



Mitochondrial damage drives T-cell immunometabolic paralysis after major surgery

Simon Hirschberger^{ID 1,2}, Martin B Müller^{1,2}, Hannah Mascolo¹, Melissa Seitz^{ID 1}, Stefan Nibler¹, David Effinger^{ID 1,2}, Kun Lu^{ID 3,4}, Joscha Büch³, Martin Bender^{ID 2}, Tobias Kammerer¹, Sven Peterß³, Karin Kleigrew⁵, Miriam Abele^{ID 5}, Teresa Barth⁶, Olga Kushnir^{1,2}, Axel Imhof^{ID 6}, Steffen Dietzel^{ID 7,8}, Bernd Wegener⁹, Ralf Sowa^{ID 10}, Frank Vogel¹¹, Peter Lamm¹¹, Roland Tomasi^{ID 2}, Kristian Unger¹², Markus Sperandio^{ID 8}, Erich Kilger², Simone Kreth^{ID 1,2}✉ & Max Hübner^{ID 1,2}

Abstract

Cytotoxic T cell (CTL) dysfunction is a hallmark of immune paralysis after major surgery, increasing susceptibility to severe nosocomial infections and contributing to mortality in critically ill patients. The mechanisms remain poorly understood. We demonstrate that reactive oxygen species (ROS) released by myeloid-derived suppressor cells (MDSC) transiently emerging after surgery, drive perioperative CTL immunoparalysis. These ROS damage CTL mitochondria, triggering secondary mitochondrial ROS amplification and overwhelming antioxidant defenses. The resulting oxidative cascade impairs oxidative phosphorylation and suppresses CTL effector function. Concurrently, stress-induced mitochondrial hyperfusion disrupts fission-dependent translocation to the immunological synapse, exacerbating bioenergetic failure. MitoTEMPO, a mitochondria-targeted antioxidant, partially mitigates these effects, highlighting mitochondrial stabilization as a potential strategy to prevent perioperative immune dysfunction.

Keywords Cytotoxic T cells; Mitochondria; Immunometabolism; Major Surgery; Immunosuppression

Subject Categories Immunology; Metabolism; Organelles

<https://doi.org/10.1038/s44321-025-00324-1>

Received 13 May 2025; Revised 29 September 2025;

Accepted 7 October 2025

Published online: 3 November 2025

Introduction

Modern surgery has undergone significant advancement in recent decades, becoming a highly specialized and effective medical tool that benefits millions of patients annually. However, its success is still significantly compromised by postoperative complications,

largely attributable to immunological dysregulation (Dobson, 2020). Surgical procedures inevitably cause tissue trauma, disrupting the body's natural defenses and triggering the release of damage-associated molecular patterns (DAMPs) from injured cells (Relja and Land, 2020). These DAMPs activate pattern recognition receptors (PRRs) on immune cells, initiating an inflammatory response. Both the acute tissue damage and the resulting immunological imbalance can lead to a systemic inflammatory response syndrome (SIRS) (Vourc'h et al, 2018). This condition not only contributes to postoperative morbidity and mortality but also promotes a prolonged state of immunosuppression, which is characterized by impaired immune responses and increased vulnerability to infections (Papadopoulos et al, 2017; Xiao et al, 2011; Margraf et al, 2020). Consequently, the postoperative immune system becomes less capable of combating future challenges, which hampers recovery and increases the risk of complications for the patient (Rankin et al, 2011; Cornell et al, 2012). This is highlighted by nosocomial infection rates ranging from 25 to 35% after major surgery (Hadaya et al, 2022; Jannasch et al, 2015). Indeed, this immunosuppressive state is believed to be a leading cause of death among critically ill patients (Boomer et al, 2011; Bain et al, 2023). The mechanisms underlying perioperative immune dysfunction remain largely unclear, and currently, there are no reliable predictive markers or therapeutic options available.

T-cell immune dysfunction is a hallmark of prolonged perioperative immunosuppression (postoperative immunosuppression of T cells, PITC), with human cytotoxic CD8⁺ T cells (CTL) being particularly susceptible (Franke et al, 2006; Markewitz et al, 1996, 1993; Albertsmeier et al, 2015; Sun et al, 2017; Ananth et al, 2016; Menges et al, 2012; Torrance et al, 2016; Pérez-Granda et al, 2024). As a result, these cells exhibit suppressed interferon signaling, impaired cytokine production, and reduced synthesis of cytotoxic proteins (Bain et al, 2023; Menges et al, 2012; Torrance et al, 2018; Ananth et al, 2016; Hotchkiss et al, 2013). This T-cell

¹Walter Brendel Center of Experimental Medicine, Research Unit Immune Function and Immune Metabolism, Ludwig-Maximilian-University (LMU), Munich, Germany.

²Department of Anaesthesiology, LMU University Hospital, LMU, Munich, Germany. ³Department of Cardiac Surgery, LMU University Hospital, LMU, Munich, Germany. ⁴DZHK (German Center for Cardiovascular Research), Partner Site Munich Heart Alliance, Munich, Germany. ⁵Bavarian Center for Biomolecular Mass Spectrometry, TUM School of Life Sciences, Technical University of Munich, Freising, Germany. ⁶Protein Analysis Unit, BioMedical Center, Faculty of Medicine, LMU, Martinsried, Germany. ⁷Core Facility Bioimaging and Walter Brendel Center of Experimental Medicine, Biomedical Center, LMU, Munich, Germany. ⁸Institute of Cardiovascular Physiology and Pathophysiology, Walter Brendel Center for Experimental Medicine, LMU, Munich, Germany. ⁹Department of Orthopaedic Surgery, Physical Medicine and Rehabilitation, LMU University Hospital, LMU, Munich, Germany. ¹⁰Department of Anesthesiology and Intensive Care Medicine, University Medical Center Goettingen, Goettingen, Germany. ¹¹Artemed Hospital Munich South, Munich, Germany. ¹²Department of Radiotherapy and Radiooncology, LMU University Hospital, LMU, Munich, Germany. ✉E-mail: simone.kreth@med.uni-muenchen.de

immunoparalysis results in an inability to effectively clear pathogens and an increased vulnerability to secondary infections (Huang et al, 2022; Jensen et al, 2018). Despite these well-documented consequences, the mechanisms driving T-cell dysfunction remain poorly understood. Given the critical importance of perioperative immunological complications, it is essential to deepen our understanding of this phenomenon.

We employed a multi-omics screening approach to identify the mechanisms underlying CTL immunosuppression following major surgery. Our data show that perioperative T-cell dysfunction is linked to systemic oxidative stress induced by transiently emerging myeloid-derived suppressor cells (MDSC). Functional mitochondrial analyses revealed that ROS released by MDSC increase ROS levels in CTL, thereby compromising mitochondrial integrity, inducing mitochondrial hyperfusion and impairing mitochondrial translocation to the immunological synapse (IS), ultimately leading to bioenergetic dysfunction of CTL.

Results

Post surgical immunosuppression (PITC) of CTL after major surgery

Immune-related postoperative complications significantly impact patient outcomes. To assess postoperative immunosuppression following major surgery, we enrolled patients scheduled for cardiothoracic (aortocoronary bypass, aortic arch replacement) and major orthopedic procedures (polysegmental spinal stabilization) and analyzed CTL at three time points (Fig. 1A): preoperatively (T1), postoperatively (T2), and on the first day after surgery (T3). Patients with preexisting conditions associated with immune dysregulation, such as cancer, autoimmune diseases, immunosuppressive therapy or active infections were excluded from the study.

At T2, CTL exhibited a dysfunctional phenotype. After T-cell-specific stimulation, transcript levels of interferon gamma (*IFNG*), granzyme B (*GZMB*), and perforin 1 (*PRF1*) mRNA were substantially reduced (Fig. 1B). Intracellular IFN- γ protein expression was significantly decreased, and IFN- γ secretion was almost completely abrogated (Fig. 1C,D). We next evaluated the cytotoxic function of CTL using Electric Cell-Substrate Impedance Sensing (ECIS) and a flow-cytometry based killing assay, revealing a significantly impaired cell lysis capacity of T2 CTL (Figs. 1E and EV1A). To assess whether the observed functional impairments were also reflected in classical activation and exhaustion markers, we compared their expression at time points T1 and T2. As expected — given the short time interval — canonical exhaustion markers remained unchanged. (Fig. EV1B). Similarly, the activation markers CD25 and CD69 showed no differences between time points (Fig. EV1B). Notably, only CD38 was significantly upregulated following major surgery (Fig. 1F), suggesting a possible link to impaired cellular metabolism (Ghosh et al, 2023).

Consistent with their reduced cytolytic capacity, T2 CTL also induced lower caspase activity in K562 cells, also suggesting diminished GZMB-dependent activation of apoptotic pathways in target cells (Fig. EV1D). In line with this finding, secretion of GZMB and PRF1 by CTL was markedly diminished (Fig. 1G). Unexpectedly, T2 CTL exhibited elevated intracellular levels of GZMB and PRF1 (Fig. 1H/I). This led us to speculate that the

transfer of cytotoxic granules (CG) to the immunological synapse and their subsequent exocytosis may be impaired, causing the retention of GZMB and PRF1 within the cell. Consistent with this, quantification of CG exocytosis, measured by plasma membrane incorporation of CD107a, showed a significant reduction in CD107a⁺ CTL at T2 (Fig. 1J). Taken together, these results demonstrate that, after major surgery, CTL display severe immunosuppression (^{PITC}CTL), which may contribute to postoperative immune-related morbidity and mortality.

Multi-omics approach reveals severe oxidative stress signature in patients after major surgery

To elucidate potential mechanisms underlying the observed ^{PITC}CTL, we performed a comprehensive analysis of patient sera using proteomics and metabolomics. Compared to T1, serum proteomes at T2 and on the first postoperative day (T3) were profoundly altered with >100 proteins exhibiting significantly differential expression, which formed clearly distinguishable clusters (Fig. EV2A). As expected, several acute-phase proteins (CRP, LTA4H, LBP, and HSPA1B) were strongly induced, indicating a systemic inflammatory state (Fig. EV2B). Moreover, we detected significantly higher expression of numerous proteins related to redox homeostasis (TALDO1, PRDX1/2/6, SELENBP1, BLVRB, and AHCY; Fig. 2A) and to the oxidative stress response (PARK7, MPO, GSTO1, GSS, GCLC, CAT, and ADH1B; Fig. 2A).

LC-MS/MS-based untargeted metabolomic analyses (TOF-MS) of human sera revealed profound changes to the metabolic fingerprint following major surgery (Figs. 2B–D and EV2C). Metabolite quantification showed reduced levels of protective metabolites, such as Trigonelline, Aminobenzamide, Bilirubin, and Citrulline, as well as metabolites involved in DNA repair (Fig. 2E). In contrast, metabolites associated with ROS production and lipid peroxidation, including Decanoylcarnitine and oxidized N-acetylcysteine (Diacetylcysteine), were significantly increased post-surgery (Fig. 2E). Functional analysis highlighted the enrichment of pathways related to redox processes and mitochondrial functions (Fig. 2F,G). Joint pathway integration of proteomic and metabolomic data corroborated changes to metabolism and redox homeostasis (Fig. 2H). These findings indicate that major surgery induces a distinct serum signature reflecting systemic oxidative stress at both metabolomic and proteomic levels.

MDSC as the predominant source of reactive oxygen species (ROS) after major surgery

We next investigated whether the observed oxidative stress following PITC could be attributed to the activation of immune cells. To address this, we quantified transcript levels of the two key ROS-producing enzymes, NADPH oxidase 2 (*NOX2*) and myeloperoxidase (*MPO*) (Winterbourn et al, 2016), in freshly isolated lymphocytes, monocytes, and neutrophils. Neither CD4⁺ nor CD8⁺ T-cells or monocytes showed upregulation of ROS-producing enzymes (Fig. 3A,B). Even neutrophils, typically known for their ROS-producing capacity, exhibited only a slight increase in *NOX2* and *MPO* mRNA. However, a marked upregulation of both enzymes was observed in the overall PBMC population, suggesting that a previously unrecognized cell entity emerging acutely after major surgery may be responsible for the systemic oxidative stress response.

