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Review article

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Targeting organelle function in T cells for cancer immunotherapy

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Abstract

Organelles are the internal batteries, gears, actuators, 3D printers and transmitters that drive cell function. Their composition and activity vary between cell types depending on functional demands. In T cells, which are key mediators of immunosurveillance and tumour eradication, organelles are relatively few and function at basal levels when cells are at rest. However, upon activation, they increase in number and size and undergo extensive remodelling to support rapid proliferation, effector differentiation and adaptation to diverse microenvironments, including the tumour microenvironment, thereby enabling efficient clearance of target cells. In this Review, we provide an overview of recent advances in our understanding of how various organelles contribute to T cell-mediated antitumour immunity. We also discuss emerging strategies to modulate organelle functions — from organelle-targeted therapies and their use as cargo delivery systems to the transfer or transplantation of native or synthetic organelles — that have the potential to enhance cancer immunotherapies involving immune-checkpoint blockade or the adoptive transfer of T cells.

Sections

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Introduction

Just as the human body relies on organs such as the heart, lungs and intestines to carry out specialized functions, cells contain organelles ('little organs') that carry out essential processes and maintain cellular functions in diverse pathophysiological contexts. For example, the ability of T cells to target tumour cells effectively within the 'hostile' tumour microenvironment (TME), where resources are scarce and T cells are outnumbered by malignant cells, depends in part on how efficiently they use and adapt their organelles to sustain their function and enable them to persist in such challenging conditions. When organelles fail to operate effectively in this metabolically and immunologically stressed environment, T cell metabolism and signalling are compromised, ultimately contributing to the development of T cell exhaustion. Thus, maintaining organelle health is crucial for preserving T cell fitness and improving the efficacy of cancer immunotherapies.

In this Review, we provide an overview of recent studies that have advanced our understanding of the roles of various T cell organelles in antitumour immunity. We focus on cytoplasmic organelles, including the cytoskeleton, mitochondria, endoplasmic reticulum, Golgi apparatus, peroxisomes and lysosomes. We discuss strategies to enhance organelle fitness and durability, ranging from passive drug treatment to synthetic gene circuits designed to optimize T cell performance. In addition, we highlight emerging platforms, such as intercellular organelle transfer and the transplantation of native and synthetic organelles to T cells, that are poised to reshape the cancer immunotherapy landscape.

Organelle biology in T cell function

In the past few years, aided by the development of new methodologies (Box 1), several studies have offered fresh perspectives on organelle biology and their roles in T cell functions (Table 1). Organelles actively coordinate key processes such as metabolism, signal transduction and stress adaptation, which are essential for sustaining T cell persistence and effector functions within the TME. As our understanding of these organelle-driven processes deepens, new therapeutic strategies are emerging that seek to reprogramme cellular metabolism, fine-tune intracellular signalling and relieve organelle stress, offering a promising route to improve the efficacy of T cell-based immunotherapies. Here, we discuss the latest breakthroughs in organelle research and explore their implications for enhancing T cell function, resilience and antitumour potential.

Mitochondria

Mitochondria are best known for their role in ATP production via oxidative phosphorylation (OXPHOS); mitochondria metabolize sugars, amino acids and fatty acids, feeding their metabolic products into the tricarboxylic acid (TCA) cycle and driving energy production through the electron transport chain (ETC)[1,2]. Like cancer cells, activated T cells rely heavily on glucose metabolism to sustain their function, proliferation and migration through tissues[3,4]. In the glucose-depleted TME, the ability of T cells to rewire cellular metabolism and use alternative fuel sources, such as fatty acids[5,6], becomes crucial for sustaining antitumour activity. However, a shift towards fatty acid oxidation (FAO) in T cells is actively opposed by TME-induced metabolic constraints, including the upregulation of acetyl-coenzyme A (acetyl-CoA) carboxylase 1 (ACC1; also known as ACACA) activity[6]. Although the signals driving this induction remain to be fully elucidated, increased ACC1 activity promotes lipid storage over catabolism, thereby limiting the availability of fatty acids for ATP generation[6]. Furthermore, regardless of nutrient availability, T cells face restrictions in TCA cycle-dependent energy production upon entering the TME. Chronic antigen stimulation in this hypoxic environment induces expression of prolyl 4-hydroxylase α-subunit 1 (P4HA1), which accumulates in the mitochondria and disrupts the TCA cycle by altering α-ketoglutarate and succinate metabolism[7], ultimately leading to T cell dysfunction. Recent evidence also highlights a role for the mitochondrial enzyme arginase 2 (ARG2) in exacerbating the metabolic suppression of T cells in the TME[8–10]. ARG2 is upregulated upon T cell activation and limits the activity of the TCA cycle by depleting the intracellular pool of arginine, which is an essential amino acid for T cell activation and survival, thereby impairing overall antitumour function[10].

It is increasingly recognized that, in addition to ATP production, mitochondria also have essential signalling functions in T cells. Reactive oxygen species (ROS) are natural byproducts of mitochondrial OXPHOS, with effects on both physiological and pathological cellular processes. In T cells, mitochondrial ROS are induced by T cell receptor (TCR)-triggered calcium influx and are required for activation of the NFAT transcription factor family and IL-2 production, as shown by the defective response to TCR triggering in cells lacking Rieske iron–sulfur protein, a subunit of complex III of the ETC that is required for electron transfer and contributes to ROS production[11]. Notably, recent findings show that disruption of complex III-derived ROS, despite preserved electron transport, leads to defective formation of both naive and memory T cells, underscoring a non-metabolic, ROS-dependent role for mitochondria in T cell differentiation[2]. Complementing these findings, in vitro studies implicated another mitochondrial enzyme, glycerol-3-phosphate dehydrogenase 2 (GPD2), as the primary source of mitochondrial ROS as well as NF-κB as the main downstream signalling pathway[12]. As GPD2 is not a core component of the mitochondrial ETC, these findings reinforce the idea that ROS production itself, rather than mitochondrial respiration, has a crucial role in T cell activation. However, chronic TCR stimulation leads to excessive ROS accumulation, oxidative stress and, ultimately, T cell exhaustion, particularly in the hypoxic TME[13–17]. Downstream of chronic TCR stimulation, PRDM1 (also known as BLIMP1) suppresses the expression of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α; also known as PPARGC1A), a master regulator of mitochondrial biogenesis and antioxidant activity, thereby impairing the ability of T cells to adapt to hypoxic environments. The resulting mitochondrial stress increases ROS levels, which can promote T cell exhaustion through multiple mechanisms, including chronic NFAT signalling[13], accumulation of depolarized mitochondria[14], and the stabilization of hypoxia-inducible factor 1α, which also drives the terminal differentiation of precursor exhausted T cells[15]. Although the various pathways leading to T cell exhaustion in the TME seem redundant, mitochondrial function — particularly with respect to ROS signalling — has a central role in orchestrating this process.

Mitochondria also function as biosynthetic factories. Mitochondrial ribosomes (mitoribosomes) tethered to the mitochondrial inner membrane synthesize proteins encoded by mitochondrial DNA (mtDNA)[18]. Recent studies have shown that mitochondrial translation influences $CD8^+$ T cells beyond the production of mitochondrial proteins and enzymes. Specifically, translation by mitochondrial ribosomes is necessary to sustain cytosolic synthesis of effector molecules, such as granzyme B (GZMB), perforin, tumour necrosis factor (TNF) and interferon-γ, by T cells[19]. Although the exact mechanism remains unresolved, it has been speculated that mitochondrial enzymes may function as RNA-binding proteins to post-transcriptionally regulate

Box 1 | New techniques for organelle analysis

A key driving force behind research into organelle-targeted therapies is the development of advanced analytical tools that enhance our understanding of organelle biology. Imaging-based techniques were the first to uncover the hidden world of intracellular organelles. A new wave of imaging software pipelines and machine learning programmes is enabling more in-depth analysis of organelles at higher throughput. Electron microscopy-based imaging remains a gold standard for analysing cellular ultrastructure, particularly in cutting-edge applications such as cryo-electron tomography, which was recently used to identify a previously unrecognized vesicular organelle complex termed the hemifusome[199]. Yet, electron microscopy requires time-consuming manual annotation of organelles, and the serial alignment of sections for large-volume 3D reconstruction remains difficult. New machine learning models, such as OpenOrganelle[200] and EMDiffuse[201], automate organelle classification and enable noise reduction and/or reconstruction to an isotropic resolution. Correlative light-electron microscopy, which combines the high resolution of subcellular structures afforded by electron microscopy with confocal fluorescence microscopy, has successfully been used to study intercellular organelle trafficking[180]. Techniques such as expansion microscopy and expansion sequencing provide super high-resolution insights into organelle structure and function[202,203], including the organization of the T cell synapse[204]. However, organelle-specific properties, such as membrane composition and structural organization, must be considered when designing expansion protocols[205]. To study organelle content, positioning and interactions, a new multi-spectral organelle imaging workflow for conventional microscopy was recently developed, called OrgaPlexing[206]. This approach revealed that different organelles, such as mitochondria, endoplasmic reticulum and peroxisomes, cluster together with lipid droplets to support lipid metabolism in activated immune cells[206]. Although OrgaPlexing simplifies previous workflows that relied on fluorescent protein-tagged organelle markers[207], it requires fixation and permeabilization of cells[206], which may create artefacts. Furthermore, all microscopy-based approaches are limited by their relatively low throughput owing to, in part, their requirement for extensive post-acquisition data processing. Automation tools such as Nellie, which uses motion capture markers for 2D and 3D live-cell imaging, can streamline organelle segmentation and tracking, providing data on organelle morphology, motility and network topology[208].

Organelles can also be characterized by flow cytometry, for example, by using specific dyes to quantify organelle content and functions such as mitochondrial polarization or lysosomal pH. One approach involves the extraction of organelles from cells by conventional fractionation methods and analysis by small particle flow cytometry. The workflow can process several thousand organelles and has been used to track the intracellular distribution kinetics of fluorescent nanoparticles to fine-tune the specificity of organelle-targeted treatments[209]. High-throughput flow cytometry combined with ultrafast imaging (imaging cytometry) enables real-time sorting of cells based on organelle morphology and spatial distribution at speeds of up to 15,000 events per second[210].

Next-generation sequencing has advanced organelle research by identifying gene signatures associated with organelle functions, such as endoplasmic reticulum stress[211] or mitochondrial activity (METAFlux)[212]. Computational tools, such as mitochondrial-enabled reconstruction of cellular interactions (MERCI)[178] and MitochondRia (MitoR)[213], leverage gene signatures to accurately identify cells that have received donor mitochondria, without the need for labelling. An advantage of these gene signatures is their ability to function as markers for the identification of cells by immune state or organelle function, even retrospectively, using publicly available data sets, which reduces the need for new experiments or clinical trials. However, a major limitation of gene signatures is that differences in cell types, disease pathologies, sequencing platforms and data analysis algorithms challenge reproducibility[214]. Similarly to other modelling systems, increasing the size of the data pool and integrating data from different sources may improve predictive accuracy, but the standardization of data input and quality control metrics will be key to achieving the full potential of using gene signatures. In mice, MitoTRACER is a new molecular approach that has been developed to permanently label cells receiving mitochondria and their progeny, enabling the study of the long-term effects on cell function and fate, even after the transferred mitochondria have degraded[176].

the expression of these effector molecules in the cytosol[19]. This could partially explain the T cell-suppressive effect of mitoribosome-targeting antibiotics[20]. In addition to mitochondrial protein synthesis, mitochondria generate building blocks that are essential for epigenetic modifications and post-translational modifications of proteins[21]. Mitochondrial metabolism regulates the availability of acetyl-CoA, which is crucial for histone acetylation[22], and of *S*-2-hydroxyglutarate, which inhibits the TET family of DNA demethylating enzymes[23,24]. In addition, mitochondria regulate the activity of sirtuins, a family of histone deacetylases, by influencing the ratio of oxidized to reduced forms of nicotinamide adenine dinucleotide (NAD^+:NADH) through OXPHOS. As a result, mitochondria have emerged as central epigenetic regulators of cell fate decisions and lineage specification[1].

Under nutrient-rich conditions, mitochondria can support both ATP production and biosynthetic functions. However, until recently, it was unclear how mitochondria coordinate their anabolic and catabolic processes in nutrient-deprived environments such as the TME. Seminal work in this area has shown that, when nutrients are limited, mitochondria undergo dynamic fusion and fission events to generate specialized subpopulations with different cellular functions[25]. A subpopulation of mitochondria enriched in cristae and ATP synthase is adapted for energy production, whereas another subpopulation, marked by filamentous clusters of pyrroline-5-carboxylate synthase, functions as a biosynthetic hub[25]. These findings could offer opportunities to enhance T cell function in cancer immunotherapy by selectively targeting specific mitochondrial subsets.

In addition to the presence of functionally distinct mitochondrial subpopulations, the asymmetric distribution of pre-existing (old) and newly synthesized (new) mitochondria during cell division has been shown to influence cell fate[26,27]. Using the MitoSnap system to track old and new mitochondria in T cells, a recent preprint reports that daughter T cells preferentially inheriting old mitochondria tend to be more

Table 1 | Role of organelles in regulating T cell function and differentiation

Organelle	Function	Pathway or mechanism	Effect on T cells and antitumour response	Refs.
Mitochondria	Generation of ATP	OXPHOS	Sustains energy supply for memory recall and antitumour function	1,2
	Lipid storage	Acetyl-coenzyme A carboxylase 1	Dysfunction and impaired antitumour response	6
	Glutarate metabolism	Glutarate and *S*-2-HG; negative regulation of epigenetic modifiers	Enhances proliferation, persistence and antitumour capacity	23,24
		Interference by P4HA1	Dysfunction and impaired antitumour response	7
	Arginine metabolism	ARG2; inhibition of TCA cycle	Impairs activation, survival and antitumour function	8,10
	ROS generation	NFAT and NF-κB	Activation, cytokine production	11,12
		Unknown	Memory formation	2
		Excessive NFAT, HIF1α, mitochondrial depolarization	Exhaustion	13–15
	Protein synthesis	Mitochondrial ribosomes	Synthesis of effector molecules	19
	Epigenetic regulation	Histone acetylation, TET enzymes, sirtuins	Differentiation	1,22–24, 198
Endoplasmic reticulum and Golgi	Calcium signalling	Phosphoenolpyruvate; inhibition of SERCA	Fine-tuning TCR signalling and activation	33
	Protein translation	MYC-dependent ribosome assembly and activity, polysome formation	Activation and proliferation; production of effector molecules	34–36
		Pyrimidine-dependent ribosome biogenesis	Rapid recall responses	37
	Stress response	ERAD pathway: SEL1L	Memory formation and polyfunctionality	40
		UPR pathway: XBP1	Terminal differentiation	42
		UPR pathway: DDIT3-dependent repression of T-bet	Exhaustion	42
		UPR pathway: CPEB4	Maintenance of effector function	43
		PRDX4-dependent ROS scavenging	Enhanced tumour control	45
	Post-translational modification	ST3GAL1-dependent glycosylation of LFA1	Sequestration in non-target tissues, decreasing antitumour efficacy	47
	Nucleic acid sensing	STING translocation and activation	Enhanced stemness and antitumour function	48–52,54
Lysosomes	Mitophagy	CISH-dependent targeting of V-ATPases	Impaired mitochondrial clearance and cell function	57
	Proteolysis	Degradation of CTLA4	Stronger antitumour responses	60
		V-ATPases and mTORC1 activation	Effector differentiation	64–66
Peroxisomes	Antioxidant activity	PEX5-dependent peroxisome assembly	ROS scavenging, proliferation in vitro	70
Cytoskeleton	Immune synapse formation	Cofilin and Wiskott–Aldrich syndrome protein	Regulation of activation threshold	79,80
	Cell tension	Cytotoxic pore formation	Increased cytotoxicity	81,82
	Nutrient uptake and trafficking	Endoplasmic reticulum stress-dependent TAGLN2 silencing	Reduced FAO and antitumour function	83

2-HG, 2-hydroxyglutarate; ARG2, arginase 2; CISH, cytokine-inducible SH2-containing protein; CTLA4, cytotoxic T lymphocyte antigen 4; ERAD, endoplasmic reticulum-associated protein degradation; FAO, fatty acid oxidation; HIF1α, hypoxia-inducible factor 1α; mTORC1, mechanistic target of rapamycin complex 1; OXPHOS, oxidative phosphorylation; P4HA1, prolyl 4-hydroxylase α-subunit 1; PEX5, peroxisome biogenesis protein 5; PRDX4, peroxiredoxin 4; ROS, reactive oxygen species; SERCA, sarcoplasmic/endoplasmic reticulum calcium-ATPase; ST3GAL1, ST3 β-galactoside alpha-2,3-sialyltransferase 1; STING, stimulator of interferon genes; TAGLN2, transgelin 2; TCA, tricarboxylic acid; TCR, T cell receptor; UPR, unfolded protein response; V-ATPases, vacuolar-type ATPases.

glycolytic and less capable of forming memory cells, whereas those T cells inheriting new mitochondria have increased long-term persistence and recall capacity[28]. Central to this process is autophagy, as $CD8^+$ T cells lacking autophagic activity have a more symmetrical distribution of old mitochondria between daughter cells. This suggests that the clearance of old mitochondria by autophagy is essential for establishing memory-committed T cells free of aged organelles, which reinforces previous findings that identified mitochondrial stasis[29] and autophagy[30] as crucial determinants of effector versus memory T cell differentiation.

