface specificity with low systemic exposure.

Engineering interfaces for adoptive cell therapy. Distinct from systemic *in vivo* nanomedicine, targeted modulation of mitochondrial interfaces during the cellular manufacturing phase provides a highly translatable strategy to imprint metabolic resilience and sustain long-term antitumor efficacy in adoptive cell therapies. Sustained efficacy of adoptively transferred tumor reactive CD8⁺ T cells relies on mitochondrial fitness integrated across organelle interfaces and mitochondria nucleus renewal programs under chronic stimulation. In solid tumors, persistent antigen exposure and microenvironmental stress drive impaired ADP coupled oxidative phosphorylation, oxidative damage, reduced mitophagy and accumulation of dysfunctional mitochondria, which promote terminal exhaustion and limit persistence (187). One engineering strategy strengthens mitochondria-ER coupling through the MFN2-SERCA2 axis, in which MFN2 stabilizes contact-dependent calcium exchange and sustains metabolic fitness in tumor infiltrating CD8⁺ T cells to improve effector function and antitumor immunity in solid tumor, providing a strategy for reinforcing contact points during adoptive manufacturing or genetic engineering (50). Mitochondria to lysosome quality control is a second leverage targetable step because repeated antigen stimulation drives progressive accumulation of damaged mitochondria that cannot be compensated by late intervention. In metabolically engineered CAR-T cells, combined semaglutide and urolithin A treatment increased autophagy through mTOR inhibition and enhanced mitophagy through ATG4B, thereby improving persistence, sustaining long term antitumor activity, and reducing cytokine release syndrome liability in preclinical tumor models (188). Related interventions further support this axis, as succinate preconditioning promoted Bcl-2/adenovirus E1B 19 kDa-interacting protein 3 (BNIP3) mediated mitophagy and preserved stem like CD8⁺ T programs that improved antitumor immunity and adoptive efficacy *in vivo* (189), and pharmacologic inhibition of the mitochondrial deubiquitylase USP30 restored mitophagy and mitochondrial fitness under chronic stimulation while rejuvenating effector function, highlighting a druggable target upstream of lysosome-directed mitochondrial disposal routes (190). In parallel, mitochondria-nucleus coupling can be engineered to secure mitochondrial renewal capacity during sustained tumor challenge. In CAR-T cells, expression of an inhibitory resistant PGC-1 α isoform reinforced mitochondrial biogenesis programs, stabilized oxidative fitness, and supported durable antitumor potency, providing a transcriptional module that cooperates with contact switch

reinforcement and mitochondria to lysosome throughput by sustaining mitochondrial replacement over time (191). Collectively, adoptive cell therapy can be potentiated by coordinated manipulation of mitochondria-ER contact dependent calcium handling, mitochondria-lysosome quality control throughput, and mitochondria-nucleus biogenesis programs, while the limited availability of selective tools for other mitochondria organelle interfaces remains a major opportunity for future identification and validation. Compared with the systemic administration of small molecules or nanoparticles, manipulating mitochondrial interfaces *ex vivo* during adoptive cell therapy manufacturing bypasses numerous of the aforementioned systemic delivery and safety bottlenecks, offering exceptional practical translation value. By confining interface modulation to the *ex vivo* expansion phase, researchers can hardwire metabolic resilience into engineered immune cells, maximizing therapeutic efficacy while might be eliminating the risk of systemic pleiotropic toxicity to the patient's native tissues. However, the broader clinical translation of all interface-targeted strategies is universally hindered by shared preclinical and diagnostic bottlenecks. The cornerstone of advancing these targeted therapies into clinical trials is rigorous safety evaluation, yet current preclinical testing models represent a profound bottleneck. Murine models often fail to faithfully recapitulate the complex immuno-metabolic architectures of human patients (192). Establishing a reliable safety evaluation path requires a paradigm shift: Bypassing exclusive reliance on simple animal models in favor of human immune-competent organoids and patient-derived immune cells (193). Furthermore, across all therapeutic modalities, a critical limitation in early-phase clinical trials is the lack of validated, non-invasive biomarkers that specifically report interface dysfunction, rather than generic mitochondrial stress. Therefore, translating these therapies requires the concurrent development of quantitative spatial proteomics and localized *in vivo* dynamic sensors (194,195) to continuously monitor off-target interface toxicities and ensure precise pharmacological dosing.