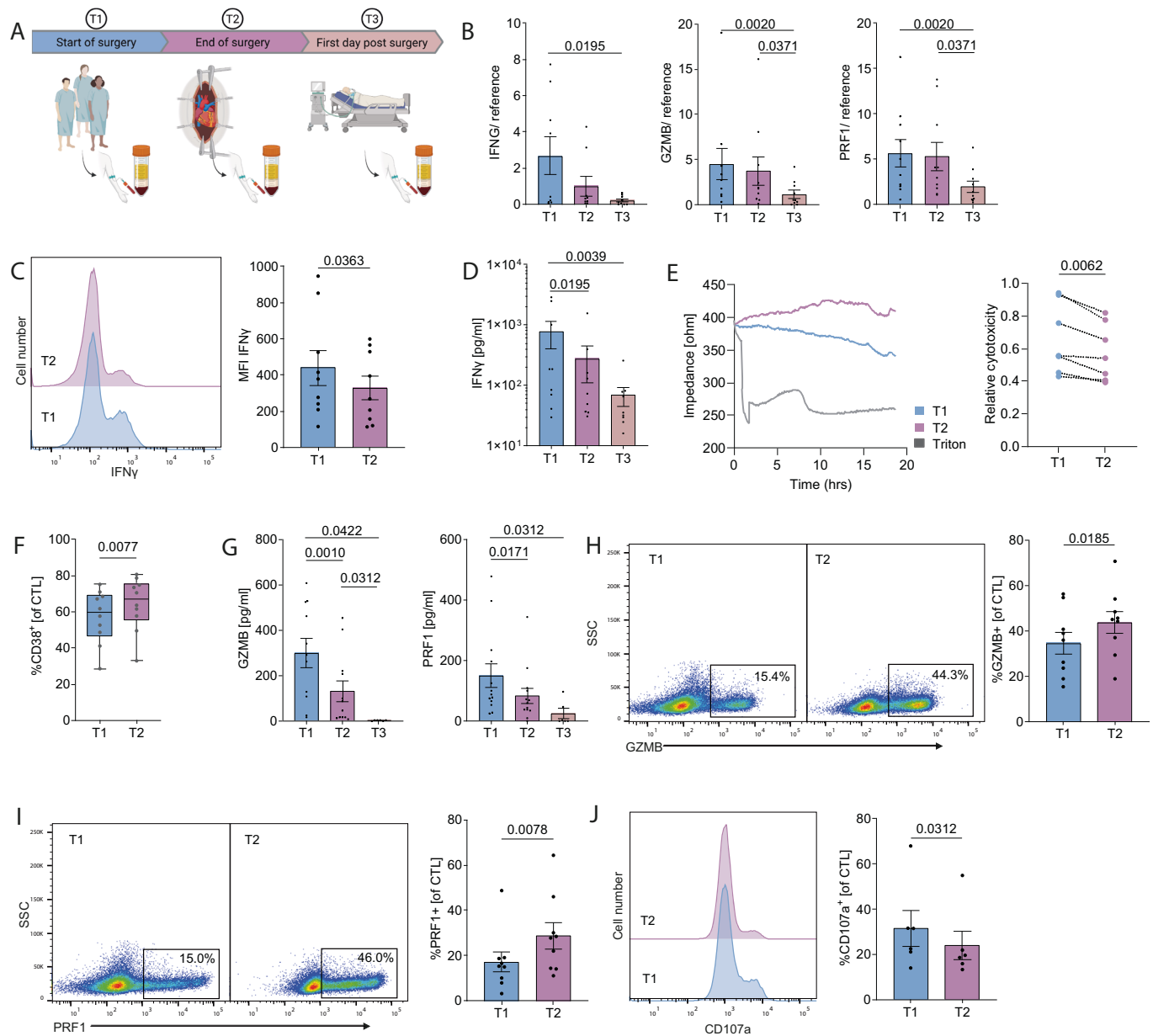


Figure 1. Post surgical immunosuppression (PITC) of CTL.

(A) Scheme of the study flow and time points of blood withdrawal during the course of major surgery. (B) mRNA expression of *IFNG* (left), *GZMB* (middle) and *PRF1* (right) in CTL (+CD3/CD28 dynabeads, stimulated for 18 h) relative to endogenous controls, $n = 9/10/10$ individual patients. (C) Flow cytometric analysis (FCA) of intracellular IFN- γ staining and quantification in CTL (+CD3/CD28 dynabeads, stimulated for 18 h; re-stimulation with PMA/Ionomycin for 4 h), $n = 9$ individual patients. (D) Quantification of IFN- γ secretion by CTL (+CD3/CD28 dynabeads, stimulated for 18 h), $n = 9$ individual patients. (E) Representative impedance plot (left) and quantification of relative CTL cytotoxicity (right) using ECIS, $n = 7$ individual patients. (F) FCA of CD38 staining and quantification in CTL, minimum = 28.6/32.9, 25%-percentile = 46.73/55.65, median = 59.65/67.05, 75%-percentile = 69.10/75.75, maximum = 75.10/80.60 (T1/T2), $n = 10$ individual patients. (G) Quantification of GZMB (left) and PRF1 (right) secretion by CTL (+CD3/CD28 dynabeads, stimulated for 18 h) as measured by ELISA, $n = 11/12/6$ (GZMB) and $13/13/6$ (PRF1) individual patients. (H) FCA of intracellular GZMB staining and quantification in CTL (+CD3/CD28 dynabeads, stimulated for 18 h; re-stimulation with PMA/Ionomycin for 4 h), $n = 9$ individual patients. (I) FCA of intracellular PRF1 staining and quantification in CTL, $n = 9$ individual patients. (J) FCA of CD107a staining and quantification in CTL (+CD3/CD28 dynabeads, stimulated for 18 h; re-stimulation with PMA/Ionomycin for 4 h), $n = 6$ individual patients. If not stated otherwise, data are represented as mean $\pm$ SEM. P values as indicated, paired t -test or Wilcoxon matched-pairs signed-rank test, as appropriate. Source data are available online for this figure.

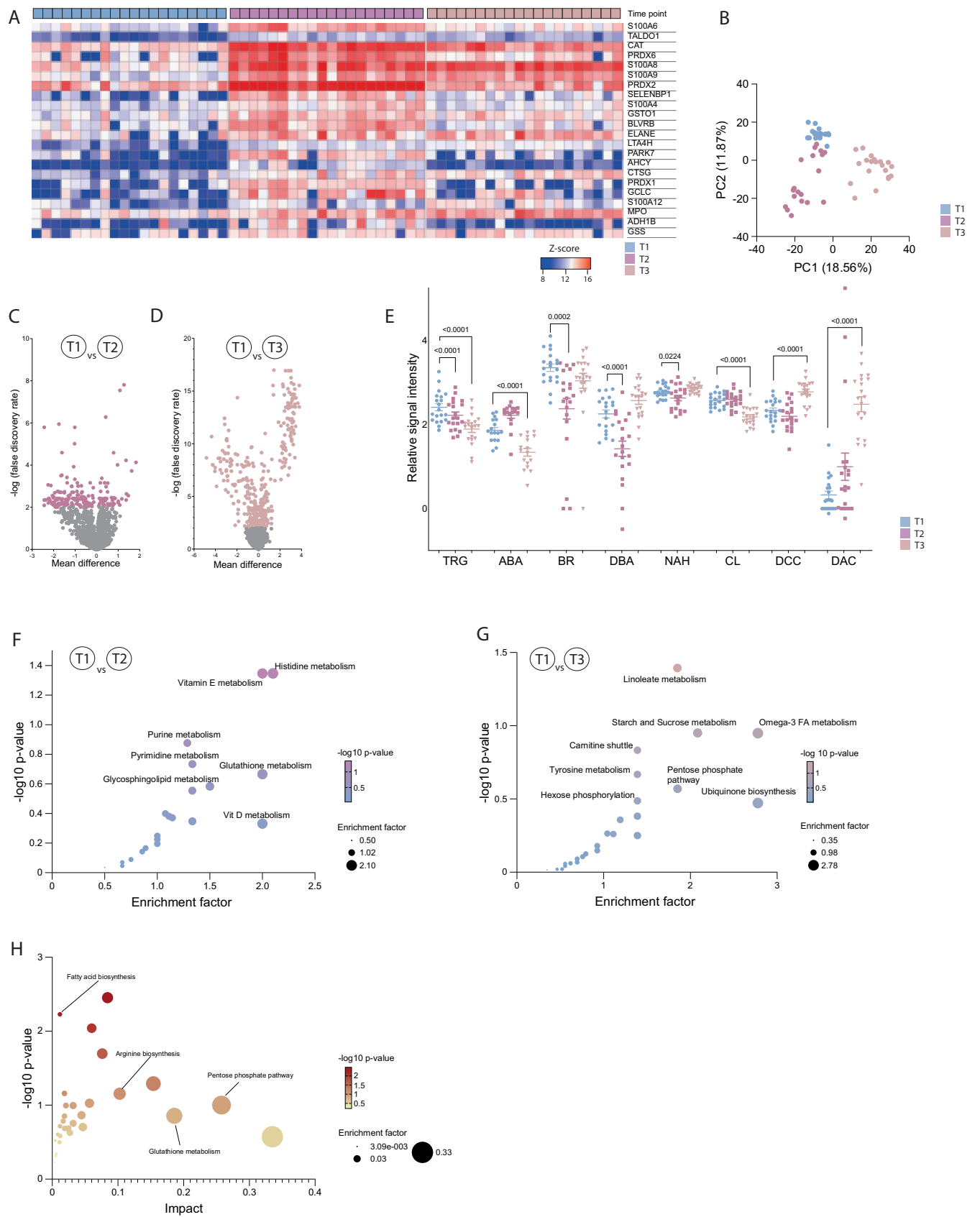


Figure 2. Serum proteomics and metabolomics reveal an oxidative stress signature after major surgery.

(A) Heat map depicting quantity of significantly differentially expressed serum proteins associated with ROS production or redox homeostasis. Z-scores and time points as indicated, $n = 20$ individual patients. (B) Principal component analysis depicting metabolic profiling results during the course of major surgery, time points as indicated. (C) Volcano plot for metabolite abundance of comparisons T1 and T2. Metabolites with $-\log \text{FDR} > 2$ are colored. (D) Volcano plot for metabolite abundance of comparisons T1 and T3. Metabolites with $-\log \text{FDR} > 2$ are colored. (E) Mass spectrometric non-targeted serum metabolome analyses during the course of major surgery, time points as indicated. TRG trigrenolline, ABA aminobezamide, BR bilirubin, DBA dihydroxybenzoic acid, NAH *N*-acetylhistidine, CL citrulline, DCC decanoylcarnitine, DAC diacetylcysteine. Relative quantification (normalized metabolite intensity, log 10, arbitrary units). Mean $\pm$ SEM with dots representing individual values, $n = 20$ individual patients, p values as indicated, paired t -test. (F) Functional analysis of untargeted metabolomics, displaying pathway enrichment results of results T1 compared to T2. (G) Functional analysis of untargeted metabolomics, displaying pathway enrichment results of results T1 compared to T3. (H) Joint pathways analysis of metabolomic and proteomic data. Source data are available online for this figure.