Together, these findings emphasize that mitochondria are not only essential for providing energy to T cells but also have crucial roles in regulating signalling and cell fate decisions, which makes them pivotal in determining the efficacy of the immune response and a promising target for therapeutic strategies in cancer immunotherapy.

Endoplasmic reticulum and Golgi

The endoplasmic reticulum (and its membrane-bound ribosomes) is responsible for the production, modification and transport of proteins, and also serves as the main intracellular calcium reservoir, having a pivotal role in regulating cellular signalling pathways essential for T cell activation and function[31]. Inositol 1,4,5-trisphosphate, generated downstream of TCR engagement, triggers the release of calcium from the

endoplasmic reticulum, initiating a transient rise in cytosolic calcium and activating store-operated calcium entry across the plasma membrane from the extracellular space, which sustains the calcium influx necessary for downstream signalling events such as calcineurin–NFAT activation[32]. The glycolytic metabolite phosphoenolpyruvate (PEP) negatively regulates sarcoplasmic–endoplasmic reticulum calcium-ATPase (SERCA; also known as ATP2A1), which actively pumps calcium from the cytosol back into the endoplasmic reticulum, thereby fine-tuning T cell activation and effector programmes. In the glucose-depleted TME, intracellular levels of PEP become limited, resulting in unchecked SERCA activity, which impairs TCR-mediated calcium–calcineurin–NFAT signalling[33].

The involvement of the endoplasmic reticulum in regulating T cell function in the TME extends beyond calcium signalling[31]. Activation of naive and memory T cells triggers MYC-dependent de novo ribosome assembly[34,35], alleviates the repression of ribosome activity[36] and promotes polysome formation, which together markedly increase protein synthesis to support metabolic reprogramming, the production of effector molecules and rapid cell divisions[35]. Notably, whereas translation is the cellular process that consumes the most ATP and is constrained by an insufficient energy supply[35], ribosome biosynthesis mainly depends on de novo pyrimidine synthesis. Continued activity of the pyrimidine synthesis pathway is required to maintain memory T cells in a state that is prepared to respond to rechallenge[37]. Cellular stresses (for example, owing to excessive demands for protein synthesis, ROS production or metabolic constraints) can interfere with protein assembly in the endoplasmic reticulum, leading to the accumulation of misfolded or improperly assembled proteins. To maintain proteostasis, the endoplasmic reticulum-associated degradation (ERAD) pathway identifies defective proteins and directs them to the ubiquitin–proteasome system for degradation. However, when misfolded proteins accumulate beyond the capacity of ERAD, the unfolded protein response (UPR) is activated to restore homeostasis. If the cellular stress remains unresolved, the UPR triggers apoptosis to eliminate severely damaged cells, which can contribute to the loss of T cells within the TME[38,39].

In the past few years, novel mechanisms that regulate endoplasmic reticulum stress responses have been discovered, identifying additional targets to enhance T cell function. SEL1L, which is a crucial component of the ERAD pathway, has been shown to be indispensable for maintaining endoplasmic reticulum homeostasis in antigen-specific $CD8^+$ T cells following acute viral infection, with roles in sustaining $CD8^+$ T cell polyfunctionality and the development of immunological memory[40]. By contrast, the UPR pathways have been implicated in driving the terminal differentiation and dysfunction of $CD8^+$ T cells. Indeed, the transcription factor XBP1, a mediator of the UPR machinery, is crucial for the formation of short-lived $KLRG1^+$ effector T cells[41], while the UPR sensor DDIT3 acts as a major repressor of transcription factor T-bet (also known as TBX21) and its downstream effector programmes, contributing to $CD8^+$ T cell exhaustion within the TME[42]. However, emerging evidence suggests that not all UPR pathway components negatively affect T cell function. For example, the UPR regulator CPEB4 has recently been identified as mitigating endoplasmic reticulum stress in $CD8^+$ T cells. Unlike canonical UPR programmes that can lead to global translation shutdown over time, the CPEB4 pathway enables T cells to endure endoplasmic reticulum stress while maintaining effector functions[43].

Similarly to the endoplasmic reticulum, the Golgi apparatus in tumour-infiltrating T cells can experience stress, particularly oxidative stress, leading to Golgi fragmentation, impaired protein processing and, ultimately, T cell dysfunction[44]. The thiol-specific peroxidase, peroxiredoxin 4, has a crucial role in alleviating Golgi stress by scavenging hydrogen peroxide, which restores the function of antitumour T cells[45].

The Golgi apparatus processes proteins received from the endoplasmic reticulum — adding post-translational modifications such as glycosylation and phosphorylation — before directing them to their final destinations such as the plasma membrane, lysosomes or secretory pathways[46]. Recent findings have shown that such post-translational modifications have a crucial role in regulating the migration and therapeutic potential of chimeric antigen receptor (CAR) T cells. Through an in vivo CRISPR screen, the sialyltransferase ST3GAL1 was identified as a key determinant of CAR T cell trafficking[47]. ST3GAL1 transfers sialic acid to, among others, the integrin LFA1, which prevents its endocytic recycling and promotes its surface accumulation on T cells. This ultimately leads to the sequestration of CAR T cells in non-target tissues such as the lungs, which can reduce their antitumour efficacy and potentially lead to off-tumour toxicities.

Activation of stimulator of interferon genes (STING) — an endoplasmic reticulum and Golgi-resident transmembrane receptor for cytoplasmic foreign or mtDNA — markedly increases the efficacy of T cell therapy, both owing to its adjuvant activity[48–50] and by promoting T cell stemness[51,52]. However, a limitation of using STING-based adjuvants in cancer immunotherapies is their potential to induce uncontrolled inflammation and T cell stress[53]. It was noted that sulfated glycosaminoglycans in the Golgi apparatus bind STING to promote its translocation and activation[54]. Indeed, the strength of the STING–sulfated glycosaminoglycan interaction was shown to determine the overall level of STING activation, which could allow for more precise regulation of STING responses in cancer immunotherapy. Together, these findings underscore that the endoplasmic reticulum and Golgi apparatus are crucial regulators of T cell resilience to stress, trafficking and function, revealing new therapeutic opportunities to enhance cancer immunotherapy.

Lysosomes

Many ingested materials, organelles and other proteins end their fate in lysosomes, which break down biomolecules for recycling or excretion using pH-sensitive hydrolytic enzymes. The low pH of lysosomes is regulated by ion channels and pumps such as vacuolar-type ATPases (V-ATPases) and transmembrane protein 175 (TMEM175)[55]. Cytokine-inducible SH2-containing protein (CISH), a negative regulator of TCR signalling[56], has been shown recently to impair lysosome function by targeting V-ATPases. Thus, increased levels of CISH reduce the clearance of damaged mitochondria by lysosomes in T cells, impairing their function[57]. Consistently, knockout of *Cish* in adoptively transferred T cells improves their ability to control tumour growth[56]. Lysosome-associated membrane protein 1 (LAMP1) and LAMP2 are glycoproteins that comprise almost half of the lysosomal membrane protein content[58] and were originally thought to protect the lysosomal membrane from autodigestion. However, we now recognize that LAMPs are also crucial regulators of lysosome biogenesis[58], lysosomal pH (through inhibition of TMEM175 (ref. 59)) and autophagy, which is essential for the maintenance of memory $CD8^+$ T cells[30].

Lysosomal degradation has also been found to regulate the turnover of cell-surface receptors. In T cells, this process has important functional consequences in regulating the cell-surface expression of immune-inhibitory receptors such as cytotoxic T lymphocyte antigen 4 (CTLA4). Recently, it was reported that TNF receptor-associated factor 6

(TRAF6) mediates the ubiquitylation and subsequent lysosomal degradation of CTLA4, a process that is enhanced by OX40 agonist-mediated upregulation of TRAF6, resulting in a stronger antitumour response[60].

Unlike in other cell types, the lysosomal contents of cytotoxic $CD8^+$ T cells (and natural killer cells) can be secreted. Their secretory lysosomes (known as lytic granules) contain cytotoxic proteins, such as GZMB and perforin, that are released upon TCR engagement through the immune synapse with target cells, inducing their apoptosis[61]. Distinct subsets of lytic granules contain either soluble GZMB (described as single-core granules) or intact supramolecular attack particles, which consist of stable core cytotoxic proteins, including GZMB, and a thrombospondin 1 shell (referred to as multi-core granules)[62]. It is hypothesized that single-core granules confer immediate cytotoxicity activity, whereas multi-core granules with latent cytotoxic proteins allow for delayed killing[62].

The discovery that lysosomes function as platforms for the activation of mechanistic target of rapamycin complex 1 (mTORC1) has widened the perspective on lysosomes from being recycling centres and depots for cytotoxic proteins to central signalling hubs[63]. Upon activation, mTORC1 translocates to lysosomes, where it promotes protein synthesis while blocking autophagy and lysosome biogenesis via inactivation of the transcription factor TFEB[64]. In T cells, lysosomal mTORC1 activity has an important role in regulating T cell differentiation as evidenced by the ability of the mTOR inhibitor rapamycin to enhance T cell memory formation[65]. A recent study showed that natural killer cell group 7 (NKG7), a lysosomal protein expressed exclusively in cytotoxic lymphocytes, interferes with the assembly and function of V-ATPases, thereby blocking mTORC1 recruitment to lysosomes[66]. Consequently, NKG7 is necessary for the generation of $CD8^+$ memory precursor T cells that are required for optimal immune responses to infections and solid tumours[66]. Furthermore, in T cells from older individuals, mTORC1 activation occurs at late endosomes instead of lysosomes, which disrupts the mTORC1–TFEB-mediated negative-feedback loop, impairing lysosome biogenesis. This change prevents the lysosomal degradation of the immune-checkpoint protein PD1, which contributes to T cell exhaustion[67]. Together, these studies show that targeting lysosome activity has the potential to boost T cell-mediated immunity.

Peroxisomes

Peroxisomes, like lysosomes, are cellular recycling centres but with a focus on fatty acids and the breakdown of ROS[68]. Clinical findings suggest that peroxisomes are important regulators of thymopoiesis given that people with Zellweger syndrome, a hereditary peroxisome deficiency, have thymic hypoplasia[69]. Peroxisome biogenesis involves the import of cytosolically synthesized proteins that contain a peroxisome-targeting sequence (PTS) — recognized by peroxisome biogenesis protein 5 (PEX5) — into the peroxisome. Deficiency of *Pex5* results in the formation of empty 'ghost' peroxisomes. Given the crucial signalling functions of ROS in T cells, peroxisomes would also be expected to have an important role in T cell signalling. Indeed, *Pex5*-deficient T cells have defective proliferation compared with wild-type T cells in vitro owing to the accumulation of high levels of ROS. However, in vivo, T cell development and responses to viral infections seem to be unaffected by the deletion of *Pex5* (ref. 70). These contradictory observations may arise from the higher oxygen levels present in vitro compared with in tissues, leading to unphysiologically high levels of oxidative stress and therefore greater reliance on the antioxidant capacity of peroxisomes in vitro[70]. Moreover, other subcellular complexes or organelles, including mitochondria and Golgi, that also have antioxidant activity may compensate for the loss of peroxisome function to a certain extent in low-stress environments. Whether peroxisome functions are redundant in the TME, which has high levels of ROS, has yet to be tested.

Cytoskeleton

The cytoskeleton is the internal scaffolding of the cell, providing structure and shape while also enabling directional intracellular transport, phagocytosis, migration and cell division. It consists of diverse protein polymers such as actin filaments, intermediate filaments and microtubules[71]. The components of the cytoskeleton are highly dynamic, constantly assembling and disassembling. Externally, through focal adhesions, the cytoskeleton connects to integrins and other transmembrane proteins, driving cell motility, mechanosensing and intercellular connections[72–75].

In T cells, the cytoskeleton not only ensures integrity of the nucleus during migration in constrained 3D environments[76] but also orchestrates the immune synapse that forms with antigen-presenting cells (APCs) and target cells[77,78]. Recent findings have shown that the cortical cytoskeleton of naive T cells has greater mechanical stiffness than that of effector T cells, which depends on the increased activity of the actin-severing enzyme cofilin in effector T cells[79]. As a result, naive T cells tend to form smaller immune synapses with APCs than do effector T cells. This may allow effector T cells to respond more rapidly while preventing the premature activation of naive T cells. Synaptic contact between patrolling T cells and APCs is sustained by cytoskeletal tension, driven by focal actin nucleation mediated by Wiskott–Aldrich syndrome protein, which degrades after successful T cell activation[80]. Cytotoxic T cells also use the cytoskeleton to exert mechanical force on target cells through the immune synapse. The increased cell tension increases perforin release from T cells, facilitating pore formation in target cells and increasing target cell killing[81]. In melanoma and breast cancer cells, myocardin-related transcription factors stiffen the filamentous actin cytoskeleton, which increases the activation and cytotoxicity of T cells and natural killer cells[82]. These findings underscore the role of cytoskeletal tension — both within T cells and externally — in fine-tuning T cell signalling and effector function.

The cytoskeleton also provides structural support to cells and guides the movement of cargo proteins within the cell. Recent findings have highlighted the cytoskeletal organizer transgelin 2 (TAGLN2) as a key regulator of the cell-surface localization of fatty acid-binding protein 5 (FABP5). Under endoplasmic reticulum stress conditions within the TME, T cells silence expression of TAGLN2, which reduces surface levels of FABP5 and shuts down fatty acid uptake and trafficking to mitochondria, leading to T cell dysfunction[83]. This exemplifies the inter-organelle coordination that is required for effective antitumour immunity.

Inter-organelle crosstalk

Organelles do not operate in isolation; rather, their coordinated functions are required for cell homeostasis. The cytoskeleton mediates the spatial positioning of organelles to enable inter-organelle collaboration in specialized tasks that would be unachievable individually. For example, the interaction of the cytoskeleton with lytic granules, using motor proteins as adaptors, is key to the formation of the immune synapse[84,85].

Membrane-bound organelles in close proximity can form membrane contact sites[86,87] stabilized by tethering proteins[88]. Advances in

imaging techniques (Box 1) have expanded our understanding of how inter-organelle membrane contact sites can influence immune cell function. For example, sites of contact between lysosomes and mitochondria have been shown to mark mitochondrial fission sites and to regulate lysosomal RAB7 hydrolysis[89], exemplifying bidirectional communication between organelles. This view has recently been further supported by organelle proteomic profiling[90], which shows that proteins that were previously considered to be organelle-specific may have multifunctional roles across distinct cellular compartments. Using targeted probes, retrograde signalling was detected at mitochondria–endoplasmic reticulum contact sites, where ROS generated from mitochondrial oxidative bursts were sensed by inositol 1,4,5-trisphosphate receptors on the endoplasmic reticulum, triggering Ca^{2+} release[91]. Additionally, recent work has shown that mitochondrial ROS are relayed at peroxisome–mitochondria contact sites, contributing to the alleviation of oxidative stress[92]. Although ROS can broadly affect multiple signalling pathways, inter-organelle membrane contact sites ensure that communication through ROS is confined to adjacent organelles and excessive ROS are scavenged, preventing cell-wide signalling events. In T cells, inter-organelle membrane contact sites facilitate, among others, lipid transfer and Ca^{2+} homeostasis and function as hubs to coordinate signalling and metabolism[93–95]. Together, these findings underscore the essential role of organelle interactions in maintaining the spatial and temporal precision required for effective cellular responses.