5. Technical methodologies for probing mitochondrial interfaces: Applications, limitations and potential biases

The rigorous translation of mitochondrial interface biology relies heavily on the precision of the methodologies used to detect and quantify these nanoscale interactions. Because organelle contacts are highly dynamic, transient and structurally complex, a major bottleneck in the field is that no single technique can simultaneously capture high-resolution architecture, rapid temporal changes, and the comprehensive proteomic landscape. To meet the rigorous standards of modern immunometabolism research and avoid overinterpreting experimental findings, it is essential to critically evaluate the specific operational principles, applicable scopes, advantages and inherent biological biases of current probing technologies developed over the past decade.

Fluorescence and super-resolution microscopy. The traditional optical diffraction limit of ~200 nm has historically hindered the precise characterization of MCSs, as the physical cleft between mitochondria and partner organelles typically

ranges from 10 to 30 nm (196). Over the past decade, the advent of advanced fluorescence and super-resolution microscopy has bridged this gap, allowing researchers to visualize these interfaces. Crucially, the accuracy of these optical techniques relies on the selection of robust, validated biomolecular markers. For tracking mitochondria-ER contacts, commonly utilized markers include the IP3R-GRP75-VDAC1 complex, MFN2, and the VAPB-PTPIP51 tethering axis, often visualized alongside bulk organelle markers such as TOMM20 for mitochondria and SEC61 β or calreticulin for the ER (197). When visualizing mitochondria-lysosome crosstalk, researchers frequently co-stain TOMM20 with late endosomal or lysosomal markers such as Rab7 or LAMP1 (55), whereas mitochondria-LD interfaces are effectively tracked using PLIN2/5 paired with mitochondrial tracking dyes (198). By leveraging these specific markers, researchers can balance spatial resolution, temporal dynamics and cell viability across a progressive toolkit of microscopy techniques.

Standard live-cell confocal microscopy, and its advanced iterations mathematically push the resolution limit down to ~120-140 nm by utilizing concentric detector arrays to reassign photon detection (199). This technique is broadly utilized for live-cell tracking of bulk inter-organelle network changes, such as macroscopic mitochondria-lysosome kissing events during autophagy marked by TOMM20 and LAMP1 overlap (55), or broad ER-mitochondria co-localization in microglia (200). Its core advantage lies in its high accessibility, deep tissue penetration and low phototoxicity, allowing for continuous, multi-hour tracking of organelle networks without inducing artificial metabolic stress. However, its primary limitation is the inability to physically resolve the nanoscale MCS gap. Due to the overlap of point-spread functions, two organelles separated too close will appear merged under diffraction-limited imaging, leading to an overestimation of actual contact surface areas and an increase false-positive interpretations of tethering (201). To achieve true super-resolution while maintaining live-cell compatibility, structured illumination microscopy (SIM) projects patterned grid light onto the sample, mathematically reconstructing the resulting moiré interference fringes to double the spatial resolution to ~100 nm in three dimensions (202). Multispectral SIM has been heavily applied to map highly dynamic, multi-organelle networks, including the spatiotemporal rewiring of ER-mitochondria (203). SIM represents the optimal compromise between spatial resolution and temporal dynamics. Its low illumination intensity minimizes photobleaching and phototoxicity, making it the preferred modality for tracking rapid, real-time 'kiss-and-run' interface dynamics in delicate, highly photosensitive primary cells such as T cells. Despite this dynamic superiority, a 100-nm resolution is still mathematically insufficient to visualize the actual 10-30 nm physical cleft, confirming close apposition but fundamentally failing to identify the ultrastructural architecture of the tethers (202). Pushing spatial resolution further, stimulated emission depletion (STED) nanoscopy utilizes a conventional excitation laser paired with a donut-shaped depletion laser, forcing peripheral fluorophores back to the ground state via stimulated emission and driving resolution down to 30-50 nm (204). STED is specifically utilized to unmask intricate sub-organelle architecture, physically distinguishing the opposing membranes of multi-organelle