In a prior study, we demonstrated that cardiac surgery with cardiopulmonary bypass leads to the acute appearance of ROS-producing myeloid-derived suppressor cells (MDSC), which negatively impact immune responses (Hübner et al, 2019). We thus hypothesized that MDSC induction might be a general response to major surgery and, accordingly, investigated their presence in our cohort of patients. As shown in Fig. 3C, major surgery also led to the emergence of CD14⁺HLA-DR⁺CD11b⁺CD15⁺ MDSC (Figs. 3C and Fig. EV3A–C), whereas minor surgeries, such as joint arthroscopic procedures, did not induce MDSC formation (Fig. EV3D,E). These findings suggest that the transient appearance of MDSC may represent a common response to major surgery.

To further characterize MDSC as the potential cause of oxidative stress after major surgery, we conducted a comprehensive analysis of freshly isolated T2 MDSC using Next-Generation Sequencing. Differential gene expression analysis revealed a unique transcriptional profile in MDSC, with over 6000 downregulated and 8000 upregulated genes compared to neutrophils, their closest relatives (Fig EV3F–H) (Veglia et al, 2021). Many of these upregulated genes are involved in ROS generation, redox homeostasis, and the antioxidative stress response (Fig. 3D). Notably, *NOX2* and *MPO* were strongly expressed in MDSC. These findings were further corroborated by qRT-PCR-based transcript quantification, which showed nearly exclusive expression of *NOX2* and *MPO* mRNA in MDSC, with transcript levels being up to nearly 50 times higher than in neutrophils (Fig. 3E,F).

A hallmark immunosuppressive mechanism of MDSC is their rapid release of ROS or ROS-producing proteins such as MPO and *NOX2* into the extracellular milieu, rather than intracellular ROS storage in granules that is mainly seen in neutrophils (Ohl and Tenbrock, 2018; Huang et al, 2023). As expected, T2 MDSC exhibited no evidence of elevated intracellular ROS levels (Fig. EV3H) as measured by CellROX-dye. In contrast, luminol-based chemiluminescence assays directly quantify extracellular ROS production. Importantly, sorted T2 MDSC displayed markedly higher luminol-induced relative luminescence compared to T2 neutrophils, indicating substantially greater extracellular ROS release and/or peroxide-dependent enzyme activity of MDSC (Fig. 3G). Together, these data identify MDSC as the dominant cellular source of surgery-induced oxidative stress, acting primarily through extracellular ROS generation.

ROS-release by MDSC compromises CTL mitochondrial function

To investigate whether oxidative stress evoked by MDSC impairs CTL function and thus contributes to postoperative immune

paralysis, we quantified intracellular ROS in ^{PITC}CTL, revealing markedly elevated levels after major surgery. (Fig. 4A). Concurrently, the antioxidative capacity, as reflected by intracellular reduced glutathione (GSH) levels, was significantly diminished (Fig. 4B).

Critical and prolonged elevation of ROS in CTL could be the result of oxidative damage to their mitochondria, resulting in dysfunctional ROS generation (Martínez-Reyes and Cuezva, 2014). To further explore this, we assessed mitochondrial membrane potential ($\Delta\psi\text{M}$) as a marker of mitochondrial integrity. ^{PITC}CTL exhibited significantly impaired $\Delta\psi\text{M}$, accompanied by a concomitant increase in mitochondrial ROS levels (Fig. 4C,D), indeed suggesting a disruption of mitochondrial coupling (Guo et al, 2013; Peoples et al, 2019). ^{PITC}CTL further exhibited a significant decrease in $\Delta\psi$ -independent MitoTracker Green labeling and a marked reduction in the expression of respiratory chain (OXPHOS) protein complexes, indicating a lower mitochondrial protein content (Fig. 4E,F). Of note, we detected a preferential decrease of complex I/IV and complex V (Fig. 4G), which is known to be associated with detrimental ROS production in conditions of mitochondrial stress (Murphy, 2009; Leadsham et al, 2013).

These mitochondrial alterations were associated with a substantial decline in mitochondrial function, reflected by a strong reduction in all parameters of respiratory capacity (Fig. 4H). Basal and maximal oxidative respiration in ^{PITC}CTL were significantly reduced (Fig. 4I,J), while spare respiratory capacity and ATP-linked respiration were also diminished (Fig. 4K,L). Notably, compromised oxidative respiration was not compensated by an increase in glycolytic capacity (Fig. EV4A). In line with this, cellular glucose uptake was significantly reduced in ^{PITC}CTL (Fig. 4M). To further confirm that MDSC-derived ROS are responsible for the observed mitochondrial dysfunction in T2 CTL, we co-cultured preoperative T1 CTL with either T1/T2 neutrophils or MDSC. Upon co-culture, T1 CTL exhibited a phenotype highly similar to T2 CTL only when co-cultivated with MDSC, characterized by impaired mitochondrial mass and decreased mitochondrial membrane integrity (Fig. 4N,O) as well as a marked increase in (m)ROS (Fig. 4P,Q). In contrast, T2/T1 neutrophils did not significantly impact CTL mitochondrial health (Fig. 4N–Q).

ROS-producing enzymes like MPO might also be secreted by MDSC, leading to ROS formation in the serum (Monteserín et al, 2001). To assess the impact of such released enzymes and other serum factors on CTL, we next incubated T1 CTL with T2 serum, which again induced a CTL phenotype of mitochondrial dysfunction characterized by markedly distorted mitochondrial mass (Fig. EV4B), compromised mitochondrial membrane potential (Fig. EV4C) and impaired mitochondrial respiratory function (Fig. EV4D–G).

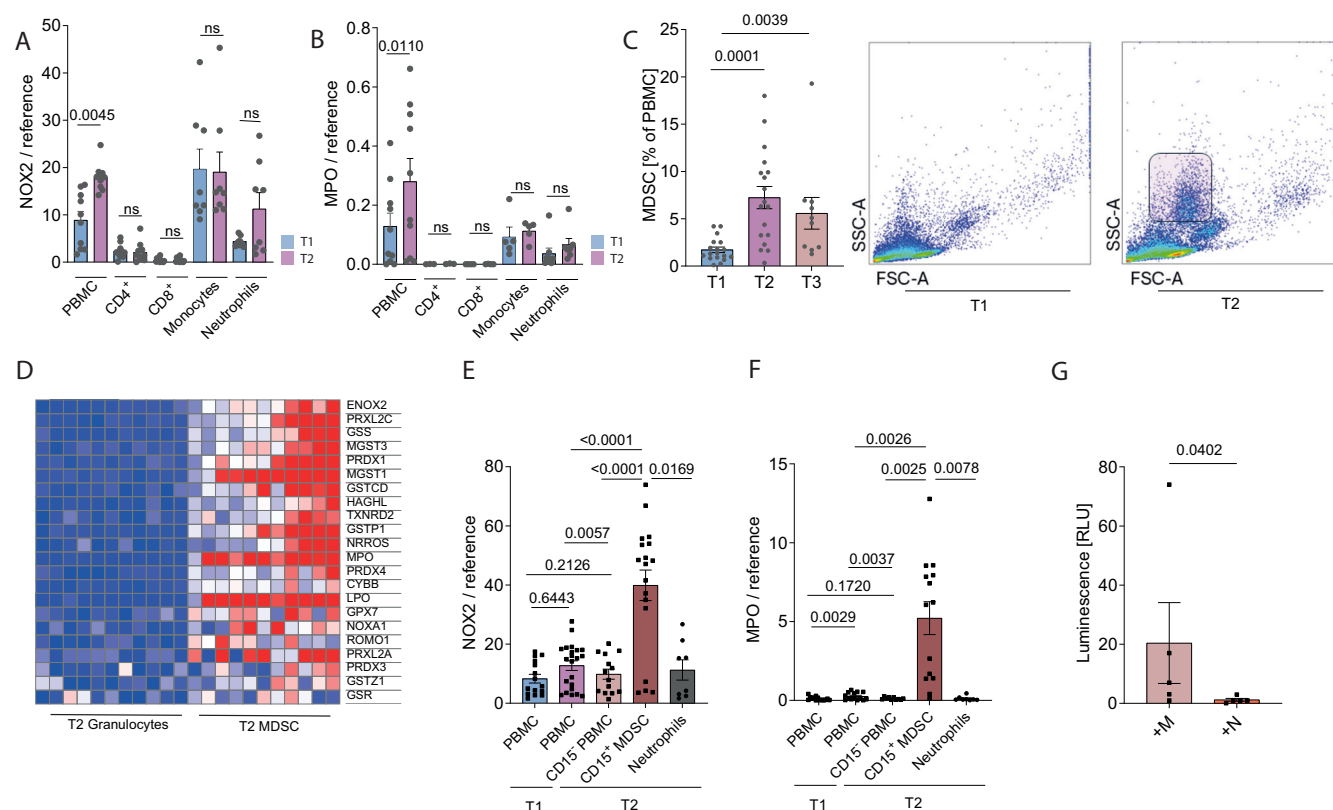


Figure 3. ROS burst after major surgery by the transient occurrence of myeloid-derived suppressor cells.

(A, B) mRNA expression of *NOX2* (A) and *MPO* (B) in PBMC, CD4⁺ T-cells, CTL, monocytes and neutrophils relative to endogenous control. Time points as indicated, $n = 10/10/11/8/8$ (*NOX2*) and $n = 10/3/4/5/7$ (*MPO*) individual patients. (C) Quantification of CD14-HLA-DR⁺CD11b⁺CD15⁺ MDSC and representative FCA before (T1) and after (T2) major surgery, $n = 18/18/10$. (D) Heat map showing quantity of significantly differentially expressed genes associated with oxidative stress of T2 MDSC compared to T2 granulocytes. Genes as indicated. Red color indicates an upregulation, and downregulated genes are indicated by blue color. (E, F) mRNA expression of *NOX2* (E) and *MPO* (F) in PBMC, CD15⁺ PBMC, CD15⁺ MDSC and granulocytes, relative to endogenous control. Time points as indicated. $n = 15/22/14/18/8$ (*NOX2*) and $n = 14/14/10/14/8$ (*MPO*) individual patients. (G) Luminol-based assay and quantification of extracellular ROS (H_2O_2 - and peroxidase-dependent ROS production) of freshly sorted T2 MDSC (+M) compared to T2 neutrophils (+N), $n = 5$ individual patients. Data were represented as mean $\pm$ SEM with dots representing individual values. *P* values as indicated, paired *t*-test, Wilcoxon matched-pairs signed-rank test or log normal Welch's *t*-test (G), as appropriate. Source data are available online for this figure.