Targeting organelles in immunotherapy

Given the crucial roles of organelles in regulating T cell function, differentiation and persistence, there is growing interest in targeting these cellular components to improve the efficacy of T cell-based immunotherapies. Approaches include pharmacological manipulation of organelle abundance and function, genetic engineering strategies (Fig. 1), and intercellular organelle transfer or organelle transplantation (Fig. 2).

Pharmacological manipulation

In recent years, stem-like T cells have been increasingly recognized as key response determinants to both immune-checkpoint blockade (ICB)[96,97] and adoptive T cell therapies[98,99]. Selecting T cells with low mitochondrial membrane potential has been shown to enrich for cells with greater 'stemness'[100], which suggests that targeting mitochondria with pharmacological agents might be an effective approach to inducing stem-like behaviour in T cells. Candidates include small-molecule inhibitors of mitochondrial enzymes or carrier proteins, which allow for organelle-selective targeting through passive mechanisms. Recent screens have identified mitochondrial isocitrate dehydrogenase 2 (IDH2) as a promising target, as its inhibition with enasidenib (Fig. 1A) in CAR T cells increases memory cell formation and sustains antitumour efficacy in vivo[101]. Enasidenib redirects glucose to the pentose phosphate pathway, which increases the oxidant buffering capacity of T cells. Concomitantly, IDH2 inhibition modulates the turnover and availability of metabolites that are essential for epigenetic modifications[102]; for example, prolonged enasidenib treatment resulted in cytosolic accumulation of citrate, a substrate for acetyl-CoA production for histone acetylation, and promoted the accessibility of gene loci associated with memory T cell formation[101,102]. Likewise, the metabolic conditioning of CAR T cells by pharmacological inhibition of mitochondrial pyruvate carrier proteins[103,104], lactate dehydrogenase A[105] or pyruvate dehydrogenase kinase 1 (PDK1)[106] (Fig. 1A) during manufacturing has been found to skew T cell differentiation towards stem-like T cells, thereby improving antitumour activity across various tumour models[103–106]. Inhibiting PDK1 with dichloroacetate has been shown to enhance mitochondrial adaptability to different carbon sources in T cells while simultaneously increasing the availability of metabolites for epigenetic modifications[106]. Other drugs have been found to counteract dysfunctional mitochondrial adaptations to the TME. For example, targeting P4HA1 with 4-hydroxy-1,10-phenanthroline-3-carboxylic ethyl ester (DPCA) restores TCA cycle flux in T cells ex vivo; accordingly, DPCA increased the efficacy of ICB therapy[7] despite prior concerns that it might reduce antigen processing and presentation by tumour cells, thereby blunting T cell recognition[107]. Similarly, blocking ACC1 reinstates FAO in T cells in the TME, promoting T cell persistence and antitumour efficacy[6] (Fig. 1Ba).

Other strategies involve boosting mitochondrial activities indirectly through the modulation of signalling pathways that regulate mitochondrial biogenesis and function. Duvelisib increases mitochondrial mass by inhibiting phosphatidylinositol 3-kinase-δ (PI3Kδ) and PI3Kγ and promoting the expression of mitofusin 2, which is a key regulator of mitochondrial fusion and elongation (Fig. 1C). The increased mitochondrial size and altered network architecture after duvelisib treatment result in $CD8^+$ CAR T cells with enhanced stemness and improved efficacy in eradicating chronic lymphocytic leukaemia cells in a humanized mouse model[108]. In addition, countering the effects of ROS with antioxidants such as N-acetylcysteine (Fig. 1D) limits the terminal differentiation of CAR T cells, yielding a more potent antitumour product[109].

Efforts are also under way to enhance the resilience of organelles to withstand the harsh conditions of the TME. In vivo administration of nicotinamide riboside, a precursor to NAD^+, stimulates mitophagy, improves mitochondrial fitness and reduces exhaustion in $CD8^+$ T cells. This intervention eliminates defective mitochondria, emphasizing the importance of mitochondrial quality in optimizing T cell-mediated antitumour responses[14]. Supplementation of ubiquinone (also known as coenzyme Q10), an essential component of the mitochondrial ETC, has been shown to reduce ROS production and oxidative stress-induced apoptosis in T cells[110] (Fig. 1E). Similarly, metformin, which is a direct inhibitor of complex I of the mitochondrial ETC (Fig. 1E), promotes $CD8^+$ T cell survival and tumour infiltration by mitigating excessive ROS accumulation caused by mitochondrial dysregulation in the hypoxic TME[111]. These findings show the potential of drug repurposing to deliver substantial benefits when integrated with immunotherapies.

Besides mitochondria, other organelles, such as lysosomes, the endoplasmic reticulum and the Golgi apparatus, have been successfully targeted to improve the fitness and antitumour function of T cells. One key challenge in the TME is the accumulation of lactic acid, which suppresses T cell-mediated antitumour immunity despite being a potential energy source for T cells[3,5,112]. Lithium carbonate — which inhibits lactic acid-induced acidification of lysosomes in $CD8^+$ T cells by competing with protons at the V-ATPase (Fig. 1F) — not only mitigates the immunosuppressive effects of lactic acid but also promotes the redistribution of lactate-importing monocarboxylate transporter 1 from the endoplasmic reticulum to mitochondrial membranes, which facilitates the use of lactate as a fuel and boosts the antitumour efficacy of $CD8^+$ T cells in a dual manner[113]. Another approach to targeting lysosomes was recently described in a study showing that D-mannose administration triggers lysosome biogenesis, leading to lysosomal degradation of the immune checkpoint PD1 and T cell reinvigoration to an extent comparable to that mediated by PD1 blockade[114].

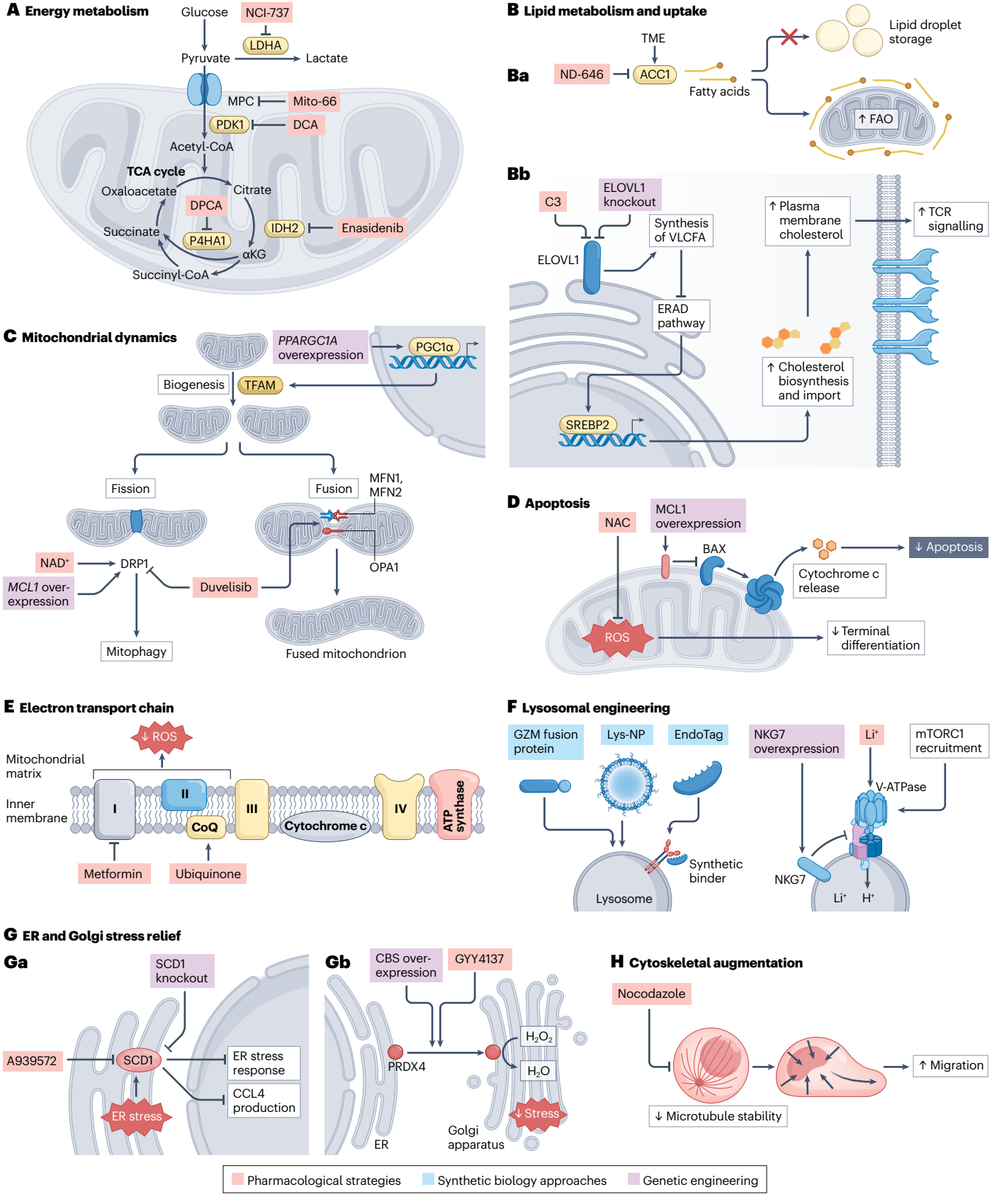
A Energy metabolism
Glucose
NCI-737
LDHA
Pyruvate
Lactate
MPC
Mito-66
PDK1
DCA
Acetyl-CoA
TCA cycle
Oxaloacetate
Citrate
DPCA
Succinate
IDH2
Enasidenib
P4HA1
αKG
Succinyl-CoA
B Lipid metabolism and uptake
Ba
TME
ND-646
ACC1
Fatty acids
Lipid droplet storage
↑ FAO
Bb
C3
ELOVL1 knockout
Synthesis of VLCFA
ELOVL1
ERAD pathway
SREBP2
↑ Plasma membrane cholesterol
↑ TCR signalling
↑ Cholesterol biosynthesis and import
C Mitochondrial dynamics
PPARGC1A overexpression
PGC1α
Biogenesis
TFAM
Fission
Fusion
MFN1, MFN2
OPA1
NAD+
DRP1
MCL1 over-expression
Duvelisib
Mitophagy
Fused mitochondrion
D Apoptosis
NAC
MCL1 overexpression
BAX
↓ Apoptosis
Cytochrome c release
ROS
↓ Terminal differentiation
E Electron transport chain
↓ ROS
Mitochondrial matrix
Inner membrane
I
II
III
IV
CoQ
Cytochrome c
ATP synthase
Metformin
Ubiquinone
F Lysosomal engineering
GZM fusion protein
Lys-NP
EndoTag
Synthetic binder
Lysosome
NKG7 overexpression
Li+
mTORC1 recruitment
V-ATPase
NKG7
Li+
H+
G ER and Golgi stress relief
Ga
SCD1 knockout
A939572
SCD1
ER stress response
CCL4 production
ER stress
Gb
CBS over-expression
GYY4137
PRDX4
H2O2
H2O
↓ Stress
ER
Golgi apparatus
H Cytoskeletal augmentation
Nocodazole
↑ Migration
↓ Microtubule stability
Pharmacological strategies
Synthetic biology approaches
Genetic engineering

Fig. 1 | Pharmacological and genetic engineering strategies to boost organelle function for improved cancer immunotherapy. Selected examples are shown; full descriptions of the mechanisms are given in the main text. **A**, Mitochondrial transporters and enzymes can be targeted pharmacologically to re-route energy-rich carbons for metabolic tuning of T cell differentiation. Blocking glycolysis with an inhibitor (NCI-737) of lactate dehydrogenase A (LDHA), blocking import through the mitochondrial pyruvate carrier (MPC) with Mito-66, or blocking pyruvate processing with the pyruvate dehydrogenase kinase 1 (PDK1) inhibitor dichloroacetate (DCA) have all been shown to promote T cell fitness. In addition, the isocitrate dehydrogenase 2 (IDH2) inhibitor enasidenib is an example of a strategy that interferes with the tricarboxylic acid (TCA) cycle. Additionally, targeting prolyl 4-hydroxylase α-subunit 1 (P4HA1), which disrupts the TCA cycle by altering α-ketoglutarate (αKG) and succinate metabolism, with 4-hydroxy-1,10-phenanthroline-3-carboxylic ethyl ester (DPCA) restores TCA cycle flux in T cells. **Ba**, Pharmacological inhibition of acetyl-coenzyme A (CoA) carboxylase 1 (ACC1), which initiates fatty acid synthesis in the tumour microenvironment (TME), with ND-646 redirects fatty acids to catabolic use through fatty acid oxidation (FAO). **Bb**, Compound 3 (C3)-mediated inhibition of elongation of very long chain fatty acids protein 1 (ELOVL1), which is involved in the synthesis of very long chain fatty acids (VLCFA), promotes endoplasmic reticulum (ER)-associated protein degradation (ERAD) pathway-mediated activation of SREBP2, inducing cholesterol synthesis. Increased cholesterol in the plasma membrane enhances T cell receptor (TCR) clustering, thereby strengthening TCR signalling. Similar effects can be induced by knocking out ELOVL1. **C**, Mitochondrial dynamics are a key regulator of T cell fitness. Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α, encoded by *PPARGC1A*) is the central regulator of mitochondrial biogenesis, which is mediated by mitochondrial transcription factor A (TFAM). Nicotinamide adenine dinucleotide (NAD^+), MCL1 overexpression and duvelisib control dynamin 1-like (DRP1) activity, which drives mitochondrial fission and mitophagy of dysfunctional mitochondria. As well as inhibiting mitochondrial fission, duvelisib enhances mitochondrial fusion through expression of mitofusin 1 (MFN1), MFN2 and OPA1. **D**, Overexpression of anti-apoptotic MCL1 protects tumour-fighting T cells from cell death by inhibiting BAX, and reactive oxygen species (ROS) scavenging with antioxidants such as N-acetylcysteine (NAC) delays terminal differentiation. **E**, Modulating the mitochondrial electron transport chain, using either metformin, which inhibits complex I, or by supplementing ubiquinone (also known as CoQ), reduces the generation of ROS and is therefore cytoprotective. **F**, Transgenically expressed fusion proteins (such as granzymes (GZM)) carrying a cell transduction domain and lysosomal-targeting sequence or CD63 aptamer-decorated nanoparticles (Lys-NP) loaded with cytotoxic effector molecules enhance the lytic potential of T cells. De novo-designed synthetic binders, so-called EndoTags, enable selective routing of surface and soluble molecules or cargo to the endolysosomal pathway. Interfering with vacuolar-type ATPase (V-ATPase) activity by overexpression of natural killer cell group 7 (NKG7) or by lithium carbonate (Li^+) alters, among others, mechanistic target of rapamycin complex 1 (mTORC1) recruitment to the lysosome. **Ga**, Stearoyl-CoA desaturase 1 (SCD1) is a mediator of the ER stress response, with detrimental effects on T cell health. These effects are abrogated by gene knockout or by pharmacological inhibition of SCD1 with A939572, which may also promote immune cell recruitment by increasing tumour cell production of the chemokine CCL4. **Gb**, Exogenous supplementation of the H_2S donor GYY4137 or bolstering endogenous H_2S production by cystathionine β-synthase (CBS) overexpression facilitates redistribution of peroxiredoxin 4 (PRDX4) from the ER to the Golgi, where it supports antioxidant capacity, thereby alleviating Golgi stress. **H**, Nocodazole destabilizes cytoskeletal microtubules, thereby promoting increased contractility and migration of T cells, for example, inside the tumour mass. Pharmacological strategies are highlighted in red, genetic engineering approaches using overexpression or knockout strategies are depicted in purple, and synthetic biology approaches are blue.

However, although promoting lysosome biogenesis in T cells can increase the degradation of undesired factors such as PD1, it may also increase the turnover of functionally crucial components such as TCR and CAR molecules, surface levels of which are tightly regulated by ubiquitylation and lysosomal degradation[115,116]. Interestingly, the introduction of a simple mutation in the CAR intracellular domain prevented its ubiquitylation and lysosomal degradation, allowing CAR molecules to be recycled to the cell surface[115]. This strategy could be combined with lysosome-targeted treatments to optimize T cell function and enhance their therapeutic efficacy.