and mitochondria interaction by resolving specific markers or identifying cristae topology (205). While STED provides true nanoscale resolution capable of resolving the actual contact gap in living or minimally fixed cells, the steep cost of this resolution is phototoxicity (206). The ultra-high-intensity depletion laser rapidly photobleaches conventional dyes and induces massive oxidative stress. This severely restricts the duration of time-lapse imaging and necessitates the use of highly specialized, photostable synthetic probes, strictly limiting its broad application in unperturbed primary immune cells. Representing the pinnacle of optical spatial resolution, single-molecule localization microscopy techniques such as STORM and PALM rely on the stochastic photoswitching of individual fluorophores, generating a composite image with near-molecular resolution of 10–20 nm (207). STORM and related single-molecule localization microscopy approaches are well suited to resolving the nanoscale organization and spatial topology of proteins at inter-organellar interfaces. STORM achieves the highest spatial resolution among all optical technologies, effectively matching the structural precision of electron microscopy while simultaneously providing specific molecular identification of the tethering components. However, the fundamental trade-off is the absolute loss of temporal dynamics, the need to acquire thousands of frames greatly prolongs acquisition time and increases phototoxicity, therefore STORM is still most often applied to chemically fixed cells and remains poorly suited to capturing rapid organellar crosstalk dynamics (207). Ultimately, for future clinical applications, such as evaluating the metabolic resilience of engineered CAR-T cells, relying on a single optical modality is insufficient. Researchers must employ a tiered approach, utilizing SIM for live-cell tracking of multi-organellar network markers, followed by STORM or STED to validate the precise molecular assembly of tethers at the nanoscale. For future clinical application, however, a significant limitation is that primary immune cells are notoriously sensitive to phototoxicity (208), which can artificially induce metabolic stress or mitophagy during prolonged observation. Furthermore, these methods rely heavily on the exogenous expression of fluorescent fusion proteins. The overexpression of these organellar-targeted tags can inadvertently alter membrane lipid curvature or artificially stabilize otherwise transient contacts, leading to false-positive interpretations of interface frequency and duration. Therefore, while super-resolution imaging excels in mapping temporal dynamics, its objectivity is often compromised by tag-induced structural artifacts and light-induced metabolic stress.

Genetically encoded contact sensors. To translate visual co-localization observed in traditional microscopy into quantifiable physical proximity, the last decade has seen the rapid evolution of genetically encoded contact sensors. These systems bridge the gap between static imaging and functional biochemistry by converting the physical narrowing of the inter-organellar cleft into a definitive, measurable signal. The first major breakthrough in this domain was the development of split-fluorescence contact sensors, most notably the split-GFP-based contact site sensor (SPLICS). The core principle of SPLICS relies on splitting a beta-barrel fluorescent protein into two non-fluorescent fragments, which are targeted to the outer membranes of opposing organelles; fluorescence

is only reconstituted when the organelles are within a strictly defined physical gap. Originally engineered to quantify ER-mitochondria contacts (209), the SPLICS architecture has since been adapted into an extensive toolkit. Researchers have subsequently extended split-fluorescence approaches to visualize a broader repertoire of inter-organellar contacts, including mitochondria-associated interfaces with lysosomes and peroxisomes (210). The paramount advantage of split-GFP sensors is their ability to provide highly specific, tunable and high-throughput readouts of contact events without requiring complex super-resolution equipment. However, the most critical limitation of early split-protein systems is the irreversible tethering artifact. Because the functional readout relies on the high-affinity physical reconstitution of the protein fragments, the sensor itself acts as an artificial molecular staple, trapping the organelles together and masking the natural disassembly of the contact site.