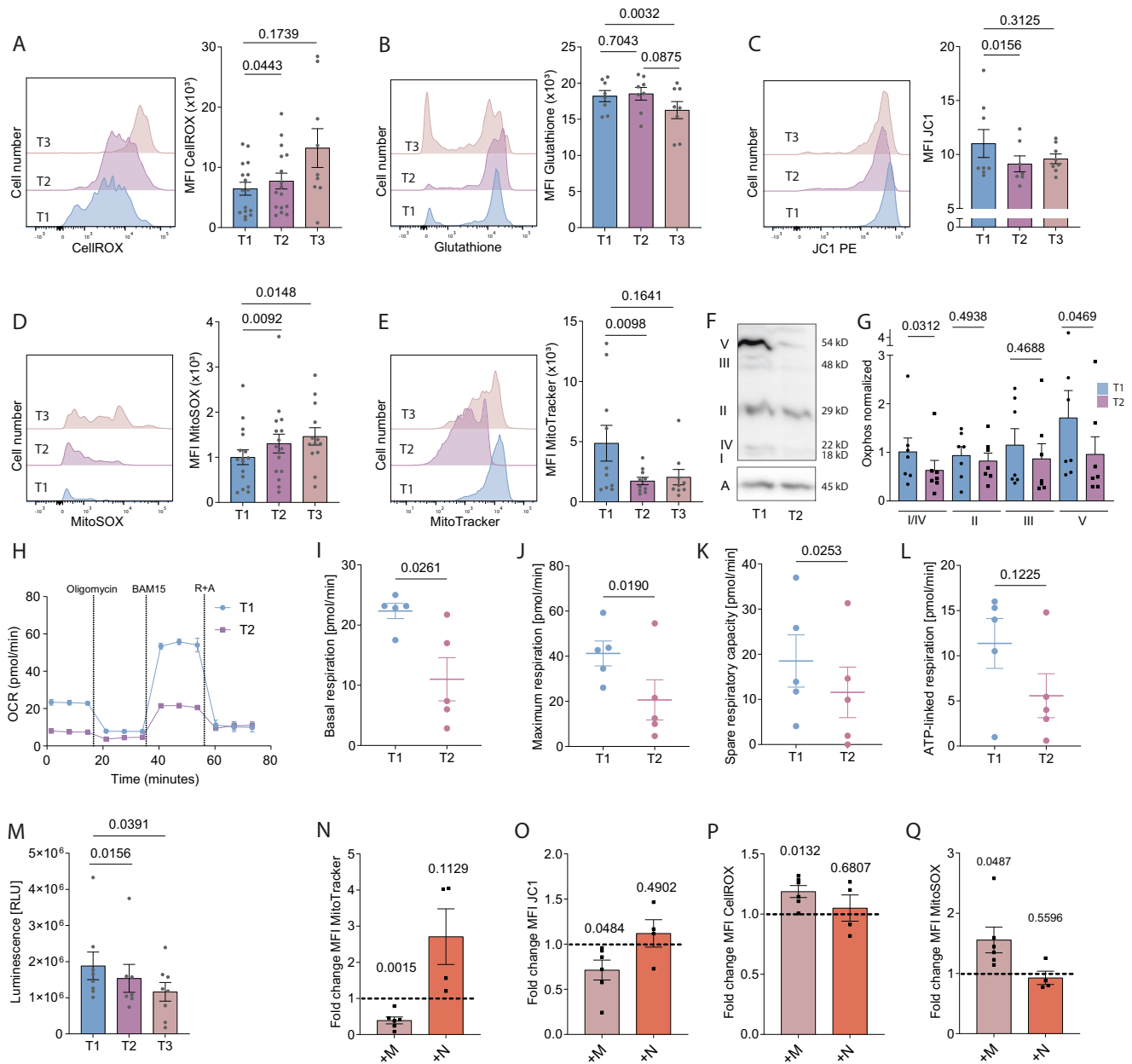
Stress-induced mitochondrial hyperfusion in response to major surgery impairs mitochondrial translocation to the immunological synapse

We next analyzed isolated mitochondria from ^{PITC}CTL at time points T1 and T2, based on our previous experiments that indicate the most profound mitochondrial damage immediately after major surgery. This analysis again revealed a significant reduction in membrane potential, protein content, and OXPHOS protein complexes (Fig. 5A–C). These findings suggest that the impaired mitochondrial fitness of ^{PITC}CTL is not solely due to a reduction in the overall mitochondrial content but also reflects dysfunction at the level of individual organelles. To preserve basal metabolic function following stress-induced damage, mitochondria are known to undergo fusion processes (Tondera et al, 2009). In isolated mitochondria of ^{PITC}CTL, we observed a significant higher protein expression of the fusion proteins Mitofusin 2 (MFN2) and Optic atrophy 1 (OPA1), while the expression of its functional counterpart, fission protein DRP1, was downregulated (Fig. 5D/E). These expression alterations suggest a shift in the balance between mitochondrial fusion and fission, favoring fusion in ^{PITC}CTL. Consistent with this, confocal microscopy

revealed distinct structural changes, with mitochondria forming large supercomplexes, resulting in a reduced mitochondrial count per cell and an increase in mitochondrial length and mean mitochondrial area (Fig. 5F–H). DRP1-dependent mitochondrial fission enables mitochondrial translocation to the T-cell immunological synapse (IS), ensuring ATP production in close proximity to the T-cell receptor (TCR) (Lisci and Griffiths, 2023; Baixauli et al, 2011). To examine this in ^{PITC}CTL, we assessed the subcellular localization of the TCR and mitochondria. As shown in Fig. 5I, we observed a significant reduction in mitochondria-TCR approximation. These findings indicate that PITC-induced oxidative stress triggers mitochondrial hyperfusion in CTL, impairing their ability to translocate to the IS.

Mitochondrial dysfunction contributes to PITC

We next aimed to determine whether isolated mitochondrial damage in intact CTL produces the same functional phenotype observed in ^{PITC}CTL, thereby providing further evidence that mitochondrial dysfunction underlies the decrease of immunometabolic fitness in ^{PITC}CTL. To investigate this, we activated human T cells from healthy donors and treated them with the mitochondria-



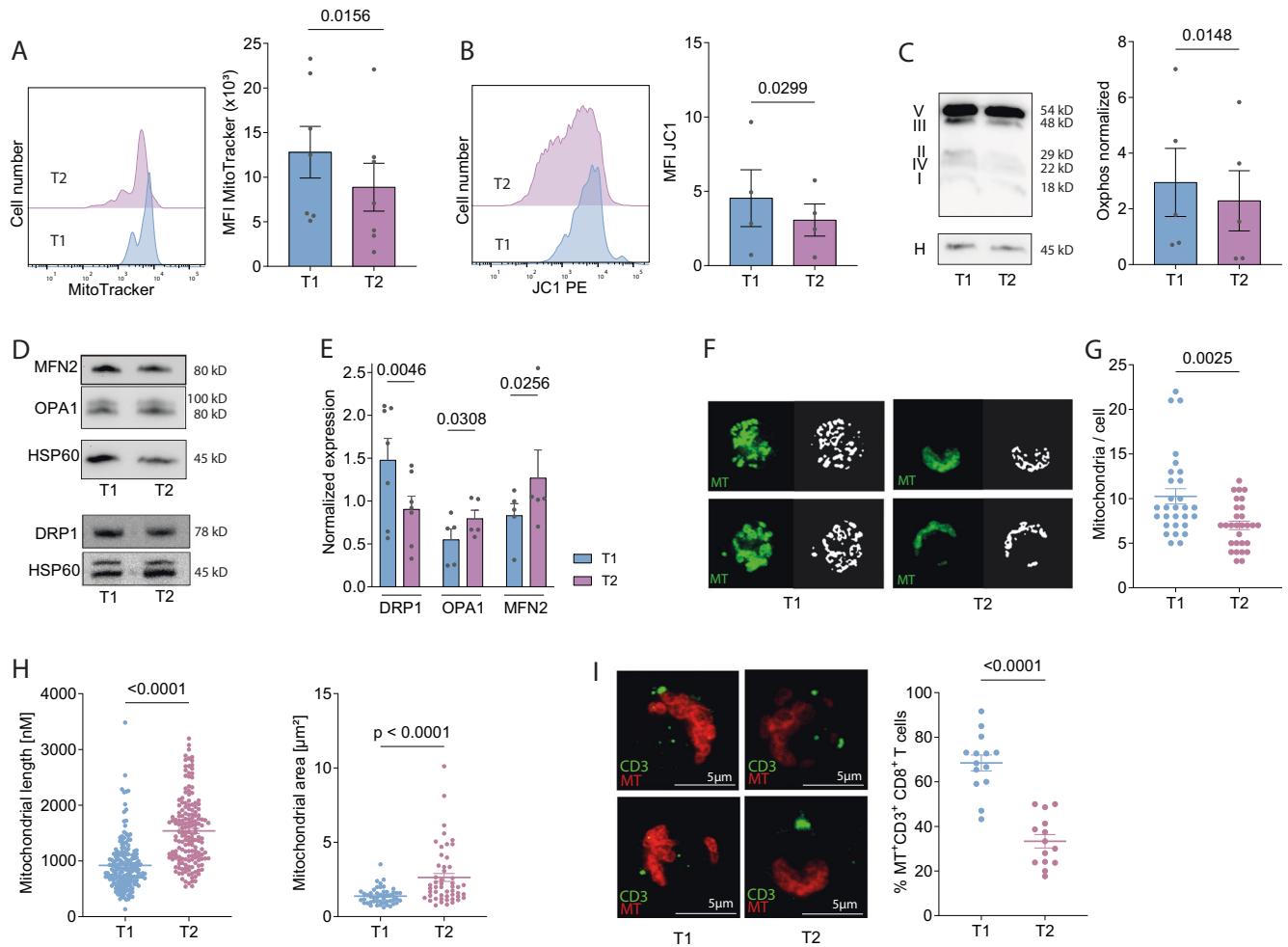


Figure 5. PITC leads to stress-induced mitochondrial hyperfusion in CD8⁺ CTL.

(A) FCA of MitoTracker green and quantification in isolated mitochondria from CTL, $n = 7$ individual patients. (B) FCA of JC1 green and quantification of red/green fluorescence in isolated mitochondria from CTL, $n = 4$ individual patients. (C) Immunoblot of OXPHOS complexes using isolated mitochondria of CTL and normalized densitometric quantification of complex V, $n = 5$ individual patients. I–V complex I–V; H heat shock protein 60 (HSP60). Subunits detected by the antibody cocktail: complex I: subunit NDUF88; complex II: subunit 30 kDa (SDHB); complex III: subunit Core 2 (UQCRC2); complex IV: subunit II (COXII); complex V: ATP synthase subunit alpha (ATP5). (D, E) Representative immunoblot of mitochondrial fusion and fission proteins of isolated mitochondria of CTL (D) and normalized densitometric quantification (E) of the respective proteins, $n = 5/4/3$ individual patients. (F) Representative projections of 3D image stacks of CTL stained with MitoTracker (MT, green, left) and the respective skeleton images (white, right), generated using mitochondrial analyzer, time points as indicated, representative of six individual patients. (G) Quantification of mitochondrial count per cell, $n = 29$ cells from three individual patients. (H) Quantification of mitochondrial length (left) and mean mitochondrial area using mitochondrial analyzer (right), $n = 180/170$ mitochondria from 14/22 cells from three individual patients (length) and $n = 50$ cells from three individual patients (area). (I) Representative projection of 3D image stacks (two per time point) of translocated mitochondria to the IS in CTL as indicated by MitoTracker (MT) Deep Red and CD3 FITC staining, and quantification of mitochondrial translocation to the CTL IS, time points as indicated, $n = 14$ from 5 individual patients. If not stated otherwise, data were represented as mean $\pm$ SEM. P values as indicated, paired t -test or Wilcoxon matched-pairs signed-rank test (A–E), and unpaired t -test or Mann–Whitney test (G–I), as appropriate. Source data are available online for this figure.

specific protonophore FCCP, which partially uncouples the respiratory chain and mildly reduces $\Delta\psi M$ ($\Delta\psi M^{\text{low}}$ CTL). A concentration of 20 nM FCCP – $\sim 1/1000$ of the typical dose required for complete uncoupling – resulted in a reduction of mitochondrial membrane potential comparable to that observed in PITC CTL, without compromising cell viability (Figs. 6A and EV5A). In $\Delta\psi M^{\text{low}}$ CTL, we observed diminished mitochondrial function, marked by impaired oxidative phosphorylation and an absence of compensatory glycolysis, resembling the metabolic changes seen in PITC CTL (Fig. 6B–D). Furthermore, mitochondrial protein content

was significantly reduced (Fig. 6E) and a shift toward mitochondrial fusion was evident, as indicated by the downregulation of DRP1 and upregulation of MFN2 expression (Fig. 6F,G). This mitochondrial dysfunction in $\Delta\psi M^{\text{low}}$ CTL led to a functional phenotype similar to that of PITC CTL, characterized by reduced T-cell proliferation and significantly lower mRNA expression and protein secretion of IFNG/IFN- γ , GZMB, and PRF1 (Fig. 6H–J). Collectively, these findings strongly support the notion that mitochondrial damage drives the decrease of immunometabolic fitness in PITC CTL.