Recent studies have highlighted opportunities to counteract TME-induced endoplasmic reticulum and Golgi stress to prevent T cell dysfunction[45,83,117,118]. Pharmacological inhibition of stearoyl-CoA desaturase 1 (SCD1), which is a key regulator of endoplasmic reticulum stress responses (Fig. 1Ga), enhances T cell function in vitro and synergizes with PD1 blockade in vivo, thereby improving antitumour efficacy in diverse mouse tumour models[117]. Similarly, reversing Golgi stress using a hydrogen sulfide donor increases T cell antioxidant capacity, protein translation and stemness (Fig. 1Gb), resulting in increased anti-tumour responses in models of both TCR-directed and CAR-redirected T cell therapy[45].

Finally, pharmacological interventions are being developed that target the cytoskeleton. Nocodazole destabilizes microtubules and has been shown to enhance T cell migration in 3D collagen matrices and tumour slices[119]. This effect is mediated by the release of microtubule-sequestered guanine nucleotide exchange factor H1 (GEF-H1; also known as ARHGEF2), which promotes robust RHO-mediated cortical contractility, driving the transition from bleb-like to pseudopodial protrusions from T cells[119] (Fig. 1H). However, it remains untested whether nocodazole negatively impacts immune synapse formation and TCR signalling. Therefore, achieving temporal control — to promote microtubule disassembly while T cells navigate the TME but reverse microtubule destabilization upon target cell engagement to allow for stable synapse formation — may be crucial for optimizing this therapeutic strategy.

Organelle-targeted drug delivery approaches

The pharmacokinetic effects of active pharmaceutical compounds could be enhanced by organelle-specific delivery mechanisms that direct subcellular protein transport or by leveraging the inherent properties of organelles. Examples of organelle-targeting sequences include the mitochondrial targeting sequence[120]; mannose 6-phosphate residues, dileucine-based motifs and tyrosine-based motifs[121], which target to lysosomes; PTS1, PTS2 or membrane PTS[122], which target to peroxisomes; KDEL (Lys-Asp-Glu-Leu) and KDEL-like endoplasmic reticulum protein retention sequences[123]; and the newly identified minimal Golgi targeting sequence[124]. These sequences can be used not only to interfere with organelle function in tumour cells[125] but also to enhance T cell function in the TME and increase the efficacy of cancer immunotherapy.

The mitochondrial targeting sequence, in combination with cell-penetrating protein domains or viral vectors, has been used successfully to deliver antioxidant proteins to mitochondria to enhance their function as well as genetic material to reverse mitochondrial genetic disorders[126–129]. This strategy has great potential for alleviating damage to mtDNA in antitumour T cells, which are particularly susceptible to DNA mutations owing to ageing, systemic chemotherapies, radiation treatments and prolonged exposure to oxidative

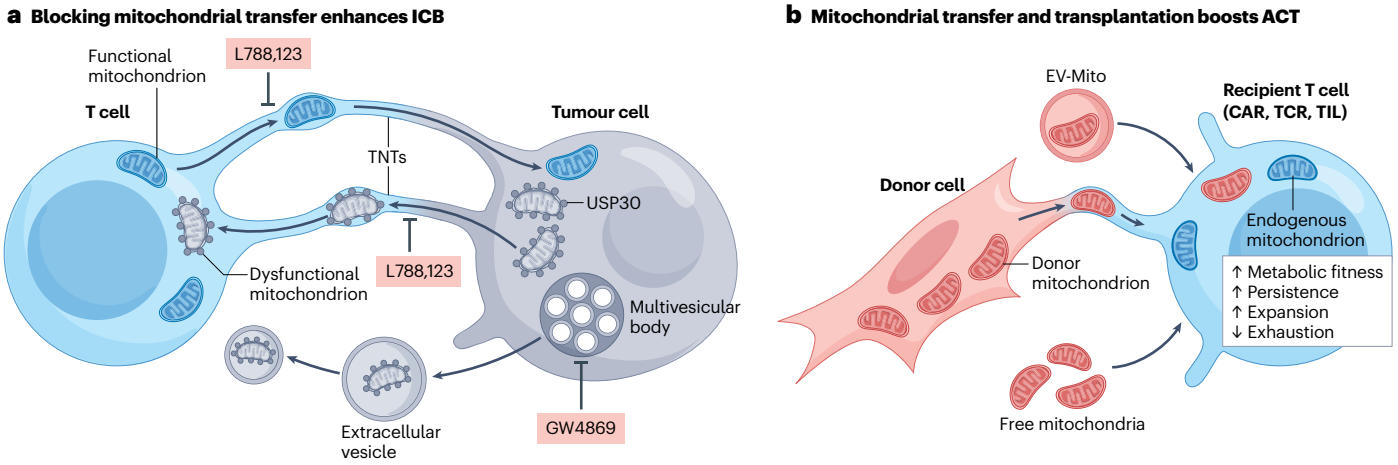

Fig. 2 | Enhancing cancer immunotherapy by targeting mitochondrial transfer or transplantation. a, Tumour cells exploit the mobility of mitochondria by 'stealing' functional mitochondria from T cells or by poisoning T cells with dysfunctional mitochondria (coated with ubiquitin-specific peptidase 30 (USP30)). Transfer occurs through tunnelling nanotubes (TNTs) or extracellular vesicles, the formation of which can be blocked pharmacologically (for example, with L788,123 or GW4869, respectively). Blocking these mechanisms has the potential to increase the efficacy of immune-checkpoint blockade (ICB) therapy. **b**, Intercellular transfer of mitochondria to T cells through TNTs or by the transplantation of free mitochondria or extracellular vesicle-embedded mitochondria (EV-mito) during the manufacture of adoptive cell therapies (ACT), such as chimeric antigen receptor (CAR) T cells, T cell receptor (TCR)-transgenic T cells and tumour-infiltrating lymphocytes (TILs), increases T cell fitness and antitumour efficacy.

stress in the TME[130–132]. Aptamers with lysosome-targeting motifs that bind the lysosome-specific protein CD63 have been used to direct the release of nanoparticles containing perforin and GZMB into the lysosomes of tumour-specific T cells, effectively 'super-weaponizing' them (Fig. 1F). Upon TCR engagement, these 'super-cytotoxic' T cells markedly reduced the tumour burden in a mouse model of breast cancer compared with non-modified tumour-specific T cells[133]. For the correction of hereditary catalase deficiency (the inability to process hydrogen peroxide and other ROS), PTS motifs have been added to catalase to facilitate its homing to peroxisomes[134]. Given the crucial roles of ROS in T cell survival and function[135], enzyme-replacement strategies targeting peroxisomes could be highly beneficial for therapies using tumour-infiltrating lymphocytes (TILs), in which dysregulated intracellular ROS levels are commonly observed[136]. Lastly, owing to the role of the endoplasmic reticulum in MHC processing and antigen loading, endoplasmic reticulum-targeting sequences have been used mainly in vaccines to increase antigen presentation to T cells[137].

Other drug-targeting strategies take advantage of the unique properties of organelles such as the negative charge of mitochondria[138], the high ROS content of the endoplasmic reticulum[139] or the acidic pH of lysosomes[140]. A well-known example of mitochondrial targeting is the antioxidant MitoQ, a ubiquinone–triphenylphosphonium conjugate with an overall positive charge that allows it to accumulate in negatively charged mitochondria. By preventing oxidative damage through ubiquinone supplementation (Fig. 1E), MitoQ has been shown to partially restore the activity of $CD8^+$ TILs in renal cell carcinoma[141] and to improve the function of exhausted hepatitis B virus-specific $CD8^+$ T cells[16]. The oxidative endoplasmic reticulum environment, particularly after exposure to stressors[142], has been pharmacologically exploited by combining endoplasmic reticulum-targeting peptides with nanoparticles capped with a ROS-cleavable boronobenzyl acid linker[143]; the endoplasmic reticulum-targeting peptide directs the nanoparticles to the endoplasmic reticulum, where the ROS-cleavable cap selectively releases the therapeutic cargo (antimicrobial peptide) in the presence of high levels of hydrogen peroxide, enabling precise treatment only of cells that are experiencing endoplasmic reticulum stress (owing to infection). In summary, organelle-specific delivery strategies offer the potential for precise, targeted therapies to correct T cell dysfunction, enhance cellular fitness and achieve improved therapeutic efficacy with remarkable specificity.

Genetic and synthetic biology tools

Organelle-targeting pharmacological approaches are limited by their off-target effects upon systemic administration and their transient impact when used ex vivo to modify organelle function during T cell manufacturing. An alternative strategy involves enhancing organelle function through genetic engineering of cells, yielding effects that are both cell-specific and more sustained. For example, overexpression of *PPARGC1A* (encoding PGC1α), which regulates mitochondrial biogenesis and antioxidant activity, has been used to boost mitochondrial activity and enhance T cell fitness and antitumour immunity[17,144,145] (Fig. 1C). This intervention favoured the development of less-differentiated central memory $CD8^+$ T cells, enabling robust recall responses upon antigen-specific rechallenge[144]. In addition, overexpression of the cytoskeletal organizer *Tagln2* enables T cells to overcome endoplasmic reticulum stress-induced dysfunction and enhances antitumour responses by restoring mitochondrial FAO[83]. Genetic silencing of glutaryl-CoA dehydrogenase leads to increased glutarate levels in CAR $CD8^+$ T cells, which inhibits α-ketoglutarate-dependent dioxygenase, thus enhancing the cytotoxicity of CAR T cells through epigenetic reprogramming[24]. Recently, CRISPR screens have identified the endoplasmic reticulum enzymes ELOVL1 (ref. 146) (Fig. 1Bb) and POFUT1 (ref. 147) as potential targets to enhance organelle function in T cell-based cancer immunotherapies. *Elovl1* deficiency indirectly increases cholesterol biosynthesis and import downstream of ERAD pathway-mediated activation of SREBP2, thereby potentiating TCR

signalling, whereas *POFUT1* deletion strengthens effector T cell responses by promoting mitochondrial OXPHOS through Notch signalling.

Several of the druggable targets that enhance the function of antitumour T cells can also be manipulated genetically to achieve a similar effect. Examples include the overexpression of cystathionine β-synthase, an enzyme involved in the biosynthesis of endogenous hydrogen sulfide, which has a similar capacity to alleviate Golgi stress in T cells as a hydrogen sulfide donor[45] (Fig. 1Ga). Disrupting the expression of P4HA1, which accumulates in mitochondria under hypoxic conditions, improves mitochondrial TCA function and promotes the expansion of a stem-like $CD8^+$ T cell population in a similar manner to administration of DPCA[7]. In addition, knocking out *Scd1* in $CD8^+$ T cells reduces endoplasmic reticulum stress responses and increases the antitumour efficacy of T cells following PD1 blockade, similarly to pharmacological inhibition of SCD1. Notably, however, whereas *Scd1* knockout can be restricted to T cells, systemic SCD1 inhibition also stimulates release of the chemokine CCL4 by cancer cells (Fig. 1Ga), which promotes dendritic cell recruitment to tumours and facilitates the activation and accumulation of $CD8^+$ T cells, thereby providing additive therapeutic benefits[117]. Genetic engineering strategies have yet to be directly compared with pharmacological interventions in the same experimental setting to determine which is superior.

The potential of lysosomal engineering is illustrated by a recent study indicating that overexpression of NKG7, which inhibits mTORC1 recruitment to lysosomes, boosts T cell infiltration into tumours[66] (Fig. 1F). Moreover, an innovative synthetic biology approach leveraging lysosomes as a payload delivery system provided proof of concept for a GZMB fusion protein that shuttles protein cargoes into T cell lytic granules for delivery to target cells via the immune synapse[148] (Fig. 1F). However, as the loading motifs that direct GZMB to lytic granules only function when contiguous in the tertiary structure[148], any fusion protein must be carefully evaluated to avoid masking this crucial structure. Bio-orthogonal, endocytosis-triggering binding proteins (EndoTags) exploit the endosomal–lysosomal pathway more efficiently through the design of binding proteins (Tags) for cellular receptors that trigger their endocytosis into lysosomes without disrupting native receptor–ligand interactions[149] (Fig. 1F), although EndoTags still need to be designed carefully for each specific target. EndoTags can guide immune-checkpoint proteins, such as PDL1 and CTLA4, to lysosomes for degradation or can enable the targeted delivery of cargo proteins to lysosomes in vivo[149]. Arming T cells, particularly hypofunctional T cells, using EndoTags coupled to cytotoxic lysosomal mediators as cargo proteins, might boost T cell effector functions and tumour control. In addition, EndoTags enhance the ligand-dependent activation of synthetic intramembrane proteolysis receptors, which primarily occurs in lysosomes, highlighting the versatility of this technology for synthetic biology applications[149,150].

Dynamic regulation of gene expression is fundamental for proper T cell development and function, which may be impaired by engineering strategies that result in constitutive overexpression or gene knockout. For example, constitutive overexpression of cystathionine β-synthase, which potentiates the oxidant buffering capacity of a cell through production of hydrogen sulfide, may interfere with ROS signalling during T cell activation[151]. Likewise, interfering with mitochondrial fission and fusion using constitutively active genetic engineering strategies could lead to undesired therapeutic outcomes given the important roles of mitochondria throughout T cell differentiation. Indeed, mitochondrial fission supports T cell migration, tissue infiltration and cytotoxicity, whereas mitochondrial fusion is essential for T cell memory phenotypes and long-term persistence[152]. Several inducible systems, with varying levels of complexity, can provide more controlled gene regulation.

For example, the inducible Tet-On system has been used to control the expression of MCL1 (ref. 153), which regulates mitochondrial fission and fusion dynamics[154], with effects on T cell phenotype and survival[155]. Placing *Mcl1* and other genes of interest under the control of a Tet-On system could be a powerful tool to dynamically modulate the metabolic profile of T cells and guide their differentiation towards specific phenotypes as required (Fig. 1C). In addition, MCL1 could be leveraged to boost T cell survival by inhibiting BAX[156] (Fig. 1D). Limitations of Tet-On and Tet-Off conditional transgene systems include the 'leakiness' of the expression system, the potential for tetracycline induction to perturb mitochondrial function[157,158] and genotoxic effects of the Tet-transactivator in activated antigen-induced T cells[159,160]. A new generation of synthetic gene circuits has been developed and tested that can control multiple genes in T cells using clinically approved small molecules[161]. Although these circuits did not incorporate genes specifically aimed at improving organelle function, the ability for clinicians to activate multiple cellular programmes sequentially is an exciting prospect, as the platform is more reflective of the dynamic temporal nature of immune cell states. An alternative, simpler approach may be the use of synthetic promoters and gene circuits that respond to stimuli in the TME, such as hypoxia or inflammation, or logic gates[162] that enable T cells to autonomously regulate their differentiation depending on external cues[163,164]. These platform technologies are in the early stages of development but hold great promise for targeted organelle engineering in the future.

Mitochondria are unique among organelles in having their own genome, which can be edited, ranging from point mutations to large deletions[129,165–168]. Whereas CRISPR-based editing systems are limited by the inefficient import of guide RNAs into mitochondria[169], protein-only nuclease techniques, such as mitochondria-targeted transcription activator-like effector nucleases[165] and mitochondria-targeted heterodimeric zinc finger nucleases[166], have shown greater efficiency in selectively editing mtDNA. Double-stranded DNA cytidine deaminase toxin A-derived cytosine base editors[167] and transcription activator-like effector-linked deaminases[168] primarily catalyse C-to-T and A-to-G base editing of mtDNA, respectively. The toolbox for precision engineering of mtDNA has recently been complemented with mitochondrial base editors, which operate in a similar manner to double-stranded DNA cytidine deaminase toxin A-derived cytosine base editors and transcription activator-like effector-linked deaminases but promise extra precision and efficiency[170]. Although mtDNA editing remains challenging, these technologies could be used to correct mtDNA mutations in TILs, which often have a high mutation burden, or to improve mitochondrial function in adoptively transferred T cells by replacing suboptimal single-nucleotide polymorphisms in their mtDNA. However, important limitations remain, including low editing efficiency and the high copy number of mtDNA within individual cells, which can result in variable levels of heteroplasmy across cells.

Organelle transfer and transplantation

In the past 15 years, a growing body of evidence has shown that organelles are far more mobile than was previously thought and can even move between cells. Owing to their endosymbiotic origin, most research in this area has focused on the transfer of mitochondria. Intercellular mitochondrial transfer was first described in 2006 in a study showing

that stromal cells could supply mitochondria to a lung cancer cell line deficient in mtDNA, thereby rescuing aerobic respiration[171]. Since then, mitochondrial transfer has become a rapidly expanding field of study. Various modes of transfer have been described, including tunnelling nanotubes and dendritic structures, extracellular vesicles, and release of free mitochondria[172].