To overcome this irreversible tethering trap and capture the true transient nature of mitochondrial crosstalk, the field progressively transitioned toward reversible genetically encoded sensors. These advanced tools utilize low-affinity fragments that dynamically associate and dissociate in strict accordance with physiological membrane distancing. For example, reversible proximity sensors such as MERLIN have expanded the field's ability to quantify dynamic mitochondria-ER interactions in living cells without enforcing irreversible tethering (211). More recently, this reversible paradigm has been extended to map mitochondria-LD contacts. By utilizing a reversible, fluorogen-activated bimolecular complementation toolkit (FABCON) based on splitFAST, researchers can quantitatively track the dynamic association and dissociation of mito-LD foci during rapid shifts in lipid biogenesis and nutrient deprivation, thereby bypassing the irreversible tethering trap of traditional GFP sensors (212). Despite these massive technological leaps, critical gaps remain in the biosensor repertoire, particularly concerning the mitochondria-nucleus axis and vesicular transport pathways. Currently, the field severely lacks dedicated and widely validated genetically encoded proximity sensors for the mitochondria-nucleus interface. Furthermore, while genetically encoded sensors excel at mapping stable membrane appositions, they are fundamentally ill-equipped to monitor MDVs or mitoEVs. Because MDVs are highly transient trafficking intermediates that bud from the mitochondria to deliver specialized cargo to lysosomes or peroxisomes, attempting to tag them with standard membrane-anchored split-proteins fails to capture their dynamic budding, target fusion and release. Consequently, precisely quantifying MDV and mitoEV dynamics still relies almost entirely on pH-sensitive fluorescent cargo trackers or biochemical fractionation, highlighting a major technical limitation and a critical opportunity for future bioengineering. Ultimately, translating the profound insights gained from these cellular sensors into clinical practice will require identifying non-invasive, systemic biomarkers that accurately reflect the interface dynamics first unmasked by these genetic tools.

Proximity-dependent biotinylation and spatial proteomics. The translation of mitochondrial interface biology relies on the precision of spatial proteomics to map the dynamic inter-organellar contactome in living cells. Traditional biochemical

fractionation protocols frequently fail to preserve weak or transient tethers (213). This prompted the development of proximity dependent biotinylation methodologies. The core principle of these techniques involves genetically fusing a promiscuous enzyme to a known interface resident protein. Upon activation, the enzyme covalently tags neighboring proteins within a defined nanometer radius of 10 to 20 nm with biotin, enabling subsequent affinity purification and mass spectrometry identification. The earliest iteration, BioID, utilized a mutant *Escherichia coli* biotin ligase, but its slow kinetics limited the study of acute metabolic shifts. To capture rapid interface dynamics, the field advanced toward APEX2, an engineered ascorbate peroxidase capable of biotinylating targets within 1 min upon the addition of hydrogen peroxide and biotin phenol (214). TurboID, an evolved biotin ligase, offers a reduced cytotoxicity associated with APEX2-mediated oxidative stress, improving the temporal resolution of spatial proteomics (215).

Researchers leverage these enzymes to decode tethering architectures across the mitochondrial network. For mitochondria-ER contacts, standard single enzyme targeting occasionally resulted in high cytosolic background. To resolve this, researchers developed split TurboID, a system where the enzyme is divided into two inactive fragments respectively targeted to the outer mitochondrial membrane and the ER. Enzymatic activity is reconstituted upon the direct physical apposition of the organelles, allowing for the enrichment of over 100 endogenous interface proteins in mammalian cells (82). This targeted enzyme logic has been expanded to mitochondria-LD interactions. Utilizing spatially restricted proximity proteomics targeted explicitly to LD and mitochondrial outer membranes in hepatic cell models revealed a multimeric architecture. This approach identified extended synaptotagmins and VAPB at LD-mitochondria tri-junctions that facilitate FA transfer during lipotoxic stress (16). For mitochondria-peroxisome proximity proteomics, a dual-purification APEX2-based system was developed to isolate mitochondrial subpopulations adjacent to peroxisomes in U2OS cells, enabling interface-enriched proteomic mapping distinct from the bulk mitochondrial network (216). Spatial proteomics is constrained by inherent biases, particularly concerning vesicular transport such as MDVs or mitoEVs. While researchers use APEX2 or TurboID targeted to the mitochondrial matrix or outer membrane to catalog internal cargo packaged into EVs prior to release, capturing the transient membrane budding interface of a vesicle remains difficult. The primary limitation of proximity labeling tools is the bystander effect. The 10 to 20 nm labeling radius is a chemical gradient, not a defined boundary. Abundant cytosolic proteins or unrelated organelles rapidly diffusing past the targeted interface during the labeling window can be inadvertently biotinylated and identified as contact residents. In the context of dynamic vesicle budding, this bystander noise obscures the specific vesiculation machinery. To translate these high throughput datasets into clinical targets, researchers are advised to cross reference spatial proteomes with orthogonal structural validation and functional screens.