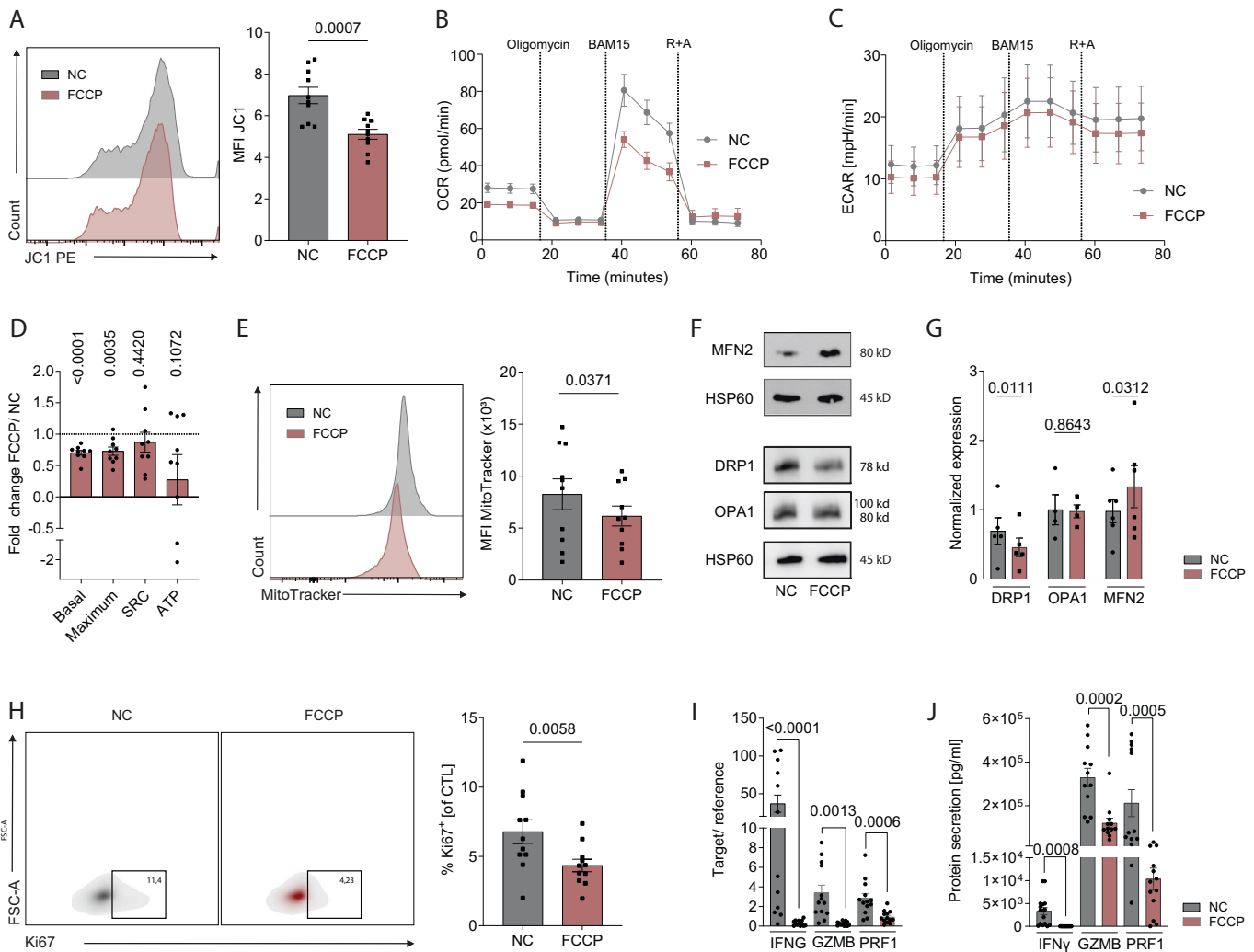


Figure 6. Mitochondrial membrane potential is required for T-cell immunocompetence.

(A) FCA of JC1 and quantification of red/green fluorescence in CTL, $n = 10$ individual biological replicates. (B) Representative Seahorse OCR plot. (C) Pooled seahorse ECAR plot, mean $\pm$ SEM from ten individual biological replicates. (D) Fold change of basal OCR, maximum OCR, spare respiratory capacity (SRC) and ATP production, $n = 10$ individual biological replicates. (E) FCA of MitoTracker green and quantification in CTL, $n = 10$ individual biological replicates. (F) Immunoblot of mitochondrial fusion and fission proteins of isolated mitochondria of CTL and (G) normalized densitometric quantification of the respective proteins, $n = 5/4/6$ individual biological replicates. (H) FCA of Ki-67 and quantification in CTL, $n = 11$ individual biological replicates. (I) mRNA expression of *IFNG*, *GZMB* and *PRF1* of CTL, relative to endogenous controls, $n = 14/13/13$ individual biological replicates. (J) Quantification of IFN- γ /GZMB/PRF1 secretion by CTL, $n = 16/12/13$ individual biological replicates. If not stated otherwise, data were represented as mean $\pm$ SEM. P values as indicated, one-sample t -test (**D**), paired t -test or Wilcoxon matched-pairs signed-rank test, as appropriate. Source data are available online for this figure.

Enhancing mitochondrial antioxidative capacity mitigates PITC

Finally, we aimed to investigate whether mitochondria-specific interception of ROS could serve as a potential strategy to mitigate PITC. To this end, $PITC$ CTL were supplemented with the mitochondria-targeted antioxidant MitoTEMPO (MT). As shown in Fig. 7A–C, $PITC$ CTL treated with MT exhibited a significant increase in mitochondrial protein content (Fig. 7A) and a marked improvement in mitochondrial function, characterized by enhanced oxidative phosphorylation and preserved glycolytic capacity (Figs. 7B and EV5B). Both basal and maximal respiration, as well as spare respiratory capacity, were significantly elevated (Fig. 7C). MT

treatment also alleviated stress-induced mitochondrial hyperfusion, as evidenced by a significant reduction in mean mitochondrial length and an increase in mitochondrial count per cell (Fig. 7D,E). Additionally, a substantial increase in mitochondrial translocation to the T-cell immunological synapse was detected (Fig. 7F,G). Functionally, MT treatment restored the effector functions of $PITC$ CTL, as demonstrated by ECIS cytotoxicity assays, which revealed a significant improvement of cytolytic capacity (Fig. 7H,I). Of note, these effects were only present under conditions of critically elevated oxidative stress, as T1 CTL supplemented with MT showed no alterations to mitochondrial mass, mitochondrial function or CTL cytotoxicity (Figs. 7B and EV5C–E). By contrast, T1 CTL that have been damaged via co-cultivation with T2 MDSC exhibit improved mitochondrial

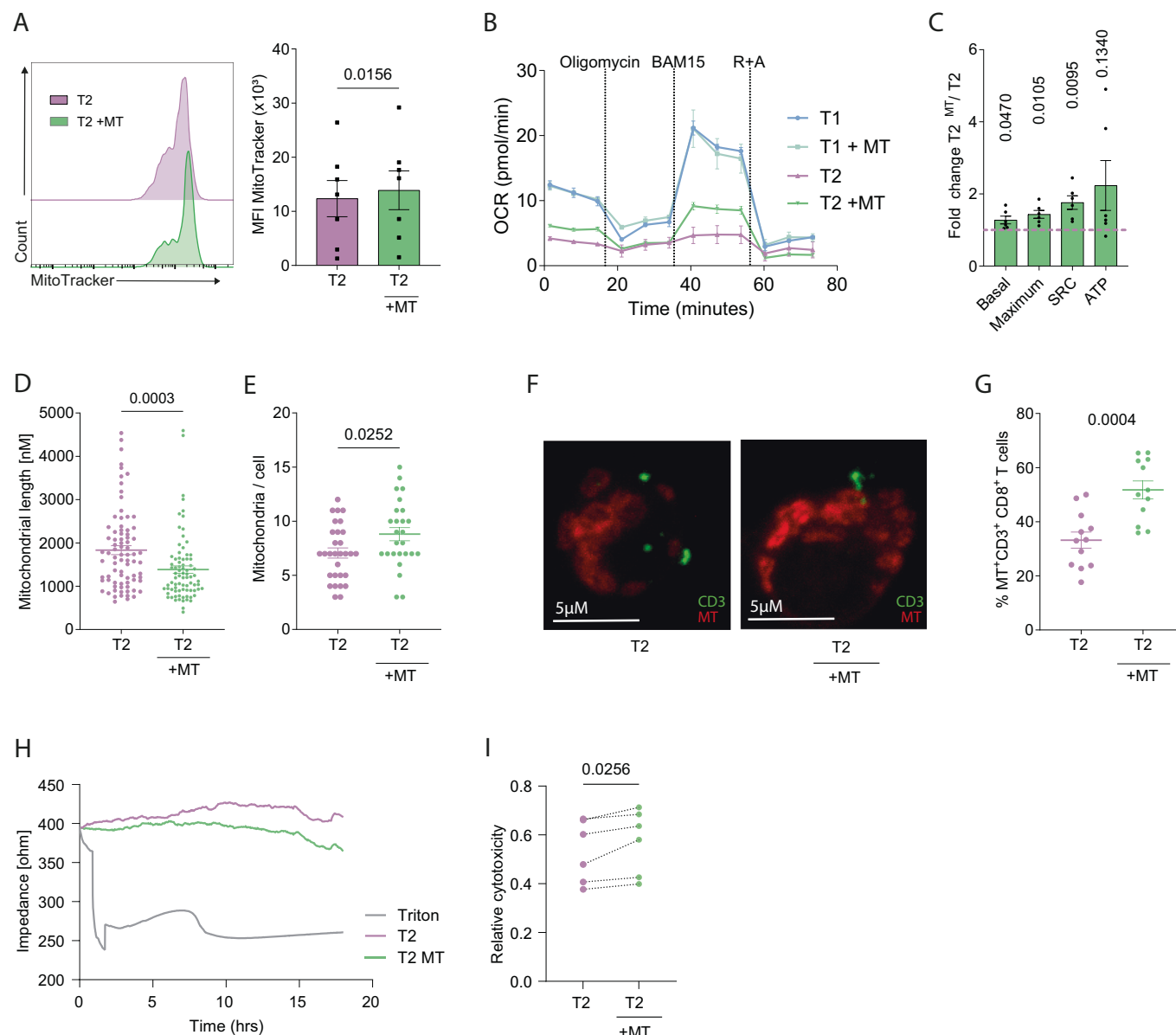


Figure 7. Enhancing mitochondrial antioxidative capacity using MitoTEMPO (MT) ameliorates PITC.

(A) FCA of MitoTracker green and quantification in CD8⁺ CTL, $n = 7$ individual patients. (B) Representative Seahorse OCR plot. (C) Fold change of basal OCR, maximum OCR, spare respiratory capacity (SRC) and ATP production, $n = 6$ individual patients. (D) Quantification of mitochondrial length, $n = 92$ mitochondria from 28 cells from three individual patients. (E) Quantification of mitochondrial count per cell, $n = 30/26$ cells from three individual patients. (F) Representative confocal microscopy image of mitochondria translocated to the TCR in CTL as indicated by MitoTracker (MT) Deep Red and CD3 FITC staining, T2 image as depicted in Fig. 5I, upper right image. (G) Quantification of mitochondrial translocation in proximity to the CTL IS, $n = 12$ from four individual patients. (H) Representative impedance plot and (I) quantification of relative CTL cytotoxicity using ECIS, $n = 6$ individual patients. If not stated otherwise, data are represented as mean $\pm$ SEM. P values as indicated, one-sample t -test (C), paired t -test or Wilcoxon matched-pairs signed-rank test (A–C, H, I), and unpaired t -test or Mann-Whitney test (D–G), as appropriate. Source data are available online for this figure.

health in response to MT treatment (Fig. EV5F). In conclusion, these findings both consolidate the detrimental role of oxidative stress for CTL immunometabolism and highlight potential therapeutic avenues.