Mitochondrial transfer has mainly been studied in the context of regenerative medicine (for example, for cardiovascular disease), tumour progression, and as a novel therapeutic platform for treating inherited mitochondrial diseases[172–176]. However, new research has highlighted its relevance in regulating immune responses against cancer. Two studies showed that cancer cells use mitochondrial transfer mechanisms to evade immune attack (Fig. 2a). On the one hand, tumour cells acquire healthy mitochondria from T cells to meet their metabolic demands and sustain their proliferation[177,178]. On the other hand, tumour cells offload their dysfunctional mitochondria to T cells, which reduces T cell fitness and impairs their antitumour function[179]. These cancer-derived mitochondria are coated with the deubiquitylase ubiquitin-specific peptidase 30 (USP30), which is a negative regulator of mitophagy, leading, over time, to mitochondrial homoplasmy through the replacement of T cell mitochondria with cancer-derived mitochondria and ultimately driving T cell exhaustion. Blocking mitochondrial transfer with inhibitors of nanotube formation or small extracellular vesicle production has been shown to boost antitumour responses to PD1 blockade, which shows the potential of disrupting this mechanism to improve the efficacy of ICB therapies (Fig. 2a). Alternatively, antitumour T cells can be 'supercharged' with exogenous mitochondria (Fig. 2b). Through the natural intercellular transfer of mitochondria[180] or by directly transplanting mitochondria[181], T cells can acquire enhanced metabolic resilience together with increased resistance to apoptosis and exhaustion to increase the efficacy of T cell-based immunotherapy. We have provided proof of concept that this strategy is highly effective across various adoptive cell therapy platforms, including CAR T cells, TCR-modified T cells and TILs. Notably, the transferred mitochondria were maintained in T cells for at least several weeks, which suggests that organelle transfer may enable long-term cellular reprogramming. By contrast, in other cell types such as endothelial cells, transferred mitochondria seem to be short-lived, although they still confer cytoprotective effects in recipient cells through the induction of mitophagy to maintain intracellular homeostasis[182].

Various platforms for mitochondrial transfer exist, including intercellular mitochondrial transfer and transplantation of extracellular vesicle-delivered mitochondria or free mitochondria, each having distinct advantages and limitations (Box 2). Regardless of the platform, mitochondrial transfer and transplantation provide unique benefits over conventional pharmacological and genetic engineering approaches targeting mitochondria as they deliver whole, intact organelles (including mtDNA) rather than targeting specific genes or pathways to enhance mitochondrial function. This strategy is likely to be particularly relevant for TIL-based therapies as these cells often contain

Box 2 | Advantages and limitations of mitochondrial transfer and transplantation

Mitochondrial transfer and transplantation have both proven effective in providing recipient cells with healthy mitochondria, thus enhancing their metabolic activity and function. Both technologies have unique advantages and specific challenges that must be carefully weighed when considering their translation to clinical settings.

Mitochondrial transfer enables a more physiological intercellular exchange of organelles but it requires complex co-culture systems, which pose challenges for good manufacturing practice standards. It is also limited by a relatively low transfer rate and difficulty in controlling the quantity of transferred mitochondria, leading to a heterogeneous cell product. In addition, donor cells may transfer other factors to recipient cells during this process, which could influence the function of recipient cells in unpredictable ways, potentially complicating therapeutic outcomes.

Mitochondrial transplantation has the advantage of being a cell-free approach, which eliminates the need for co-culture of donor and acceptor cells. This method allows for precise control over the quantity of mitochondria that are added, ensuring a more consistent and reproducible delivery of organelles to target cells. However, the natural uptake of isolated mitochondria by recipient cells is typically low. To overcome this limitation, various strategies have been developed to increase the internalization of mitochondria, including centrifugation-based methods such as MitoCeption[215], pressure-driven techniques such as MitoPunch[216], encapsulation within artificial lipid membranes[217] or microinjections[218]. A major disadvantage of mitochondrial transplantation is the potential for donor mitochondria to become damaged or lose function during the extraction process or when exposed to environmental factors in the media during application. Damaged mitochondria and free mtDNA in the cytoplasm can trigger innate immune signalling pathways[219] that could potentially mask the intended therapeutic effect.

One study found that mitochondria had greater persistence when transferred through cell–cell interactions rather than through microinjection of isolated mitochondria[218], which indicates that using endogenous transfer mechanisms is superior to artificial manipulation for the long-term engraftment of donor mitochondria[215,217–219]. A hybrid approach that combines the benefits of both mitochondrial transplantation and intercellular mitochondrial transfer is the use of extracellular vesicle-delivered mitochondria (EV-Mito). Like mitochondrial transplantation, EV-Mito is a cell-free method, eliminating the need for direct co-culture of donor and recipient cells. At the same time, similarly to mitochondrial transfer, EV-Mito preserve the structural and functional integrity of mitochondria as they are released from cells through endogenous mechanisms rather than harsh chemical extraction. EV-Mito are enclosed by a phospholipid bilayer membrane, which protects mitochondria against the extracellular environment and enzymatic degradation[220,221]. However, a limitation of EV-Mito is that microvesicles can function as a cellular release route for damaged mitochondria[175,222–224] as well as healthy mitochondria[225,226], with small microvesicles (~40–200 nm) tending to be enriched for mitochondrial fragments[172,227]. As for mitochondrial transfer, EV-Mito may also transfer other factors to recipient cells, which could influence the therapeutic effect. Therefore, stringent quality control measures are essential for screening and sorting EV-Mito.

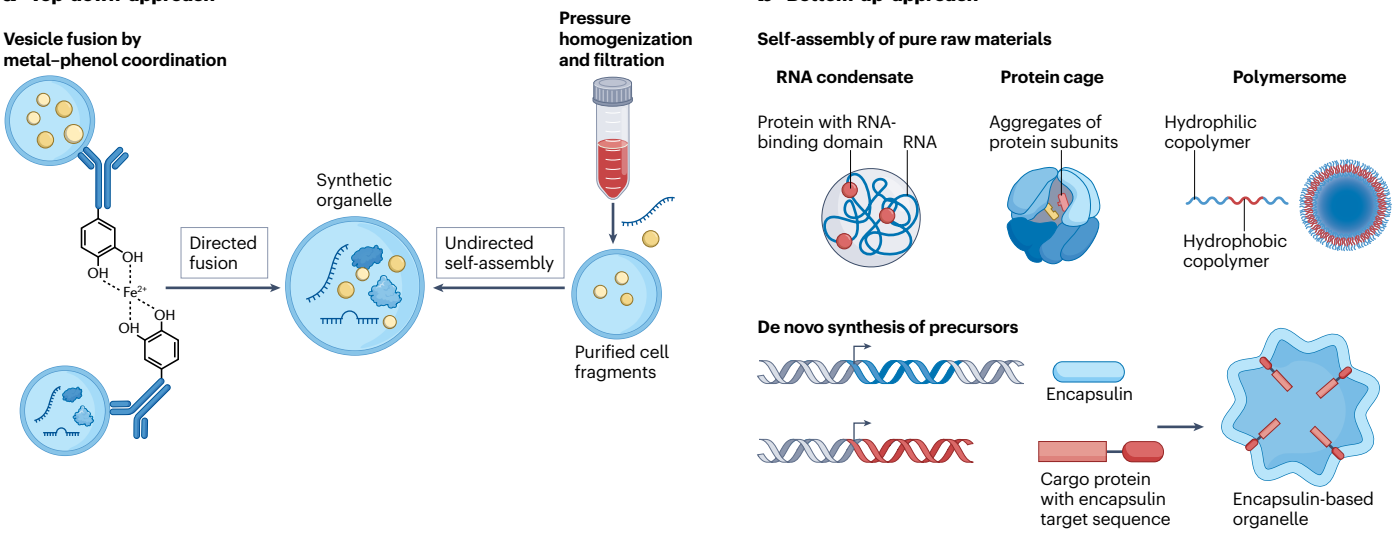

Fig. 3 | Generation of synthetic organelles. a, 'Top-down' approaches for organelle synthesis use biologically produced material mixed with varying compositions of macromolecules, nucleic acids, proteins and protein complexes, together with (intact) vesicles. These are combined in a directed or undirected manner to form organelles that can carry out dedicated functions. Examples include the directed, metal–phenol coordinated fusion of extracellular vesicles or the undirected, spontaneous self-assembly of purified cell fragments. **b**, 'Bottom-up' approaches to organelle production use self-assembling, pure raw materials (RNA, protein or polymers) or de novo-synthesized precursor molecules, which can exploit the full physicochemical space of biomaterials and biocompatible materials. Features such as hydrophobicity, affinity and complementarity determine the shape, uptake and stability of the synthetic organelles, whereas the cargo molecules on the organelle surface and in its lumen endow it with specialized functions or direct its subcellular localization. Encapsulins are bacteria-derived structural proteins that can self-assemble in living cells to create organelle-like structures; they can be paired with engineered cargo proteins designed to self-target the encapsulin-based organelles.

damaged mitochondria or dysfunctional mitochondria transferred from cancer cells[13,14,17,179]. Further studies to elucidate the mechanisms of both transferring and maintaining mitochondria in recipient cells will help in scaling up the process to achieve clinically relevant numbers of transferred mitochondria.

In addition to mitochondria, other organelles have also been shown to transfer between different cell types. For example, a recent study provided the first documented evidence of cell-to-cell peroxisome transfer from bone marrow stromal cells to haematopoietic stem and progenitor cells (HSPCs) in both in vitro and in vivo models of HSPC transplantation[183]. A similar transfer of peroxisomes also occurred between HSPCs, which suggests that intercellular peroxisome exchange may have a crucial role in maintaining stem cell function and survival within the bone marrow niche. Interestingly, the transferred peroxisomes could rescue recipient cells from radiation-induced, ROS-mediated cell death in vitro[183]. Although these findings are preliminary, they provide an exciting opportunity to explore peroxisome transfer as a means to improve T cell resilience and survival.

Synthetic organelles

Synthetic biology is now enabling the creation of synthetic organelles that can either mimic the functions of native organelles or carry out new cellular tasks. There are two main approaches to creating synthetic organelles: a 'top-down' strategy in which organelles are derived from pre-existing components of live cells and modified for specific purposes (Fig. 3a), and a 'bottom-up' strategy, whereby organelles are synthesized de novo from raw materials (such as lipids, enzymes and DNA) (Fig. 3b).

Following a 'top-down' strategy, cell-derived exosomes can be used as a template to engineer synthetic organelles owing to their biocompatibility and cell permeability[184]. For example, exosomes preloaded with different enzymes (such as ATP synthase and bacterial bo3 oxidase) were fused to create a nanoreactor capable of generating ATP inside a living cell, effectively functioning as an artificial mitochondria-like organelle[184]. Another example involves using the self-assembly properties of membrane components to create artificial organelles with OXPHOS capacity. Researchers disrupted the membranes of neural stem cells using high-pressure homogenization and allowed the components to self-assemble into nanovesicles rich in OXPHOS complexes. These nanovesicles were readily taken up by a neural cell line, in which they enhanced mitochondrial function[185].

The alternative, 'bottom-up' strategy has been used to design synthetic organelles ranging from simple RNA condensates to capsosomes, polymersomes, liposomes and protein cages[186,187]. A recent example is a semipermeable polymersomal nanoparticle comprising a biodegradable copolymer surface decorated with cell-penetrating peptides and loaded with catalase[188]. When introduced into HEK293T cells or skin fibroblasts from patients with mitochondrial complex I deficiency, the catalase-loaded polymersomes helped to protect the cells from ROS-mediated damage[188].

As synthetic organelles are much larger than single proteins, a major technical challenge is their efficient delivery into cells. This issue of cellular uptake can be circumvented by instead using a single-cell nanoencapsulation process to coat cells with liposome-based synthetic organelles, referred to as exorganelles[189]. Exorganelles have been tested for several applications, including stimuli-responsive payload release,

Glossary

Autophagy
A cellular process by which cells break down and recycle their proteins and organelles to maintain intracellular homeostasis. The specific removal of mitochondria through autophagy is known as mitophagy.

Chimeric antigen receptor
(CAR). A synthetic surface receptor that typically consists of three key components: an extracellular antigen-recognition domain derived from a single-chain antibody variable fragment; an intracellular co-stimulatory domain (for example, CD28 or 4-1BB); and a CD3ζ cytoplasmic signalling domain for cell activation.

CRISPR screen
A genome-wide screening approach using the CRISPR–Cas9 gene editing system in combination with libraries of guide RNAs to delete or activate large numbers of genomic loci. Cells can be selected based on phenotype, and the corresponding guide RNA that is integrated into the genome can be identified by sequencing.

Epigenetic modifications
Modifications that alter gene expression and phenotype without affecting the DNA sequence. Epigenetic mechanisms include DNA methylation and modifications to histones such as methylation, acetylation or lactylation.

Heteroplasmy
The presence of at least two versions of mitochondrial genomes within one cell. Different genomes may carry mutations, and microheteroplasmy (<5% mutations) is common in eukaryotic cells.

Homoplasmy
Complete identity of all copies of the mitochondrial (or plastid) genome within one cell. Some tumour cells carry homoplasmically mutated mitochondrial genomes.

Immune synapse
The interface between an immune cell and a target cell (for example, a cancer cell) or an antigen-presenting cell (for example, a dendritic cell, macrophage or B cell).

Mechanistic target of rapamycin complex 1
(mTORC1). A multi-protein complex formed by the interaction of mTOR with DEPTOR, RPTOR, AKT1S1 and MLST8. mTORC1 is a crucial nutrient, energy and redox sensor within the cell. It regulates protein synthesis, cellular growth and metabolism by integrating signals from environmental cues such as nutrient availability and cellular energy levels.

MitoSnap
A transgenic mouse model system that enables tracking of mitochondria originating from the mother cell through the permanent fluorescent labelling of a SnapSubstrate that is specifically targeted to mitochondria by SYNJ2BP. Sequential labelling of SnapTag-expressing cells with different fluorescently labelled SnapSubstrates allows for the identification and sorting of distinct cell populations based on patterns of organelle inheritance.

Polysome
A polysome, or polyribosome, is a complex formed when multiple ribosomes simultaneously translate a single mRNA molecule. This arrangement allows for efficient, high-throughput protein synthesis.

Stem-like T cells
Minimally differentiated T cells that share characteristics with stem cells, including self-renewal and the capacity to differentiate into various functional T cell subsets. These cells can emerge following acute infections, where they are known as stem cell memory T cells, or in response to chronic inflammation and cancer, where they are referred to as precursor exhausted T cells.

Synthetic intramembrane proteolysis receptors
Engineered, Notch-based receptors activated by synthetic, bio-orthogonal or natural soluble ligands. Receptor activation depends on endocytosis and endosome acidification to elicit a cellular response.

T cell exhaustion
A dysfunctional T cell state caused by chronic infection or cancer that is marked by reduced proliferation, impaired effector function, increased expression of inhibitory receptors, and distinct transcriptional and epigenetic changes.

Tet-On system
A tetracycline-inducible bacteria-derived gene expression system. It consists of a reverse tetracycline transactivator that, in the drug (tetracycline)-bound state, binds to a tetracycline response element to induce expression of the downstream gene.

Tumour-infiltrating lymphocytes
(TILs). The heterogeneous population of lymphocytes found in a tumour. Their relative abundance, differentiation and functions depend on type, stage and location of the cancer. T cells isolated from TILs can be activated and expanded to large numbers ex vivo before re-infusion into patients with cancer for therapeutic purposes.

Tumour microenvironment
(TME). Tissue at the tumour site, consisting of cancer cells, blood vessels, immune cells and surrounding stromal cells, that, depending on the type of cancer, is often immunosuppressive and has physicochemical properties of hypoxia and low pH.

Unfolded protein response
(UPR). An endoplasmic reticulum stress response triggered by the misfolding of proteins inside the cell. The response can initiate repair mechanisms to correct misfolding such as upregulating chaperones, reducing overall protein synthesis to alleviate the burden, and promoting the degradation of misfolded proteins. If these repair mechanisms fail to resolve the stress, the UPR triggers cell apoptosis.