Ultrastructural imaging: EM and Cryo-ET. Establishing the definitive physical architecture and absolute membrane

distances of inter organelle contact sites necessitates the sub-nanometer resolution of ultrastructural imaging. Conventional electron microscopy utilizes heavy metal staining and electron scattering to map cellular architecture at nanometer resolution. Researchers apply conventional TEM to quantify the physical gap between mitochondria and other organelles. For example, researchers utilized electron microscopy alongside structured illumination to identify dynamic mitochondria-lysosome contact sites, demonstrating that these specific interfaces regulate mitochondrial fission events via localized GTPase activity (55). The primary advantage of conventional TEM is its high spatial resolution capable of distinguishing individual lipid bilayers. However, a notable limitation is its reliance on chemical fixation and dehydration. These processing steps introduce osmotic stress and membrane blebbing, potentially shrinking or expanding the perceived contact gap and creating morphological biases (217). Furthermore, conventional electron microscopy provides a static snapshot, lacking the temporal resolution to capture dynamic tethering events.

To overcome the limitations of two-dimensional cross sections, the field advanced toward three-dimensional volume electron microscopy techniques such as focused ion beam scanning electron microscopy. This technique sequentially mills the sample surface with gallium ions and images the newly exposed face, allowing computational reconstruction of large cellular volumes (218). Focused ion beam scanning electron microscopy is widely applied to map the complex three-dimensional architecture of multi-organelle junctions. Researchers utilize this technique to reconstruct holistic cellular maps encompassing the ER, mitochondria, LDs, lysosomes, peroxisomes, and the nucleus, quantifying how mitochondria intricately associate with LDs to facilitate efficient FA transfer during metabolic shifts (219). The core advantage of focused ion beam scanning electron microscopy is its ability to contextualize organelle interactions within broad cellular volumes, revealing extensive contact networks rather than isolated focal points. Despite this spatial advantage, the technique remains destructive and relies on heavy metal staining, which limits specific molecular identification of the tethering proteins involved.

Representing the pinnacle of ultrastructural preservation, cryo-electron tomography captures samples in vitrified ice at cryogenic temperatures. This approach preserves biological structures in near native states without chemical fixatives, followed by tilt series imaging to reconstruct high-resolution three-dimensional tomograms. Cryo-electron tomography is increasingly applied to map the native three-dimensional architecture of MCSs, including mitochondria-associated interfaces, under near-native conditions (220). Additionally, researchers utilize electron tomography to observe the biogenesis of mitochondrial-derived compartments, demonstrating that these vesicular structures emerge directly from mitochondrial tubules at specific ER contact sites to sequester distinct cargo (221). The defining advantage of cryo-electron tomography is the ability to visualize molecular tethers and membrane curvature in an unperturbed physiological context. However, the technique faces notable limitations. The imaging depth is severely restricted to thin cellular regions, often requiring complex cryogenic focused ion beam milling prior to

imaging (222). This technical complexity reduces throughput and limits sample sizes. Looking toward future clinical applications, validating therapeutic interventions targeting mitochondrial interfaces relies on an integrated structural approach. Researchers are encouraged to combine the high throughput spatial mapping of volume electron microscopy with the native structural preservation of cryo-electron tomography. This complementary strategy helps mitigate the individual biases of each imaging modality, ensuring that newly identified interface regulators or mitochondria-derived vesicular pathways represent genuine physiological targets rather than sample preparation artifacts.

6. Conclusions and perspectives

The mitochondria-centered organelle networking functions as a unifying integrative model for immune regulation, in which spatially organized interfaces and mitochondrial trafficking routes enforce a critical level of governance of immunometabolism and inflammatory signaling (223,224). It integrates contact-dependent signaling with vesicle-mediated redistribution of mitochondrial cargo to explain how mitochondria partner organelle coupling coordinates Ca^{2+} flux, lipid exchange, redox control, mitochondrial quality control, and epigenetic adaptation to shape innate and adaptive immune states (225–227). It is proposed that interface architecture, tether composition, organelle positioning and mitochondrial vesicle flux collectively determine whether activation driven stress is limited and resolved or propagated into persistent immune dysfunction. By integrating shared and context specific interface remodeling across infection and sepsis, cancer, autoimmune and chronic inflammatory disorders, it links organelle network failure to endolysosomal routing defects, nucleic acid sensing, and maladaptive inflammatory program. Finally, therapeutic relevance was dissected, emphasizing that progress will depend on interface resolved profiling, rigorous validation in human immune systems, and development of selective tools capable of perturbing defined contact sites or mitochondrial trafficking checkpoints (105,228).