Discussion

Dysfunction of cytotoxic T cells following major surgery (^{PITC}CTL) is recognized as a hallmark of postoperative immune paralysis

(Albertsmeier et al, 2015; Sun et al, 2017; Ward et al, 2008), with significant clinical consequences, including increased rates of nosocomial infections, prolonged ICU stays, and elevated perioperative mortality (Hadaya et al, 2022; Torrance et al, 2016; Pérez-Granda et al, 2024; Hadaya et al, 2021). The underlying mechanisms of this phenomenon remain poorly understood. In the present study, severe systemic oxidative stress, produced by transiently emerging myeloid-derived suppressor cells (MDSC) was identified as a key driver of this dysfunction. MDSC-derived

reactive oxygen species (ROS) caused a critical increase in ROS levels within CTL, overwhelming their antioxidative defenses and resulting in mitochondrial damage, ultimately leading to decreased metabolic fitness of CTL.

In this study, we selected patients scheduled for major surgery who did not have preexisting conditions associated with immune dysregulation, such as cancer, autoimmune diseases, immunosuppression, or active infections. We demonstrated that postoperative CTL of these patients indeed exhibited clear signs of immunoparalysis, as evidenced by decreased expression of IFNG, PRF1, and GZMB, and impaired cytotoxicity. To evaluate the root cause of this disorder, we employed a multi-omics approach that identified a systemic serum signature indicative of severe oxidative stress. We identified acutely emerging MDSC as the primary source of ROS after major surgery. Other immune cell types contributed minimally or not at all to ROS production in this scenario. Lymphocytes and monocytes generally produce low levels of ROS, while neutrophils are known to generate ROS-bursts as part of their antimicrobial defense against pathogens, rather than during sterile inflammation (Dale et al, 2008; Taylor et al, 2013; El-Benna et al, 2016; Botha et al, 1995; Lumsdaine et al, 2014). Consistently, we did not observe activation of the key ROS-producing enzymes in these leukocyte populations and found markedly higher ROS levels produced by MDSC than by pre- or postoperative neutrophils.

MDSC are commonly observed in chronic inflammatory conditions such as cancer and autoimmunity, where they are thought to suppress immune responses through the production of ROS and other immunosuppressive molecules (Beury et al, 2016; Groth et al, 2019). In the perioperative setting, only one study has previously reported the presence of MDSC following the use of cardiopulmonary bypass (Hübner et al, 2019). However, the current study expands on this by demonstrating that MDSC emerge after major surgery, regardless of the specific surgical procedure. In contrast, minor surgeries do not lead to a significant increase in MDSC numbers. The origins and precise mechanisms underlying MDSC release remain speculative. One may believe that these cells are released from the bone marrow, potentially serving an evolutionary function in response to life-threatening injuries outside of a sterile environment. In such a context, MDSC may help maintain a balance in the inflammatory response, preventing excessive immune activation that could be detrimental.

However, in the sterile environment of major surgery, excessive ROS production by MDSC may impair CTL function, thereby contributing to the postoperative immunosuppression observed. Our findings show a significant increase in ROS levels in ^{PITC}CTL, while ROS-producing enzymes were not induced. Additionally, we observed a depletion of reduced glutathione (GSH), a critical antioxidant, indicating the exhaustion of the cellular compensatory mechanisms.

In CTL, ROS levels must be tightly regulated. At low concentrations, ROS function as essential signaling molecules for immune cell activation (Picard and Shirihai, 2022; Hirschberger et al, 2021). However, excessive ROS levels induce mitochondrial damage by inactivating multiple enzymes of the electron transport chain (ETC), thereby impairing oxidative phosphorylation (OXPHOS) (Guo et al, 2013; Vardhana et al, 2020). Furthermore, elevated ROS levels can damage the mitochondrial membrane, exacerbating OXPHOS dysfunction and triggering the release of

ROS from the mitochondrial matrix, which perpetuates a vicious cycle of mitochondrial damage (Zorov et al, 2014). In this study, we identified all the hallmark features of ROS-induced mitochondrial damage in ^{PITC}CTL: A reduction in mitochondrial mass, decreased expression of ETC proteins, impaired OXPHOS function, and compromised mitochondrial membrane integrity.

Furthermore, we observed signs of stress-induced mitochondrial hyperfusion (SIMH) in ^{PITC}CTL. Specifically, we detected elevated levels of fusion proteins, reduced mitochondrial expression of the fission protein Drp1, a decrease in the mitochondrial count per cell, and an increase in the mean mitochondrial length of ^{PITC}CTL. SIMH is a well-documented response to critical cellular damage, by which mitochondria merge partially damaged or dysfunctional organelles into larger supercomplexes (Das and Chakrabarti, 2020; Youle and van der Bliek, 2012; Buck et al, 2016). This process helps to maintain basal metabolic function and mitigate stress-induced damage; however, it occurs at the expense of mitochondrial mobility due to the downregulation of DRP1.

Drp1 has been shown to be essential for mitochondrial movement toward the T-cell immunological synapse, which is necessary to meet the high energy demands at this immune hub during T-cell activation (Lisci and Griffiths, 2023; Baixeli et al, 2011; Desdín-Micó et al, 2018; Quintana et al, 2007; Huppa and Davis, 2003). We found significantly reduced co-localization of mitochondria with the TCR in ^{PITC}CTL, indicating a substantial impairment in mitochondrial translocation. Consequently, ^{PITC}CTL not only show a massive decrease in mitochondrial oxidative phosphorylation but might also fail to localize their ATP synthesis to the potential area of highest demand. This bioenergetic failure likely contributes to impaired effector functions, including both the production and release of cytolytic granules (CG), as trafficking of these CG to the cell membrane is based on high ATP-consuming dynein-dependent microtubule–cytoskeleton interactions (Stinchcombe et al, 2006; de Saint Basile et al, 2010). Consistent with this, ^{PITC}CTL showed impaired release of CG.

The exact mechanism by which MDSC-derived ROS induce this mitochondrial damage in CTL after major surgery remains to be elucidated. However, co-culture of postoperative MDSC with healthy CTL and incubation with postoperative serum both induced a similar phenotype of mitochondrial damage, indicating that MDSC-derived ROS or ROS-producing enzymes initiate this process. This likely triggers a cell-intrinsic amplification of mitochondrial ROS, worsening damage and suppressing CTL function. Thus, we propose a two-step model of postoperative CTL suppression: (i) primary damage from MDSC-derived factors, and (ii) secondary ROS amplification within CTL due to mitochondrial dysfunction.

This hypothesis was further validated through ex vivo experiments, where low-dose application of the protonophore FCCP to CTL from healthy donors successfully reproduced the ^{PITC}CTL phenotype, which was characterized by all previously described hallmark features, including collapse of the mitochondrial membrane potential, impairment of mitochondrial metabolism, enhanced mitochondrial fusion, and subsequent CTL effector dysfunction. In contrast, ex vivo treatment of ^{PITC}CTL with the mitochondria-specific antioxidant MitoTEMPO partially reversed all aspects of mitochondrial dysfunction and partially restored immune effector functions. Notably, MitoTEMPO treatment of T1 CTL co-cultivated with MDSC was also able to ameliorate

mitochondrial damage, thereby suggesting a potential avenue for future therapeutic intervention.

Collectively, this study offers the first detailed insights into the mechanisms driving CTL immunometabolic dysfunction following major surgery, laying the groundwork for the development of therapeutic and preventive strategies aimed at improving post-operative immune function and, consequently, enhancing clinical outcomes.

Methods

Reagents and tools table

Reagent/resource	Reference or source	Identifier or catalog number
Experimental models		
n/a	n/a	n/a
Recombinant DNA		
n/a	n/a	n/a
Antibodies		
CD3	SCBT	sc-20047
AF488 anti-mouse	Invitrogen	A21042
CD38 APC	Miltenyi	130-113-991
CD69 FITC	Miltenyi	130-112-801
CD223 APC	Biolegend	369211
CD366 FITC	Biolegend	345021
CD25 PE	Biolegend	302605
CD15 PE	Biolegend	301905
CD279 BV421	Biolegend	329920
HLA-DR Pacific Blue	Biolegend	307623
CD14 PerCp	Biolegend	325631
CD11b FITC	Biolegend	301329
CD8PerCp	Biolegend	344747
CD8 BV421	Biolegend	344707
CD107a PE	Biolegend	328609
GZMB BV421	Biolegend	396413
Perforin 1 APC	Biolegend	308111
Interferon-g FITC	Biolegend	502506
CD366 FITC	Biolegend	345021
CD223 APC	Biolegend	369211
CD274 BV421	Biolegend	329920
Total OXPHOS Human WB Antibody Cocktail	Abcam	ab110411
MFN2	Cell Signaling Technology	67589
HSP60	Cell Signaling Technology	4870
DRP1	Cell Signaling Technology	5391
Phospho-DRP1	Cell Signaling Technology	3455

Reagent/resource	Reference or source	Identifier or catalog number
OPA1	Cell Signaling Technology	11925
b-Actin	Cell Signaling Technology	5125
Oligonucleotides and other sequence-based reagents		
UPL Probe #26 (PD1) from UPL-Set Probes #1-90	Roche	04683633001
UPL Probe #59 (LAG)	Roche	04688562001
UPL Probe #3 (TIM3) from UPL-Set Probes #1-90	Roche	04683633001
UPL Probe #54	Roche	04688511001
TBP RT-PCR Assay Cy3	Bio-Rad	10031231
RPL13A RT-PCR Assay FAM	Bio-Rad	12001950
PCR primers	This study	Table EV1
Chemicals, enzymes and other reagents		
Human TruStain FcX Receptor Blocking Solution	Biolegend	422302
Mounting Medium	lbbi	50001
Mitotracker Deep Red	Cell Signaling Technology	8778
Mitotracker Green	Cell Signaling Technology	9074
Fixation/Permeabilization Concentrate	Invitrogen	00-5123-43
Fixation/Permeabilization Diluent	Invitrogen	00-5223-56
Fixation/Permeabilization Buffer	Invitrogen	00-8333-56
Cell Activation Cocktail	Biolegend	42330
MitoSOX Red	Thermo Fisher	M36008
CellROX Green	Thermo Fisher	C10492
ThiolTracker	Thermo Fisher	T10095
JC1 Mitochondrial Membrane Potential Assay Kit	Cayman Chemical	701560
LightCycler 480 Probesmaster	Roche	04887301001
Ficoll	Sigma	10771
CD8 Microbeads	Miltenyi	130-045-201
CD15 Microbeads	Miltenyi	130-046-601
StraightFrom whole blood CD15 Microbeads	Miltenyi	130-091-058
Mitochondria Isolation Kit, Human	Miltenyi	130-094-833
RPMI 1640	Sigma	R0883-100ML
Seahorse XF RPMI Medium	Agilent	103576-100
Seahorse XF Calibrant	Agilent	103059-000
MitoStress Test kit	Agilent	103015-100
MitoTEMPO	SCBT	sc-221945
Calcein AM	Sigma	C1359
SYTOX AADvanced	Thermo Fisher	A35135
CFSE Cell Division Tracker Kit	Biolegend	423801
Ficoll		