ROS scavenging and magnetization. Importantly, the nanoencapsulation process did not affect CD3 expression in Jurkat T cells[189]. However, further studies are required to determine whether CD3 or other surface receptors and signalling pathways are altered by the exorganelle coating. An alternative approach involves an expression system that produces encapsulins, bacteria-derived structural proteins that can self-assemble in living cells, to create organelle-like structures. These structures were then paired with engineered cargo proteins designed to self-target the encapsulin-based organelles in HEK293T cells[190]. If such a self-assembling organelle could be stably transduced, it would overcome the crucial limitation of the short persistence of synthetic organelles. However, the potential immunogenicity of bacteria-derived proteins may limit the clinical translation of this technology. So far, synthetic organelles that have been reported in the literature are simplistic in nature (for example, comprising a single enzymatic cascade). However, given the rapid advances in synthetic organelle research, the field holds immense promise.

Conclusions and future directions

Organelle-targeted therapies are advancing rapidly, propelled by new methodologies and a deeper understanding of organelle biology.

Researchers are discovering previously unrecognized roles of organelles, which broadens our understanding of their cellular functions. In addition, emerging data increasingly highlight that organelles do not operate in isolation but instead form interconnected hubs that enable new functions.

From a translational perspective, several organelle-targeting small molecules, such as duvelisib, metformin and enasidenib, are already approved for clinical use for other indications, which might help to accelerate their transition to the clinic for organelle targeting in cancer immunotherapy. Whereas systemic drug delivery can be beneficial in cases such as lithium carbonate and SCD1 inhibitors, which act synergistically on both T cells and tumour cells, in other cases, it may result in unintended off-target effects on other cell types or other organelles within the same cell. In such cases, more controlled drug delivery to specific organelles using targeting peptides or by exploiting the physicochemical properties of organelles may increase the specificity of the drugs.

Genetic engineering offers the potential for stable and cell-restricted modification of T cell organelles, but the dynamic nature of immune cells requires adaptable approaches to gene expression that are not provided by constructs inducing constitutive gene expression. A new generation of gene constructs, ranging from the simple Tet-On system to more complicated gene circuits that can sense and respond to environmental cues, is providing better spatial and temporal control over organelle activities.

When selecting a potential therapeutic strategy for organelle targeting in antitumour T cells, several factors must be considered, including the construct design, the differentiation state of the cell, the source of the cell product, the administration of pre-conditioning regimens to the patient and the co-administration of drugs. For example, the choice of co-stimulatory domains in the CAR construct for CAR T cell therapy can markedly affect organelle function. Including the 4-1BB domain in the CAR enhances mitochondrial respiratory capacity, promoting FAO and mitochondria biogenesis in T cells, whereas the CD28 domain drives T cells towards an effector state[191]. Moreover, cell products derived from TILs are typically more senescent or exhausted, with greater organelle damage, than cells obtained from peripheral blood. In the case of TILs, dysfunctional organelles may impair cellular responses to certain treatments or drugs. Immune-checkpoint inhibitors (such as antibodies to PD1, a molecule whose signalling causes severe cristae alterations in mitochondria[192] and impairs remodelling of the actin cytoskeleton[193]) or interleukins (such as IL-10, IL-15 and IL-21, which promote mitochondria biogenesis and FAO[194–196]) can also affect organelle function in T cells and should be considered when paired with organelle-targeting therapies. Ultimately, as for other cancer therapies, there is no universal strategy to improve the efficacy of T cell therapies, underscoring the need for a personalized approach. To this end, identifying and validating predictive biomarkers of organelle function will be essential for stratifying patients based on their likelihood of benefiting from specific interventions. For example, mitochondrial haplogroups are emerging as a predictor of responses to immune-checkpoint inhibitors[197]. Such precision will not only improve therapeutic efficacy but also minimize unnecessary toxicity. Looking ahead, a deeper understanding of the organelle machinery that drives T cell function, coupled with a toolkit for fine-tuning these processes, will undoubtedly improve patient outcomes in cancer in the future.

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References

1. Suomalainen, A. & Nunnari, J. Mitochondria at the crossroads of health and disease. *Cell* **187**, 2601–2627 (2024).
2. Steinert, E. M. et al. Mitochondrial respiration is necessary for CD8$^+$ T cell proliferation and cell fate. *Nat. Immunol.* **26**, 1267–1274 (2025).
3. Heuser, C., Renner, K., Kreutz, M. & Gattinoni, L. Targeting lactate metabolism for cancer immunotherapy — a matter of precision. *Semin. Cancer Biol.* **88**, 32–45 (2023).
4. Simula, L. et al. Mitochondrial metabolism sustains CD8$^+$ T cell migration for an efficient infiltration into solid tumors. *Nat. Commun.* **15**, 2203 (2024).
5. Kaymak, I. et al. Carbon source availability drives nutrient utilization in CD8$^+$ T cells. *Cell Metab.* **34**, 1298–1311.e6 (2022).
6. Hunt, E. G. et al. Acetyl-CoA carboxylase obstructs CD8$^+$ T cell lipid utilization in the tumor microenvironment. *Cell Metab.* **36**, 969–983.e10 (2024).
7. Ma, S. et al. Targeting P4HA1 promotes CD8$^+$ T cell progenitor expansion toward immune memory and systemic anti-tumor immunity. *Cancer Cell* **43**, 213–231.e9 (2025).
8. Geiger, R. et al. L-arginine modulates T cell metabolism and enhances survival and anti-tumor activity. *Cell* **167**, 829–842.e13 (2016).
9. Kishton, R. J., Sukumar, M. & Restifo, N. P. Arginine arms T cells to thrive and survive. *Cell Metab.* **24**, 647–648 (2016).
10. Martí i Líndez, A. A. et al. Mitochondrial arginase-2 is a cell-autonomous regulator of CD8$^+$ T cell function and antitumor efficacy. *JCI Insight* **4**, e132975 (2019).
11. Sena, L. A. et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity* **38**, 225–236 (2013).
12. Murphy, M. P. & Siegel, R. M. Mitochondrial ROS fire up T cell activation. *Immunity* **38**, 201–202 (2013).
13. Scharping, N. E. et al. Mitochondrial stress induced by continuous stimulation under hypoxia rapidly drives T cell exhaustion. *Nat. Immunol.* **22**, 205–215 (2021).
14. Yu, Y.-R. et al. Disturbed mitochondrial dynamics in CD8$^+$ TILs reinforce T cell exhaustion. *Nat. Immunol.* **21**, 1540–1551 (2020).
Seminal studies by Scharping et al. (2021) and Yu et al. (2020) were instrumental in shaping our understanding of the role of mitochondrial dysfunction in driving T cell exhaustion.
15. Wu, H. et al. Mitochondrial dysfunction promotes the transition of precursor to terminally exhausted T cells through HIF-1α-mediated glycolytic reprogramming. *Nat. Commun.* **14**, 6858 (2023).
16. Fisicaro, P. et al. Targeting mitochondrial dysfunction can restore antiviral activity of exhausted HBV-specific CD8 T cells in chronic hepatitis B. *Nat. Med.* **23**, 327–336 (2017).
17. Scharping, N. E. et al. The tumor microenvironment represses T cell mitochondrial biogenesis to drive intratumoral T cell metabolic insufficiency and dysfunction. *Immunity* **45**, 374–388 (2016).
18. Itoh, Y. et al. Mechanism of membrane-tethered mitochondrial protein synthesis. *Science* **371**, 846–849 (2021).
19. Lisci, M. et al. Mitochondrial translation is required for sustained killing by cytotoxic T cells. *Science* **374**, eabe9977 (2021).
20. Almeida, L. et al. Ribosome-targeting antibiotics impair T cell effector function and ameliorate autoimmunity by blocking mitochondrial protein synthesis. *Immunity* **54**, 68–83.e6 (2021).
21. Martínez-Reyes, I. & Chandel, N. S. Mitochondrial TCA cycle metabolites control physiology and disease. *Nat. Commun.* **11**, 102 (2020).
22. Soriano-Baguet, L. & Brenner, D. Metabolism and epigenetics at the heart of T cell function. *Trends Immunol.* **44**, 231–244 (2023).
23. Tyrakis, P. A. et al. S-2-hydroxyglutarate regulates CD8$^+$ T-lymphocyte fate. *Nature* **540**, 236–241 (2016).
24. Minogue, E. et al. Glutarate regulates T cell metabolism and anti-tumour immunity. *Nat. Metab.* **5**, 1747–1764 (2023).
25. Ryu, K. W. et al. Cellular ATP demand creates metabolically distinct subpopulations of mitochondria. *Nature* **635**, 746–754 (2024).
This study sheds light on how the machinery required for competing oxidative and reductive reactions in mitochondria is segregated into distinct mitochondrial populations under nutrient stress.
26. Katajisto, P. et al. Asymmetric apportioning of aged mitochondria between daughter cells is required for stemness. *Science* **348**, 340–343 (2015).
27. Hinge, A. et al. Asymmetrically segregated mitochondria provide cellular memory of hematopoietic stem cell replicative history and drive HSC attrition. *Cell Stem Cell* **26**, 420–430.e6 (2020).
28. Borsa, M. et al. Inheritance of old mitochondria controls early CD8$^+$ T cell fate commitment and is regulated by autophagy. Preprint at *bioRxiv* https://doi.org/10.1101/2024.01.29.577412 (2024).
29. Adams, W. C. et al. Anabolism-associated mitochondrial stasis driving lymphocyte differentiation over self-renewal. *Cell Rep.* **17**, 3142–3152 (2016).
30. Xu, X. et al. Autophagy is essential for effector CD8$^+$ T cell survival and memory formation. *Nat. Immunol.* **15**, 1152–1161 (2014).
31. Schwarz, D. S. & Blower, M. D. The endoplasmic reticulum: structure, function and response to cellular signaling. *Cell. Mol. Life Sci.* **73**, 79–94 (2016).
32. Trebak, M. & Kinet, J. P. Calcium signalling in T cells. *Nat. Rev. Immunol.* **19**, 154–169 (2019).
33. Ho, P. C. et al. Phosphoenolpyruvate is a metabolic checkpoint of anti-tumor T cell responses. *Cell* **162**, 1217–1228 (2015).
34. Araki, K. et al. Translation is actively regulated during the differentiation of CD8$^+$ effector T cells. *Nat. Immunol.* **18**, 1046–1057 (2017).