Several limitations hamper mechanistic inference and clinical translation. Organelle contacts are transient and heterogeneous at the nanoscale yet are frequently assessed by qualitative co-localization or indirect reporters that fail to discriminate contact identity, lifetime, or functional effect (229,230). Moreover, commonly used tagging and imaging strategies can perturb native architectures and introduce technical confounders. These issues are exacerbated in complex tissues, where proximity labeling and spatial proteomics remain challenged by background labeling, context-dependent effective labeling distance, and limited *in vivo* deployability, collectively restricting causal interpretation in human immune pathophysiology (82,231). Clinical translation will require stringent target prioritization and validation in human relevant systems. Numerous interface components are pleiotropic and embedded within essential calcium and lipid homeostasis, which can narrow therapeutic windows and impose strong dependence on cell type, disease stage and tissue context. In addition, biomarkers that report interface dysfunction remain less developed than global mitochondrial stress signatures.

An important limitation of current models of mitochondrial interface regulation is that these structures are often discussed as relatively static, although available evidence indicates that their organization and function are modified by physiological and pathological context. The clearest evidence currently concerns ERMCS, also termed MAMs, which regulate Ca^{2+} transfer, phospholipid metabolism, inflammatory activation, antiviral responses and ER stress-related signaling in diverse cell types, including innate immune cells (226). Sex-related regulation should be interpreted cautiously. Available studies support that sex hormones, particularly estrogen, modulate mitochondrial bioenergetics, redox signaling and fusion-fission regulators including MFN1, MFN2, OPA1 and DRP1. However, most of these data come from cardiovascular and renal systems, and direct evidence for sex-dependent remodeling of mitochondrial interfaces in immune cells remains limited (232,233). Aging provides more direct evidence that interface states are not fixed. ITPR2-dependent ER-to-mitochondria Ca^{2+} transfer has been shown to promote senescence, whereas *Itpr2* deficiency reduces ER-mitochondria contacts and is associated with attenuated aging phenotypes in mice, supporting a role for contact-dependent Ca^{2+} signaling in age-related dysfunction (234). Consistent with the view that remodeling is context dependent, therapy-induced senescence has been reported to increase MERCS' contact surface while reducing ER-mitochondria Ca^{2+} flux because of decreased IP3R expression and reduced IP3R1-VDAC1 interaction (235). A recent review further conclude that MAMs undergo morphological and functional alterations during aging and senescence (236). The tissue and TME represents another important regulatory layer. Current evidence indicates that hypoxia, oxidative stress, nutrient limitation and metabolite accumulation reprogram mitochondrial metabolism in infiltrating immune cells within the TME, whereas direct quantitative evidence for remodeling of specific mitochondrial contact sites in these cells remains limited (237). In parallel, aging and diet further reshape the metabolic architecture of the tumor-immune microenvironment, thereby changing the conditions under which mitochondrial signaling operates in immune cells (238). Taken together, the available literature supports physiological plasticity of mitochondrial interfaces, but in immune cells this conclusion is currently best substantiated for ER-mitochondria contacts, while direct evidence for other interface classes remains insufficient. Progress is therefore likely to depend on integrated validation strategy that combine immune competent organoids and patient derived immune cells with minimally perturbative contact reporters, quantitative sensors for local calcium, lipid flux and redox dynamics, and orthogonal spatial proteomics to connect nanoscale organization with stratification features and therapeutic response (239,240). Future work should enable a transition from correlative evidence to actionable intervention by expanding chemical and delivery platform that enrich pharmacology at defined contact sites or mitochondria derived trafficking checkpoints, and by integrated these capabilities into adoptive cell manufacturing strategies designed to preserve interface states required for persistence and function under chronic stimulation. Limited druggability across several mitochondria organelle interfaces should be interpreted as a tool gap rather than evidence limited

physiological importance and supports systematic target discovery and validation across additional contact systems and trafficking routes that remain underexplored in immunological conditions.

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Authors' contributions

YW, HY and LX conceived the overall structure of the manuscript and drafted the article. LY provided technical and material support and contributed to manuscript preparation. YZ and QY provided critical revisions and editorial comments. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

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