Reagent/resource	Reference or source	Identifier or catalog number
SuperScript IV Reverse Transcriptase	Thermo Fisher	18090050
Random Hexamers	Qiagen	79236
Oligo-dT Primers	Qiagen	79723
miRneasy RNA extraction kit	Qiagen	217084
Caspase-Glo 3/7 Assay	Promega	G8090
IFN- γ ELISA	Biologend	430104
GZMB ELISA	Biologend	439204
PRF1 ELISA	Mabtech	3465-1HP-2
Glucose Uptake Glo Assay	Promega	J1342
FCCP	Cayman Chemical	15218IF
Poly-L-Lysine	Sigma	P4707
Complete Protease Inhibitor Cocktail	Roche	4693116001
Protease Inhibitor Cocktail	Cell Signaling Technology	5871
Phosphatase Inhibitor Cocktail	Cell Signaling Technology	5870
Cell Lysis Buffer	Cell Signaling Technology	9803
Mini Protean TGX Western Blot Gels	Bio-Rad	456-1094
Clarity Western ECL	Bio-Rad	1705061
Horseradish peroxidase	Biologend	405210
K562 cell line (human)	ATCC	CCL-243
Panc-1 cell line (human)	DSMZ	ACC783
Software		
ImageJ	Open source	n/a
Leica Application Suite X	Leica	n/a
Graphpad Prism, V10.6.1	Siemens AG	n/a
FlowJo	BD	n/a
Proteo Wizard	Proteo Wizard	n/a
MetaboAnalyst	MetaboAnalyst	n/a
Perseus	MaxQuant	n/a
Spectronaut	Biognosys	n/a
Other		
Seahorse XF HS Mini Analyzer	Agilent	S7852A
Confocal Microscope SP8	Leica	n/a
ECIS zeta	Applied biophysics	n/a
Vi-Cell XR Cell Counter	BD	383556
NanoDrop2000	Thermo Fisher	ND-2000
LightCycler 480	Roche	05015243001
Facs Canto II	BD	641889
MacsQuant 10	Miltenyi	130-096-343
AutoMACS Pro Separator	Miltenyi	130-092-245
UHPLC System	Nexera	n/a

Reagent/resource	Reference or source	Identifier or catalog number
TripleTOF 6600	AB Sciex	n/a
FilterMax F3	Molecular Devices	F3-001
8 Well microscope slides	Ibidi	80841
Trans-Blot Turbo Transfer System	Bio-Rad	1704150

Methods and protocols

Study design and patient recruitment

Patients undergoing elective major surgery were enrolled. Surgical procedures consisted of cardiac surgery with the use of cardio-pulmonary bypass, off-pump cardiac surgery, major orthopedic surgery and minor orthopedic surgery. The study was approved by the local ethics committee of the Ludwig-Maximilians-University Munich (Nr. 17-241 and 22-0384) and sample size estimation was conducted in the respective ethics applications. Written informed consent was obtained from all patients. In addition to informed consent and the WMA Declaration of Helsinki, the experiments also confirmed to the principles set out in the Department of Health and Human Services Belmont Report.

Age <18 years, emergency surgery, pregnancy, known malignancies, acute inflammatory processes prior to surgery, previous organ transplantation, immune system dysfunction or immunosuppression, and preoperative steroid treatment were considered as exclusion criteria.

Anesthesia was performed following internal protocols of the clinic: no premedication was applied; general anesthesia was induced using sufentanil 0.5–1 μ g/kg, propofol 2 mg/kg and rocuronium 0.5–1 mg/kg. Administration of 0.7–1.2 μ g/kg/h sufentanil and either 1.5–2 vol% sevoflurane or 6–8 mg/kg/h propofol was used to maintain anesthesia. For intraoperative muscle relaxation, fractionated i.v. application of rocuronium was applied. Pressure-controlled mechanical ventilation was used for all patients after endotracheal intubation with tidal volumes of ~6–8 ml/kg body weight. Blood was obtained after induction of anesthesia (T1), at the end of the surgery (T2) and on the first postoperative day on the intensive care unit (T3). Heparinized blood was processed immediately after collection.

Graphics

Figure 1A and synopsis were created with BioRender.com.

Peripheral blood mononuclear cell isolation and T-cell stimulation

Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation (Histopaque 1077, Sigma-Aldrich). A ViCell analyzer (Beckman Coulter) was used to assess cell number and viability. PBMC were suspended in RPMI 1640 (Invitrogen) supplemented with 200 mg/dl glucose, 10% heat-inactivated fetal calf serum (Biochrom), 1% HEPES (Sigma-Aldrich) and 1% L-glutamine (Life Technologies). PBMC were incubated at 37 °C and 5% CO₂ for 18 h. T-cell stimulation was performed using CD3/CD28 Dynabeads (Thermo Fisher Scientific) according to the manufacturer's instructions with a bead-to-cell ratio of 1:8.

Impact of mitochondrial depolarization on T-cell immunity

To investigate the impact of impaired mitochondrial integrity on T-cell immunometabolism, PBMC were isolated, seeded and stimulated as described above. FCCP (Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone) was added to a final well concentration of 20 nM. For vehicle controls (NC), equal amounts of JC1 assay buffer were used (FCCP and JC1 Assay Buffer: Cat. No. 701560, Cayman Chemical).

Rescue of mitochondrial antioxidative capacity

To assess the immunometabolic impact of elevated antioxidant capacity, T2 PBMC were stimulated with anti-CD3/CD28 beads (Thermo Fisher) as described above and incubated with 1 μ M MitoTEMPO (Cayman Chemical). Through the combination of the piperidine nitroxide TEMPO and the lipophilic cation triphenyl-phosphonium, MitoTEMPO can easily pass through membranes and accumulate in the mitochondria at high concentrations.

CD8⁺ T-cell isolation

Prior to T-cell isolation, PBMC were harvested and CD3/CD28 Dynabeads were magnetically removed. Magnetic cell separation was used to isolate cytotoxic CD8⁺ T-cells (Human CD8 MicroBeads, #130-045-201 | CD8⁺ T Cell Isolation Kit, human, #130-096-495; Miltenyi Biotec) on an AutoMACS Pro Separator (#130-092-545, Miltenyi Biotec) according to the manufacturer's protocol.

Next-generation Sequencing (NGS)

NGS was performed by Novogene Company Limited (Cambridge, UK) according to the company's specific protocol. In brief, sequencing libraries were generated using the QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina (Lexogen). The PCR Add-on Kit for Illumina (Lexogen) served to determine the optimal number of amplification cycles. Amplification was conducted according to the manufacturer's protocol. Quality and quantity of sequencing libraries were determined using the Quanti-iT PicoGreen dsDNA Assay Kit (Invitrogen) and the Bioanalyzer High Sensitivity DNA Analysis Kit (Agilent Technologies). Sequencing of libraries was performed on an Illumina HiSeq4000 sequencing machine (Illumina). Individually barcoded libraries were pooled and distributed across lanes of the same flow-cell aiming for approximately ten million paired-end reads per sample.

Analysis of NGS data

Downstream analysis was performed using a combination of programs including STAR, HTseq, Cufflink and wrapped scripts. Alignments were parsed using the Tophat program and differential expressions were determined through DESeq2/edgeR. Gene Ontology (GO) and Kyoto Encyclopedia Of Genes And Genomes (KEGG) enrichment were implemented by the ClusterProfiler. Reference genome and gene model annotation files were downloaded from the genome website browser (NCBI/UCSC/Ensembl) directly. Indexes of the reference genome were built using STAR and paired-end clean reads were aligned to the reference genome using STAR (v2.5). HTSeq v0.6.1 was used to count the read numbers mapped of each gene. Next, fragments per kilobase per million mapped reads (FPKM) of each gene was calculated based on gene length and mapped read counts.

Differential expression analysis between two conditions/groups (two biological replicates per condition) was performed using the DESeq2 R package (2.1.6.3) and the edgeR R package (3.16.5). The resulting *P* values were adjusted using the Benjamini and Hochberg's approach for controlling the false-discovery rate (FDR). Genes with an adjusted *P* value <0.05 found by DESeq2 were assigned as differentially expressed. Prior to differential gene expression analysis, for each sequenced library, the read counts were adjusted by the edgeR program package through one scaling normalized factor.

To identify the correlation between differences in gene expression, samples were clustered using expression level FPKM to see the correlation using hierarchical clustering distance method with the function of heat map, SOM (Self-organization mapping) and means using silhouette coefficient to adapt the optimal classification with default parameter in R.

GO enrichment analysis of differentially expressed genes was implemented by the clusterProfiler R package, in which gene length bias was corrected. GO terms with corrected *P* value less than 0.05 were considered significantly enriched by differential expressed genes. Statistical enrichment of differential expression genes in KEGG pathways was assessed by the clusterProfiler R package.

Cytotoxicity assay

Isolated primary human CD8⁺ T cells obtained at time points T1 or T2 were co-cultivated with calcein-prelabeled Panc-1 human pancreas ductal adenocarcinoma cells or calcein-labeled K562 lymphoblasts, using 8 μ M calcein AM (#C1359, Sigma-Aldrich). Lysis-dependent increase of calcein fluorescence in the supernatant was quantified on a Filtermax F3 (excitation filter: 480 nm; emission filter: 520 nm | molecular devices). 1% Triton served as a positive control. Calculation of relative cell lysis capacity was performed using the formula [(test release – spontaneous release)/(maximum release – spontaneous release)] $\times$ 100.

For impedance-based assessment of cytotoxicity, 2×10^5 Panc-1 cells were seeded on gelatin precoated electric cell-substrate impedance sensing array, containing eight-wells with 40 gold electrodes per well (ECIS 8W10E + PET; Ibidi). Impedance was measured with multiple frequencies mode using the ECIS[®]Z system (Applied BioPhysics). Attachment of the cells to the well surface was indicated by impedance plateau generation. Subsequently, T1/T2 CTL were added in a 5:1 ratio and impedance drop was measured for 24 h. 1% Triton X-100 served as a positive control. The area under the curve (AUC) of the measured impedance at 4000 Hz was determined using Graphpad Prism 10. Relative cell lysis was evaluated using the formula (positive control AUC/test AUC) $\times$ 100. Target cell lines were authenticated by DSMZ.

For flow cytometric assessment of cytotoxicity, CTL obtained at time points T1 or T2 were co-cultivated with K562 lymphoblasts for 3 h in a ratio of 5:1. Afterwards cells have been stained using Sytox AADvanced and CD8 BV421 and cytotoxicity has been quantified on a MACSQuant 10 Flow Cytometer (Miltenyi Biotec).

Caspase-3/7 activity assay

Caspase-3 and -7 activity was measured using the Caspase-Glo[®] 3/7 Assay (G8091, Promega Corporation), according to the manufacturer's instructions. Briefly, after co-incubation with CD8⁺ T cells, 5,000 K562 target cells were seeded in 100 μ L of culture medium in white-walled 96-well plates. Subsequently,

100 μ L of Caspase-Glo® 3/7 Reagent was added to each well. Plates were shaken at 400 rpm for 30 s and incubated at room temperature for 1 h. Luminescence was measured using a FilterMax F3 plate reader (Molecular Devices). Blanks containing culture medium and reagent without cells were included to determine background luminescence. Measurements were performed in triplicate.

ELISA

To quantify cytokine secretion, enzyme-linked immunosorbent assay (ELISA) was performed according to the protocol of the respective manufacturer (IFN γ : #430104; Granzyme B: #439207; Biolegend, San Diego, CA, USA | Perforin: #3465-1HP-2, Mabtec). Absorbance was measured on a Filtermax F3 using a plate-specific standard curve.

Oxygen consumption rate and extracellular acidification rate

Functional evaluation of mitochondrial oxidative phosphorylation and cellular glycolysis was conducted by extracellular flux analysis using a Seahorse HS mini (Agilent). About 150,000 T cells were plated on an eight-well HS mini plate (#103723-100, Agilent) precoated with poly-L-lysine (Biochrom, #L7240) in a final volume of 180 μ L Assay Medium (containing Seahorse RPMI supplemented with 1 mM sodium pyruvate, 2 mM glutamine and 5.5 mM glucose). All experiments were conducted in duplicates or triplicates. Quantification of extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) was performed in response to MitoStress Test (#103015-100, Agilent). To this end, final well concentrations of 1 μ M Oligomycin, 1 μ M FCCP/2.5 μ M BAM15 and 0.5 μ M rotenone/antimycin A were consecutively added through designated seahorse cartridge compound delivery ports.