35. Sinclair, L. V. & Cantrell, D. A. Protein synthesis and metabolism in T cells. *Annu. Rev. Immunol.* **43**, 343–366 (2025).
36. Wolf, T. et al. Dynamics in protein translation sustaining T cell preparedness. *Nat. Immunol.* **21**, 927–937 (2020).
37. Claiborne, M. D. et al. Persistent CAD activity in memory CD8$^+$ T cells supports rRNA synthesis and ribosomal biogenesis required at rechallenge. *Sci. Immunol.* **7**, eabh4271 (2022).
38. Todd, D. J., Lee, A.-H. & Glimcher, L. H. The endoplasmic reticulum stress response in immunity and autoimmunity. *Nat. Rev. Immunol.* **8**, 663–674 (2008).
39. Nair, K. A. & Liu, B. Navigating the landscape of the unfolded protein response in CD8$^+$ T cells. *Front. Immunol.* **15**, 1427859 (2024).
40. Correa-Medero, L. O. et al. ER-associated degradation adapter Sel1L is required for CD8$^+$ T cell function and memory formation following acute viral infection. *Cell Rep.* **43**, 114156 (2024).
41. Kamimura, D. & Bevan, M. J. Endoplasmic reticulum stress regulator XBP-1 contributes to effector CD8$^+$ T cell differentiation during acute infection. *J. Immunol.* **181**, 5433–5441 (2008).
42. Cao, Y. et al. ER stress-induced mediator C/EBP homologous protein thwarts effector T cell activity in tumors through T-bet repression. *Nat. Commun.* **10**, 1280 (2019).
43. Fernández-Alfara, M. et al. Antitumor T-cell function requires CPEB4-mediated adaptation to chronic endoplasmic reticulum stress. *EMBO J.* **42**, e111494 (2023).
This study elucidates how T cells use CPEB4 to counteract physiological endoplasmic reticulum stress and maintain function by avoiding UPR-mediated dysfunction.
44. Mennerich, D., Kellokumpu, S. & Kietzmann, T. Hypoxia and reactive oxygen species as modulators of endoplasmic reticulum and Golgi homeostasis. *Antioxid. Redox Signal.* **30**, 113–137 (2019).
45. Oberholtzer, N. et al. H2S-Prdx4 axis mitigates Golgi stress to bolster tumor-reactive T cell immunotherapeutic response. *Sci. Adv.* **10**, eadp1152 (2024).
46. Wilson, C. et al. The Golgi apparatus: an organelle with multiple complex functions. *Biochem. J.* **433**, 1–9 (2011).
47. Hong, Y. et al. ST3GAL1 and βII-spectrin pathways control CAR T cell migration to target tumors. *Nat. Immunol.* **24**, 1007–1019 (2023).
48. Uslu, U. et al. The STING agonist IMSA101 enhances chimeric antigen receptor T cell function by inducing IL-18 secretion. *Nat. Commun.* **15**, 3933 (2024).
49. Xu, N. et al. STING agonist promotes CAR T cell trafficking and persistence in breast cancer. *J. Exp. Med.* **218**, e20200844 (2021).
50. Wang, X. et al. DNA sensing via the cGAS/STING pathway activates the immunoproteasome and adaptive T-cell immunity. *Embo J.* **42**, e110597 (2023).
51. Richter, F., Paget, C. & Apetoh, L. STING-driven activation of T cells: relevance for the adoptive cell therapy of cancer. *Cell Stress.* **7**, 95–104 (2023).
52. Li, W. et al. cGAS-STING-mediated DNA sensing maintains CD8$^+$ T cell stemness and promotes antitumor T cell therapy. *Sci. Transl. Med.* **12**, eaay9013 (2020).
53. Motedayen Aval, L., Pease, J. E., Sharma, R. & Pinato, D. J. Challenges and opportunities in the clinical development of STING agonists for cancer immunotherapy. *J. Clin. Med.* **9**, 3323 (2020).
54. Fang, R. et al. Golgi apparatus-synthesized sulfated glycosaminoglycans mediate polymerization and activation of the cGAMP sensor STING. *Immunity* **54**, 962–975.e8 (2021).
The study identifies sulfated glycosaminoglycans as being essential for the translocation of STING from the endoplasmic reticulum to the Golgi apparatus and for modulating its activation.
55. Mindell, J. A. Lysosomal acidification mechanisms. *Annu. Rev. Physiol.* **74**, 69–86 (2012).
56. Palmer, D. C. et al. Cish actively silences TCR signaling in CD8$^+$ T cells to maintain tumor tolerance. *J. Exp. Med.* **212**, 2095–2113 (2015).
57. Jin, J. et al. CISH impairs lysosomal function in activated T cells resulting in mitochondrial DNA release and inflammaging. *Nat. Aging* **3**, 600–616 (2023).
58. Eskelinen, E.-L. Roles of LAMP-1 and LAMP-2 in lysosome biogenesis and autophagy. *Mol. Asp. Med.* **27**, 495–502 (2006).
59. Zhang, J. et al. Lysosomal LAMP proteins regulate lysosomal pH by direct inhibition of the TMEM175 channel. *Mol. Cell* **83**, 2524–2539.e7 (2023).
60. Yu, J. et al. The OX40-TRAF6 axis promotes CTLA-4 degradation to augment antitumor CD8$^+$ T-cell immunity. *Cell. Mol. Immunol.* **20**, 1445–1456 (2023).
61. Isaaz, S., Baetz, K., Olsen, K., Podack, E. & Griffiths, G. M. Serial killing by cytotoxic T lymphocytes: T cell receptor triggers degranulation, re-filling of the lytic granules and secretion of lytic proteins via a non-granule pathway. *Eur. J. Immunol.* **25**, 1071–1079 (1995).
62. Chang, H.-F. et al. Identification of distinct cytotoxic granules as the origin of supramolecular attack particles in T lymphocytes. *Nat. Commun.* **13**, 1029 (2022).
63. Sancak, Y. et al. Ragulator-rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* **141**, 290–303 (2010).
64. Roczniak-Ferguson, A. et al. The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. *Sci. Signal.* **5**, ra42 (2012).
65. Araki, K. et al. mTOR regulates memory CD8 T-cell differentiation. *Nature* **460**, 108–112 (2009).
66. Ham, H. et al. Lysosomal NKG7 restrains mTORC1 activity to promote CD8$^+$ T cell durability and tumor control. *Nat. Commun.* **16**, 1628 (2025).
67. Jin, J. et al. Activation of mTORC1 at late endosomes misdirects T cell fate decision in older individuals. *Sci. Immunol.* **6**, eabg0791 (2021).
This work uncovers how ageing reshapes mTORC1 compartmentalization, altering its association with lysosomes and endosomes to influence T cell differentiation.
68. Di Cara, F., Savary, S., Kovacs, W. J., Kim, P. & Rachubinski, R. A. The peroxisome: an up-and-coming organelle in immunometabolism. *Trends Cell Biol.* **33**, 70–86 (2023).
69. Gilkrist, K. W., Opitz, J. M., Gilbert, E. F., Tsang, W. & Miller, P. Letter: immunodeficiency in the cerebro-hepato-renal syndrome of Zellweger. *Lancet* **1**, 164–165 (1974).
70. Muri, J., Corak, B., Matsushita, M., Baes, M. & Kopf, M. Peroxisomes are critical for the development and maintenance of B1 and marginal zone B cells but dispensable for follicular B cells and T cells. *J. Immunol.* **208**, 839–850 (2022).
71. Vicente-Manzanares, M. & Sánchez-Madrid, F. Role of the cytoskeleton during leukocyte responses. *Nat. Rev. Immunol.* **4**, 110–122 (2004).
72. Martín-Cófreces, N. B., Baixauli, F. & Sánchez-Madrid, F. Immune synapse: conductor of orchestrated organelle movement. *Trends Cell Biol.* **24**, 61–72 (2014).
73. Quintana, A. et al. T cell activation requires mitochondrial translocation to the immunological synapse. *Proc. Natl Acad. Sci. Usa.* **104**, 14418–14423 (2007).
74. Butte, M. J., Stein, J. V. & Delon, J. The cytoskeleton in T cell migration and activation. *Front. Immunol.* **13**, 1057533 (2022).
75. Wang, N., Butler, J. P. & Ingber, D. E. Mechanotransduction across the cell surface and through the cytoskeleton. *Science* **260**, 1124–1127 (1993).
76. Shen, C. et al. A Dock8-dependent mechanosensitive central actin pool maintains T cell shape and protects the nucleus during migration. *Sci. Immunol.* **10**, eadt9239 (2025).
77. Douanne, T. & Griffiths, G. M. Cytoskeletal control of the secretory immune synapse. *Curr. Opin. Cell Biol.* **71**, 87–94 (2021).
78. Hammer, J. A., Wang, J. C., Saeed, M. & Pedrosa, A. T. Origin, organization, dynamics, and function of actin and actomyosin networks at the T cell immunological synapse. *Annu. Rev. Immunol.* **37**, 201–224 (2019).
79. Thauland, T. J., Hu, K. H., Bruce, M. A. & Butte, M. J. Cytoskeletal adaptivity regulates T cell receptor signaling. *Sci. Signal.* **10**, eaah3737 (2017).
80. Kumari, S. et al. Cytoskeletal tension actively sustains the migratory T-cell synaptic contact. *EMBO J.* **39**, e102783 (2020).
81. Basu, R. et al. Cytotoxic T cells use mechanical force to potentiate target cell killing. *Cell* **165**, 100–110 (2016).
82. Tello-Lafoz, M. et al. Cytotoxic lymphocytes target characteristic biophysical vulnerabilities in cancer. *Immunity* **54**, 1037–1054.e7 (2021).
83. Hwang, S.-M. et al. Transgelin 2 guards T cell lipid metabolism and antitumour function. *Nature* **635**, 1010–1018 (2024).
84. Combs, J. et al. Recruitment of dynein to the Jurkat immunological synapse. *Proc. Natl Acad. Sci. USA* **103**, 14883–14888 (2006).
85. Hooikaas, P. J. et al. Kinesin-4 KIF21B limits microtubule growth to allow rapid centrosome polarization in T cells. *eLife* **9**, e62876 (2020).
86. Scorrano, L. et al. Coming together to define membrane contact sites. *Nat. Commun.* **10**, 1287 (2019).
87. Voeltz, G. K., Sawyer, E. M., Hajnóczky, G. & Prinz, W. A. Making the connection: how membrane contact sites have changed our view of organelle biology. *Cell* **187**, 257–270 (2024).
88. Eisenberg-Bord, M., Shai, N., Schuldiner, M. & Bohnert, M. A tether is a tether is a tether: tethering at membrane contact sites. *Dev. Cell* **39**, 395–409 (2016).
89. Wong, Y. C., Ysselstein, D. & Krainc, D. Mitochondria–lysosome contacts regulate mitochondrial fission via RAB7 GTP hydrolysis. *Nature* **554**, 382–386 (2018).
90. Hein, M. Y. et al. Global organelle profiling reveals subcellular localization and remodeling at proteome scale. *Cell* **188**, 1137–1155.e20 (2025).
91. Booth, D. M., Várnai, P., Joseph, S. K. & Hajnóczky, G. Oxidative bursts of single mitochondria mediate retrograde signaling toward the ER. *Mol. Cell* **81**, 3866–3876.e3862 (2021).
92. DiGiovanni, L. F. et al. ROS transfer at peroxisome-mitochondria contact regulates mitochondrial redox. *Science* **389**, 157–162 (2025).
93. Wang, J. et al. Targeting membrane contact sites to mediate lipid dynamics: innovative cancer therapies. *Cell Commun. Signal.* **23**, 89 (2025).
94. Martinvalet, D. The role of the mitochondria and the endoplasmic reticulum contact sites in the development of the immune responses. *Cell Death Dis.* **9**, 336 (2018).
95. Bantug, G. R. et al. Mitochondria-endoplasmic reticulum contact sites function as immunometabolic hubs that orchestrate the rapid recall response of memory CD8$^+$ T cells. *Immunity* **48**, 542–555.e6 (2018).
96. Siddiqui, I. et al. Intratumoral Tcf1$^+$PD-1$^+$CD8$^+$ T cells with stem-like properties promote tumor control in response to vaccination and checkpoint blockade immunotherapy. *Immunity* **50**, 195–211.e10 (2019).
97. Sade-Feldman, M. et al. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* **175**, 998–1013.e20 (2018).
98. Gattinoni, L. et al. A human memory T cell subset with stem cell-like properties. *Nat. Med.* **17**, 1290–1297 (2011).
99. Wang, Y. et al. Characteristics of premanufacture CD8$^+$ T cells determine CAR-T efficacy in patients with diffuse large B-cell lymphoma. *Signal. Transduct. Target. Ther.* **8**, 409 (2023).
100. Sukumar, M. et al. Mitochondrial membrane potential identifies cells with enhanced stemness for cellular therapy. *Cell Metab.* **23**, 63–76 (2016).
101. Si, X. et al. Mitochondrial isocitrate dehydrogenase impedes CAR T cell function by restraining antioxidant metabolism and histone acetylation. *Cell Metab.* **36**, 176–192.e10 (2024).
A mitochondria-focused compound screening that identifies IDH2 as an enasidenib-targetable checkpoint; IDH2 limits T cell memory by suppressing antioxidant metabolism and histone acetylation.

102. Jaccard, A. et al. Reductive carboxylation epigenetically instructs T cell differentiation. *Nature* **621**, 849–856 (2023).
103. Wenes, M. et al. The mitochondrial pyruvate carrier regulates memory T-cell differentiation and antitumor function. *Cell Metab.* **34**, 731–746.e9 (2022).
104. Wenes, M. et al. A novel mitochondrial pyruvate carrier inhibitor drives stem cell-like memory CAR T cell generation and enhances antitumor efficacy. *Mol. Ther. Oncol.* **32**, 200897 (2024).
105. Hermans, D. et al. Lactate dehydrogenase inhibition synergizes with IL-21 to promote $CD8^+$ T cell stemness and antitumor immunity. *Proc. Natl Acad. Sci. USA* **117**, 6047–6055 (2020).
106. Frisch, A. T. et al. Redirecting glucose flux during in vitro expansion generates epigenetically and metabolically superior T cells for cancer immunotherapy. *Cell Metab.* **37**, 870–885.e8 (2025).
107. Galluzzi, L., Guilbaud, E. & Garg, A. D. Mitochondrial succinate feeds T cell exhaustion in cancer. *Cancer Cell* **43**, 168–170 (2025).
108. Funk, C. R. et al. PI3Kδ/γ inhibition promotes human CART cell epigenetic and metabolic reprogramming to enhance antitumor cytotoxicity. *Blood* **139**, 523–537 (2022).
109. Pilipow, K. et al. Antioxidant metabolism regulates $CD8^+$ T memory stem cell formation and antitumor immunity. *JCI Insight* **3**, e122299 (2018).
110. Gollapudi, S. & Gupta, S. Reversal of oxidative stress-induced apoptosis in T and B lymphocytes by Coenzyme Q10 (CoQ10). *Am. J. Clin. Exp. Immunol.* **5**, 41 (2016).
111. Finisguerra, V. et al. Metformin improves cancer immunotherapy by directly rescuing tumor-infiltrating CD8 T lymphocytes from hypoxia-induced immunosuppression. *J. Immunother. Cancer* **11**, e005719 (2023).
112. Watson, M. J. et al. Metabolic support of tumour-infiltrating regulatory T cells by lactic acid. *Nature* **591**, 645–651 (2021).
113. Ma, J. et al. Lithium carbonate revitalizes tumor-reactive $CD8^+$ T cells by shunting lactic acid into mitochondria. *Nat. Immunol.* **25**, 552–561 (2024).
This study provides deep mechanistic insights into how lithium carbonate modulates lysosomal function to counteract lactic acid-induced immunosuppression in the TME, making lactate available to $CD8^+$T cells as an alternative energy source.
114. Dong, W. et al. D-mannose targets PD-1 to lysosomal degradation and enhances T cell-mediated anti-tumor immunity. *Cancer Lett.* **591**, 216883 (2024).
115. Li, W. et al. Chimeric antigen receptor designed to prevent ubiquitination and downregulation showed durable antitumor efficacy. *Immunity* **53**, 456–470.e6 (2020).
116. Wang, H. et al. Tonic ubiquitylation controls T-cell receptor: CD3 complex expression during T-cell development. *EMBO J.* **29**, 1285–1298 (2010).
117. Katoh, Y. et al. Inhibition of stearoyl-CoA desaturase 1 (SCD1) enhances the antitumor T cell response through regulating β-catenin signaling in cancer cells and ER stress in T cells and synergizes with anti-PD-1 antibody. *J. Immunother. Cancer* **10**, e004616 (2022).
The study reveals a novel pharmacological approach to alleviate endoplasmic reticulum stress in T cells by inhibiting SCD1, thereby enhancing their antitumour efficacy.
118. Hurst, K. E. et al. Endoplasmic reticulum stress contributes to mitochondrial exhaustion of $CD8^+$ T cells. *Cancer Immunol. Res.* **7**, 476–486 (2019).
119. Tabdanov, E. D. et al. Engineering T cells to enhance 3D migration through structurally and mechanically complex tumor microenvironments. *Nat. Commun.* **12**, 2815 (2021).
120. von Heijne, G. Mitochondrial targeting sequences may form amphiphilic helices. *EMBO J.* **5**, 1335–1342 (1986).
121. Braulke, T. & Bonifacino, J. S. Sorting of lysosomal proteins. *Biochim. Biophys. Acta Mol. Cell Res.* **1793**, 605–614 (2009).
122. Gould, S. J., Keller, G.-A., Hosken, N., Wilkinson, J. & Subramani, S. A conserved tripeptide sorts proteins to peroxisomes. *J. Cell Biol.* **108**, 1657–1664 (1989).
123. Griffiths, G. et al. Localization of the Lys, Asp, Glu, Leu tetrapeptide receptor to the Golgi complex and the intermediate compartment in mammalian cells. *J. Cell Biol.* **127**, 1557–1574 (1994).
124. Navarro, A. P. & Cheeseman, I. M. Identification of a Golgi-localized peptide reveals a minimal Golgi-targeting motif. *Mol. Biol. Cell.* **33**, ar110 (2022).
125. Yang, J., Griffin, A., Qiang, Z. & Ren, J. Organelle-targeted therapies: a comprehensive review on system design for enabling precision oncology. *Signal. Transduct. Target. Ther.* **7**, 379 (2022).
126. Kang, Y. C. et al. Cell-penetrating artificial mitochondria-targeting peptide-conjugated metallothionein 1A alleviates mitochondrial damage in Parkinson's disease models. *Exp. Mol. Med.* **50**, 1–13 (2018).
127. Yu, H. et al. Gene delivery to mitochondria by targeting modified adenoassociated virus suppresses Leber's hereditary optic neuropathy in a mouse model. *Proc. Natl Acad. Sci. USA* **109**, E1238–E1247 (2012).
128. Chuah, J.-A., Matsugami, A., Hayashi, F. & Numata, K. Self-assembled peptide-based system for mitochondrial-targeted gene delivery: functional and structural insights. *Biomacromolecules* **17**, 3547–3557 (2016).
129. Fu, Y. et al. Engineering mtDNA deletions by reconstituting end joining in human mitochondria. *Cell* **188**, 2778–2793.e21 (2025).
130. Escrig-Larena, J. I., Delgado-Pulido, S. & Mittelbrunn, M. Mitochondria during T cell aging. *Semin. Immunol.* **69**, 101808 (2023).
131. Das, R. K., O'Connor, R. S., Grupp, S. A. & Barrett, D. M. Lingering effects of chemotherapy on mature T cells impair proliferation. *Blood Adv.* **4**, 4653–4664 (2020).
132. Kam, W. W. & Banati, R. B. Effects of ionizing radiation on mitochondria. *Free Radic. Biol. Med.* **65**, 607–619 (2013).
133. Zhao, Q. et al. Target reprogramming lysosomes of $CD8^+$ T cells by a mineralized metal-organic framework for cancer immunotherapy. *Adv. Mater.* **33**, e2100616 (2021).
134. Sheikh, F. G., Pahan, K., Khan, M., Barbosa, E. & Singh, I. Abnormality in catalase import into peroxisomes leads to severe neurological disorder. *Proc. Natl Acad. Sci. USA* **95**, 2961–2966 (1998).
135. Bhattacharyya, S. & Saha, J. Tumour, oxidative stress and host T cell response: cementing the dominance. *Scand. J. Immunol.* **82**, 477–488 (2015).
136. Shah, R., Ibis, B., Kashyap, M. & Boussiotis, V. A. The role of ROS in tumor infiltrating immune cells and cancer immunotherapy. *Metab. Clin. Exp.* **151**, 155747 (2024).
137. Shi, Y. et al. A vaccination with boosted cross presentation by ER-targeted antigen delivery for anti-tumor immunotherapy. *Adv. Healthc. Mater.* **10**, e2001934 (2021).
138. Huang, Y. et al. The involvement of the mitochondrial membrane in drug delivery. *Acta Biomater.* **176**, 28–50 (2024).
139. Malhotra, J. D. & Kaufman, R. J. Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? *Antioxid. Redox Signal.* **9**, 2277–2294 (2007).
140. He, X., Li, J., An, S. & Jiang, C. pH-sensitive drug-delivery systems for tumor targeting. *Ther. Deliv.* **4**, 1499–1510 (2013).
141. Siska, P. J. et al. Mitochondrial dysregulation and glycolytic insufficiency functionally impair CD8 T cells infiltrating human renal cell carcinoma. *JCI Insight* **2**, e93411 (2017).
142. Lee, J. & Song, C. H. Effect of reactive oxygen species on the endoplasmic reticulum and mitochondria during intracellular pathogen infection of mammalian cells. *Antioxidants* **10**, 872 (2021).
143. Zhao, Y. et al. Pathogen infection-responsive nanoplatform targeting macrophage endoplasmic reticulum for treating life-threatening systemic infection. *Nano Res.* **15**, 6243–6255 (2022).
144. Dumauthioz, N. et al. Enforced PGC-1α expression promotes CD8 T cell fitness, memory formation and antitumor immunity. *Cell. Mol. Immunol.* **18**, 1761–1771 (2021).
145. Lontos, K. et al. Metabolic reprogramming via an engineered PGC-1α improves human chimeric antigen receptor T-cell therapy against solid tumors. *J. Immunother. Cancer* **11**, e006522 (2023).
146. Pretto, S. et al. A functional single-cell metabolic survey identifies Elovl1 as a target to enhance $CD8^+$ T cell fitness in solid tumours. *Nat. Metab.* **7**, 508–530 (2025).
147. Huang, H. et al. In vivo CRISPR screening reveals nutrient signaling processes underpinning $CD8^+$ T cell fate decisions. *Cell* **184**, 1245–1261.e21 (2021).
148. Woodsworth, D. J., Dreolini, L., Abraham, L. & Holt, R. A. Targeted cell-to-cell delivery of protein payloads via the granzyme-perforin pathway. *Mol. Ther. Methods Clin. Dev.* **7**, 132–145 (2017).
149. Huang, B. et al. Designed endocytosis-inducing proteins degrade targets and amplify signals. *Nature* **638**, 796–804 (2025).
150. Piraner, D. I. et al. Engineered receptors for soluble cellular communication and disease sensing. *Nature* **638**, 805–813 (2025).
151. Tavassolifar, M. J., Vodjgani, M., Salehi, Z. & Izad, M. The influence of reactive oxygen species in the immune system and pathogenesis of multiple sclerosis. *Autoimmune Dis.* **2020**, 5793817 (2020).
152. Bai, R. & Cui, J. Mitochondrial immune regulation and anti-tumor immunotherapy strategies targeting mitochondria. *Cancer Lett.* **564**, 216223 (2023).
153. Ali Hosseini Rad, S. M., Poudel, A., Tan, G. M. Y. & McLellan, A. D. Optimisation of Tet-On inducible systems for sleeping beauty-based chimeric antigen receptor (CAR) applications. *Sci. Rep.* **10**, 13125 (2020).
154. Morciano, G. et al. Intersection of mitochondrial fission and fusion machinery with apoptotic pathways: role of Mcl-1. *Biol. Cell* **108**, 279–293 (2016).
155. Kim, E. H., Neldner, B., Gui, J., Craig, R. W. & Suresh, M. Mcl-1 regulates effector and memory CD8 T-cell differentiation during acute viral infection. *Virology* **490**, 75–82 (2016).
156. Tripathi, P., Koss, B., Opferman, J. T. & Hildeman, D. A. Mcl-1 antagonizes Bax/Bak to promote effector $CD4^+$ and $CD8^+$ T-cell responses. *Cell Death Differ.* **20**, 998–1007 (2013).
157. Chatzispyrou, I. A., Held, N. M., Mouchiroud, L., Auwerx, J. & Houtkooper, R. H. Tetracycline antibiotics impair mitochondrial function and its experimental use confounds research. *Cancer Res.* **75**, 4446–4449 (2015).
158. Moullan, N. et al. Tetracyclines disturb mitochondrial function across eukaryotic models: a call for caution in biomedical research. *Cell Rep.* **10**, 1681–1691 (2015).
159. Ottina, E. et al. DNA-binding of the Tet-transactivator curtails antigen-induced lymphocyte activation in mice. *Nat. Commun.* **8**, 1028 (2017).
160. Schmitt, A., Schulze-Osthoff, K. & Hailfinger, S. Correspondence: T cells are compromised in tetracycline transactivator transgenic mice. *Cell Death Differ.* **25**, 634–636 (2018).
161. Li, H. S. et al. Multidimensional control of therapeutic human cell function with synthetic gene circuits. *Science* **378**, 1227–1234 (2022).
162. Tang, L., Pan, S., Wei, X., Xu, X. & Wei, Q. Arming CAR-T cells with cytokines and more: innovations in the fourth-generation CAR-T development. *Mol. Ther.* **31**, 3146–3162 (2023).
163. Greenshpan, Y. et al. Synthetic promoters to induce immune-effectors into the tumor microenvironment. *Commun. Biol.* **4**, 143 (2021).
164. He, H. et al. Conditioned CAR-T cells by hypoxia-inducible transcription amplification (HiTA) system significantly enhances systemic safety and retains antitumor efficacy. *J. Immunother. Cancer* **9**, e002755 (2021).

165. Bacman, S. R., Williams, S. L., Pinto, M., Peralta, S. & Moraes, C. T. Specific elimination of mutant mitochondrial genomes in patient-derived cells by mitoTALENs. *Nat. Med.* **19**, 1111–1113 (2013).
166. Gammage, P. A., Rorbach, J., Vincent, A. I., Rebar, E. J. & Minczuk, M. Mitochondrially targeted ZFNs for selective degradation of pathogenic mitochondrial genomes bearing large-scale deletions or point mutations. *EMBO Mol. Med.* **6**, 458–466 (2014).
167. Mok, B. Y. et al. A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing. *Nature* **583**, 631–637 (2020).
168. Cho, S. I. et al. Targeted A-to-G base editing in human mitochondrial DNA with programmable deaminases. *Cell* **185**, 1764–1776.e12 (2022).
169. Schmiderer, L., Yudovich, D., Oburoglu, L., Hjort, M. & Larsson, J. Site-specific CRISPR-based mitochondrial DNA manipulation is limited by gRNA import. *Sci. Rep.* **12**, 18687 (2022).
170. Zhang, X. et al. Precise modelling of mitochondrial diseases using optimized mitoBEs. *Nature* **639**, 735–745 (2025).
mtDNA editing had previously been constrained to nuclease-based methods; the studies by Mok et al. (2020), Cho et al. (2022) and Zhang et al. (2025) demonstrate precise, non-destructive base editing as a breakthrough alternative.
171. Spees, J. L., Olson, S. D., Whitney, M. J. & Prockop, D. J. Mitochondrial transfer between cells can rescue aerobic respiration. *Proc. Natl Acad. Sci. USA* **103**, 1283–1288 (2006).
172. Borcherding, N. & Brestoff, J. R. The power and potential of mitochondria transfer. *Nature* **623**, 283–291 (2023).
173. Berridge, M. V. et al. Horizontal mitochondrial transfer in cancer biology: potential clinical relevance. *Cancer Cell* **43**, 803–807 (2025).
174. Nakai, R. et al. Mitochondria transfer-based therapies reduce the morbidity and mortality of Leigh syndrome. *Nat. Metab.* **6**, 1886–1896 (2024).
175. Nicolás-Ávila, J. A. et al. A network of macrophages supports mitochondrial homeostasis in the heart. *Cell* **183**, 94–109.e23 (2020).
176. Hoover, G. et al. Nerve-to-cancer transfer of mitochondria during cancer metastasis. *Nature* **644**, 252–262 (2025).
177. Saha, T. et al. Intercellular nanotubes mediate mitochondrial trafficking between cancer and immune cells. *Nat. Nanotechnol.* **17**, 98–106 (2022).
178. Zhang, H. et al. Systematic investigation of mitochondrial transfer between cancer cells and T cells at single-cell resolution. *Cancer Cell* **41**, 1788–1802.e10 (2023).
179. Ikeda, H. et al. Immune evasion through mitochondrial transfer in the tumour microenvironment. *Nature* **638**, 225–236 (2025).
180. Baldwin, J. G. et al. Intercellular nanotube-mediated mitochondrial transfer enhances T cell metabolic fitness and antitumor efficacy. *Cell* **187**, 6614–6630.e21 (2024).
Studies by Ikeda et al. (2025) and Baldwin et al. (2024) highlight the dynamic mitochondrial exchange between T cells and mesenchymal stromal cells or tumour cells, unveiling new therapeutic opportunities to enhance T cell fitness and antitumour immunity.
181. Court, A. C. et al. Survival advantage of native and engineered T cells is acquired by mitochondrial transfer from mesenchymal stem cells. *J. Transl. Med.* **22**, 868 (2024).
182. Lin, R.-Z. et al. Mitochondrial transfer mediates endothelial cell engraftment through mitophagy. *Nature* **629**, 660–668 (2024).
183. Durose, W. et al. First documentation of cell-to-cell peroxisome transfer in hematopoietic stem and progenitor cells: a method to relieve stress? *Blood* **140**, 1686–1687 (2022).
184. Kumar, S. et al. Programmed exosome fusion for energy generation in living cells. *Nat. Catal.* **4**, 763–774 (2021).
185. Wang, J. et al. Human neural stem cell-derived artificial organelles to improve oxidative phosphorylation. *Nat. Commun.* **15**, 7855 (2024).
186. Oerlemans, R. A., Timmermans, S. B. & van Hest, J. C. Artificial organelles: towards adding or restoring intracellular activity. *ChemBioChem* **22**, 2051–2078 (2021).
187. Simmel, F. C. Synthetic organelles. *Emerg. Top. Life Sci.* **3**, 587–595 (2019).
188. Van Oppen, L. M. et al. Biodegradable synthetic organelles demonstrate ROS shielding in human-complex-I-deficient fibroblasts. *ACS Cent. Sci.* **4**, 917–928 (2018).
189. Yang, S. et al. Construction of liposome-based extracellular artificial organelles on individual living cells. *Angew. Chem. Int. Ed. Engl.* **64**, e202415823 (2025).
190. Sigmund, F. et al. Bacterial encapsulins as orthogonal compartments for mammalian cell engineering. *Nat. Commun.* **9**, 1990 (2018).
This study provides compelling proof-of-concept for a 'bottom-up' approach to genetically encode synthetic organelles that can self-assemble within the cell, enabling applications such as molecular imaging and beyond.
191. Kawalekar, O. U. et al. Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells. *Immunity* **44**, 380–390 (2016).
192. Ogando, J. et al. PD-1 signaling affects cristae morphology and leads to mitochondrial dysfunction in human $CD8^+$ T lymphocytes. *J. Immunother. Cancer* **7**, 151 (2019).
193. Paillon, N. et al. PD-1 inhibits T cell actin remodeling at the immunological synapse independently of its signaling motifs. *Sci. Signal.* **16**, eadh2456 (2023).
194. van der Windt, G. J. et al. Mitochondrial respiratory capacity is a critical regulator of $CD8^+$ T cell memory development. *Immunity* **36**, 68–78 (2012).
195. Loschinski, R. et al. IL-21 modulates memory and exhaustion phenotype of T-cells in a fatty acid oxidation-dependent manner. *Oncotarget* **9**, 13125–13138 (2018).
196. Guo, Y. et al. Metabolic reprogramming of terminally exhausted $CD8^+$ T cells by IL-10 enhances anti-tumor immunity. *Nat. Immunol.* **22**, 746–756 (2021).
197. Monson, K. R. et al. Inherited mitochondrial genetics as a predictor of immune checkpoint inhibition efficacy in melanoma. *Nat. Med.* **31**, 2385–2396 (2025).
198. Henning, A. N., Roychoudhuri, R. & Restifo, N. P. Epigenetic control of $CD8^+$ T cell differentiation. *Nat. Rev. Immunol.* **18**, 340–356 (2018).
199. Tavakoli, A., Hu, S., Ebrahim, S. & Kachar, B. Hemifusomes and interacting proteolipid nanodroplets mediate multi-vesicular body formation. *Nat. Commun.* **16**, 4609 (2025).
200. Heinrich, L. et al. Whole-cell organelle segmentation in volume electron microscopy. *Nature* **599**, 141–146 (2021).
This study introduces deep learning models capable of registering 35 organelle classes in microscopy images across diverse resolutions and cell types, with open-source tools made publicly available for broader research use.
201. Lu, C. et al. Diffusion-based deep learning method for augmenting ultrastructural imaging and volume electron microscopy. *Nat. Commun.* **15**, 4677 (2024).
202. Alon, S. et al. Expansion sequencing: spatially precise in situ transcriptomics in intact biological systems. *Science* **371**, eaax2656 (2021).
203. Louvel, V. et al. iU-ExM: nanoscopy of organelles and tissues with iterative ultrastructure expansion microscopy. *Nat. Commun.* **14**, 7893 (2023).
204. Lemaître, F. et al. Unveiling the molecular architecture of T cells and immune synapses with cryo-expansion microscopy. Preprint at *bioRxiv* https://doi.org/10.1101/2025.04.15.648816 (2025).
205. Büttner, M. et al. Challenges of using expansion microscopy for super-resolved imaging of cellular organelles. *ChemBioChem* **22**, 686–693 (2021).
206. Zimmermann, J. A. et al. Functional multi-organelle units control inflammatory lipid metabolism of macrophages. *Nat. Cell Biol.* **26**, 1261–1273 (2024).
207. Valm, A. M. et al. Applying systems-level spectral imaging and analysis to reveal the organelle interactome. *Nature* **546**, 162–167 (2017).
208. Lefebvre, A. E. Y. T. et al. Nellie: automated organelle segmentation, tracking and hierarchical feature extraction in 2D/3D live-cell microscopy. *Nat. Methods* **22**, 751–763 (2025).
209. Garcia Romeu, H., Deville, S. & Salvati, A. Time- and space-resolved flow-cytometry of cell organelles to quantify nanoparticle uptake and intracellular trafficking by cells. *Small* **17**, e2100887 (2021).
210. Schraivogel, D. et al. High-speed fluorescence image-enabled cell sorting. *Science* **375**, 315–320 (2022).
This work redefines live-cell sorting by introducing high-speed subcellular cytometry as a tool for profiling and selecting cells based on organelle morphometry.
211. Song, D. et al. Identification of an endoplasmic reticulum stress-related gene signature to evaluate the immune status and predict the prognosis of hepatocellular carcinoma. *Front. Genet.* **13**, 850200 (2022).
212. Huang, Y. et al. Characterizing cancer metabolism from bulk and single-cell RNA-seq data using METAFlux. *Nat. Commun.* **14**, 4883 (2023).
213. Jiang, A., Lyu, C. & Zhao, Y. Predicting T cell mitochondria hijacking from tumor single-cell RNA sequencing data with MitoR. *Mathematics* **13**, 673 (2025).
214. Liu, W., He, H. & Chicco, D. Gene signatures for cancer research: a 25-year retrospective and future avenues. *PLoS Comput. Biol.* **20**, e1012512 (2024).
215. Caicedo, A. et al. MitoCeption as a new tool to assess the effects of mesenchymal stem/stromal cell mitochondria on cancer cell metabolism and function. *Sci. Rep.* **5**, 9073 (2015).
216. Patananan, A. N. et al. Pressure-driven mitochondrial transfer pipeline generates mammalian cells of desired genetic combinations and fates. *Cell Rep.* **33**, 108562 (2020).
217. Nakano, T., Nakamura, Y., Park, J.-H., Tanaka, M. & Hayakawa, K. Mitochondrial surface coating with artificial lipid membrane improves the transfer efficacy. *Commun. Biol.* **5**, 745 (2022).
218. Gäbelein, C. G. et al. Mitochondria transplantation between living cells. *PLoS Biol.* **20**, e3001576 (2022).
219. Zhong, Z. et al. New mitochondrial DNA synthesis enables NLRP3 inflammasome activation. *Nature* **560**, 198–203 (2018).
220. Ikeda, G. et al. Mitochondria-rich extracellular vesicles from autologous stem cell–derived cardiomyocytes restore energetics of ischemic myocardium. *J. Am. Coll. Cardiol.* **77**, 1073–1088 (2021).
221. Marcoux, G. et al. Platelet-derived extracellular vesicles convey mitochondrial DAMPs in platelet concentrates and their levels are associated with adverse reactions. *Transfusion* **59**, 2403–2414 (2019).
222. Suh, J. et al. Mitochondrial fragmentation and donut formation enhance mitochondrial secretion to promote osteogenesis. *Cell Metab.* **35**, 345–360.e7 (2023).
223. Rosina, M. et al. Ejection of damaged mitochondria and their removal by macrophages ensure efficient thermogenesis in brown adipose tissue. *Cell Metab.* **34**, 533–548.e12 (2022).
224. Hayakawa, K. et al. Transfer of mitochondria from astrocytes to neurons after stroke. *Nature* **535**, 551–555 (2016).
225. van der Vlist, M. et al. Macrophages transfer mitochondria to sensory neurons to resolve inflammatory pain. *Neuron* **110**, 613–626.e9 (2022).
226. Peruzzotti-Jametti, L. et al. Neural stem cells traffic functional mitochondria via extracellular vesicles. *PLoS Biol.* **19**, e3001166 (2021).
227. Wu, S. et al. Extracellular vesicles meet mitochondria: potential roles in regenerative medicine. *Pharmacol. Res.* **206**, 107307 (2024).

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J.G.B., C.H.-L. and L.G. conceived and wrote the manuscript. J.G.B. and C.H.-L. prepared the figures. L.G. contributed to refining the figures. All authors have read and approved the article.

Competing interests
J.G.B. and L.G. have a patent application for the use of mitochondrial transfer technology in cancer immunotherapies. L.G. has consulting agreements with Lyell Immunopharma. L.G. is a scientific advisor and a stockholder of CellRep. C.H.-L. declares no competing interests.

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