Quantification of cellular glucose uptake

Glucose uptake was measured using the Glucose Uptake Glo Assay (Promega) according to the manufacturer's instructions. Briefly, 150,000 cells were incubated with 1 mM 2-deoxyglucose (2-DG) for 10 min at room temperature. After incubation, 50 μ L of each sample was transferred to a white luciferase plate. Control samples were prepared without 2-DG. Stop buffer, neutralization buffer, and luciferase detection reagent were added sequentially, and samples were incubated for 30 min at room temperature in the dark. Luminescence was measured using a SoftMax instrument, and raw data were exported for analysis. All samples were measured in duplicate.

Mitochondrial membrane potential

Mitochondrial membrane potential $\Delta\psi$ M was assessed using the membrane permeable lipophilic cationic carbocyanine dye JC1 according to the manufacturer's protocol (#701560, Cayman Chemical). Data were acquired on a FACS Canto II flow cytometer (BD Biosciences, Franklin Lakes, USA). In mitochondria with intact membrane potential, $\Delta\psi$ M-dependent JC1 accumulation leads to the formation of J-aggregates, emitting red fluorescence (~590 nm). Depolarization of the mitochondrial membrane potential results in lower cellular concentrations of the dye, forming green fluorescent monomeric forms of JC1 (~529 nm). $\Delta\psi$ M is depicted as the mean fluorescence intensity ratio of red/green. The uncoupling agent FCCP (Carbonyl cyanide-p-trifluoromethoxyphenylhydrazine), causing almost complete loss of $\Delta\psi$ M at doses of 20 μ M, served as a negative control.

Quantification of reactive oxygen species (ROS)

CellROX Green and MitoSOX red (C10492|M36008; Thermo Fisher Scientific) were used to quantify intracellular and mitochondrial ROS levels, respectively. Experiments were performed according to the manufacturer's instructions. Briefly, 2×10^5 cells were incubated in RPMI supplemented with 500 nM CellROX green for 30 min at 37 °C and 5% CO₂. For MitoSOX, cells were incubated in prewarmed HBSS supplemented with 0.2 μ M MitoSOX red for 15 min at 37 °C and 5% CO₂. Cells were immediately analyzed on a FACS Canto II flow cytometer (BD Biosciences). To estimate the peroxidase-mediated production of H₂O₂ in sorted cell populations, 10–20 $\times 10^5$ cells have been seeded in a 96-well plate and incubated with medium:luminol in a 4:1 ratio at room temperature for 5 min. Wells with medium only and wells with luminol, horse reddish peroxidase and H₂O₂ served as a negative or positive control, respectively. Luminescence was measured using a SoftMax instrument.

Antioxidant capacity

To evaluate cellular antioxidant capacity via quantification of intracellular reduced glutathione (GSH), 2×10^5 cells were incubated in PBS supplemented with 10 μ M ThiolTracker (T10095, Thermo Fisher) according to the manufacturer's protocol. Data were acquired on a FACS Canto II flow cytometer.

Analysis of mitochondrial mass

$\Delta\psi$ M-independent MitoTracker Green FM (#9074, Cell Signaling Technology) was used for flow cytometric determination of mitochondrial mass (2×10^5 cells, 200 nM MitoTracker, incubation: 15 min, 37 °C in the dark). Mitochondrial mass was obtained by quantification of mean fluorescence intensity (MFI) green on a FACS Canto II flow cytometer (BD Biosciences).

Flow cytometry

For extracellular CD8⁺ T cell staining, 2×10^5 PBMC were preincubated with 2.5 μ L Human TruStain FcX™ Fc Receptor Blocking Solution (#422302, BioLegend), followed by incubation with the designated antibody (PerCP anti-human CD8 (#344707 BioLegend), for a duration of 30 min on ice, protected from light. Intracellular assessment of Interferon- γ , Granzyme B and Perforin 1 was performed using FITC anti-human Interferon γ (#502506, BioLegend), BV421 anti-human Granzyme B (#396413, BioLegend) and APC anti-human Perforin 1 (#308111 BioLegend) after fixation and permeabilization using eBioscience™ Fixation/Permeabilization Concentrate, Diluent and Buffer (#00-5123-43 | #00-5223-56 | #00-8333-56, Invitrogen) as to the manufacturer's protocol.

Detection of MDSC was performed using 2×10^5 PBMC, preincubated with 2.5 μ L Human TruStain FcX™ Fc Receptor Blocking Solution (#422302, BioLegend). Cells were stained with PE anti-human CD15, Pacific Blue anti-human HLA-DR, PerCP anti-human CD14, and FITC anti-human CD11b (#301905, #307623, #325631, #301329; BioLegend).

Quantification of T-cell activation and exhaustion marker (CD25, Ce38, CD69, PD1, TIM3, and LAG3) was performed on magnetically separated CTL using the antibodies specified in the reagent and tools table. About 2×10^5 CTL were incubated with the designated antibodies for a duration of 30 min on ice, protected from light.

For quantification of cytolytic granules exocytosis, detection of plasma membrane incorporation of CD107a on CTL was performed. CTL were incubated as described above. After 20 h, CD107a PE (#328608, Invitrogen) was added and cells were stimulated using 1x Cell Activation Cocktail (#423301, Biolegend). After 1 h, 1:1000 Brefeldin A (#00-4506-51, Invitrogen) was added and cells were incubated for an additional 3 h at 37 °C. Afterwards, cells were washed and fixation, permeabilization and intracellular staining was performed as described above. For all extracellular stainings, antibodies have been diluted 1:100, for intracellular staining, a 1:40 dilution has been used.

Cells were analyzed on a FACS Canto II (BD Biosciences, USA) and on a MACSQuant 10 (Miltenyi, Germany). Data analyses were performed using the software FlowJo v10 (FlowJo, Ashland, USA).

Isolation of mitochondria

Mitochondria were isolated from primary human CD8⁺ T cells using the Mitochondria Isolation Kit, human (#130-094-532, Miltenyi Biotec) according to the manufacturer's protocol. In brief, isolated CD8⁺ T cells were resuspended in lysis buffer and homogenized using a 26G needle. Homogenized mitochondria were magnetically labeled with Anti-TOM22 Microbeads. Magnetic separation was performed on an AutoMACS Pro Separator.

Confocal microscopy

For microscopic imaging, CTL were incubated as described above. Subsequently, cells were harvested and seeded at a density of 0.25×10^6 in eight-well microscope slides (#80841, ibidi) coated with 0.01% poly-L-Lysine (#P4707, Sigma-Aldrich). Microscopy slides were centrifuged at 300×g for 5 min and incubated for 10 min at 37 °C to allow attachment of cells. After fixation with ice-cold 100% methanol (incubation for 20 min. at −20 °C), cells were washed with PBS and stained with MitoTracker Deep Red (#8778, Cell Signaling Technologies) according to manufacturer's instructions, fixed with ice-cold methanol at −20 °C for 20 min. Thereafter, cells were washed, incubated with 5% BSA for 1 h and immunostained with 1:50 anti-CD3 primary antibody (#SC20047, Santa Cruz Biotechnologies), and subsequent 1:2000 anti-mouse Alexa Fluor 488 antibody (#A21042, Invitrogen). Chamber inserts were removed and slides were mounted with mounting medium (ibidi, #50001). Confocal images were recorded on a Leica SP8 microscope based on an upright DM6 stand using solid state laser excitation at 488 and 638 nm with a Leica DFC365FX with Sony ICX285 interline CCD chip. Hybrid photo detectors with detection bands of 500–550 nm and 650–700 nm were used. Images were acquired with a 63×1.4 or a 40x PL APO oil immersion objective at a scan speed of 200 or 400 Hz with a pixel size of 0.075 μm and a frame size of 1024 × 1024 (for measuring mitochondrial length) or with a pixel size of 0.4 μm and a frame size of 512 × 512 (for determining mitochondrial translocation to the immune synapse). For each image, 3D stacks were recorded (Z-Stack size: 0.3–0.5 μm). Analysis was performed on projections of the image stacks. For image visualization, Leica Application Suite X (LASX) was used. Data has been uploaded to the BioStudies depository (www.ebi.ac.uk/biostudies, accession no. S-BIAD2197).

Evaluation of mitochondrial translocation to the immunological synapse and mitochondrial length

Mitochondrial translocation to the immunological synapse of cytotoxic T cells was determined by (1) approximation of CD3

(T-cell Receptor, TCR) and MitoTracker (mitochondria) and by (2) concentration of mitochondria to the immunological synapse in contrast to dispersed mitochondrial staining throughout the cell. CTL were directly isolated after blood withdrawal, activated using CD3/CD28 dynabeads at a bead:cell ratio of 1:8 and incubated at 37 °C and 5% CO₂ for 20 h. Immediately prior to fixation and staining as described above, dynabeads were magnetically removed. For each patient and each time point, at least three Z-stacks with maximum projection were evaluated by three individual scientists. Mean values of polarized/non-polarized cells per image were then used for statistical analysis. Evaluation of mitochondrial length has been performed as previously described (Triolo et al, 2023). In short, after acquisition of confocal images as specified above, Fiji software was used to generate 3D image projections, and the line function was applied to draw individual lines across each mitochondrion identified. Mitochondrial count per cell and mean mitochondrial area per cell were additionally quantified using the Mitochondrial Analyzer ImageJ plugin as previously described (Chaudhry et al, 2020).

LC-MS/MS-based untargeted metabolomics

For analyses of serum metabolites, blood was withdrawn after induction of anesthesia (T1), at the end of surgery (T2) and on the first postoperative day (T3). S-Monovette® Serum tubes (Cat.# 02.226.160, Sarstedt, Germany) were stored at an upright position for 30 min at room temperature to allow coagulation. Serum was collected at 15 °C, 2750 g/min for 10 min., immediately frozen and stored at −80 °C until analysis.

Untargeted metabolome analysis was carried out on a Nexera UHPLC system connected to a Q-TOF mass spectrometer (TripleTOF 6600, AB Sciex). Frozen samples were thawed, 25 μl of each sample were mixed with 125 μl MeOH and incubated for 10 min at 10 °C with shaking at 1000 rpm. After 5 min sonication, samples were centrifuged for 10 min at 13000 rpm at 10 °C. Chromatographic separation was achieved by using a HILIC UPLC BEH Amide 2.1 × 100, 1.7 μm column with 0.4 mL/min flow rate. The mobile phase consisted of 5 mM ammonium acetate in water (eluent A) and 5 mM ammonium acetate in acetonitrile/water (95/5, v/v) (eluent B). The following gradient profile was used: 100% B from 0 to 1.5 min, 60% B at 8 min and 20% B at 10 min to 11.5 min and 100% B at 12 to 15 min. About 5 μL aliquots per sample were injected into the UHPLC-TOF-MS. The autosampler was cooled to 10 °C and the column oven heated to 40 °C. A quality control (QC) sample—pooled from all samples—was injected after every ten samples. MS settings in the positive mode were: Gas 1 55, Gas 2 65, Curtain gas 35, Temperature 500 °C, Ion Spray Voltage 5500, and declustering potential 80. Changes for the negative mode were ion spray voltage −4500 and declustering potential −80. For both modes, the mass range of the TOF-MS scans were 50–2000 *m/z* and the collision energy was ramped from 15 to 55 V.

The “msconvert” from Proteo Wizard (Kessner et al, 2008) served to convert raw files to mzXML (de-noised by centroid peaks). Data processing and feature identification was performed via the bioconductor/R package xcms (Smith et al, 2006). More specifically, the matched Filter algorithm was used to identify peaks (full width at half maximum set to 7.5 s). Peaks were then grouped into features using the “peak density” method (Smith et al, 2006). Area under the peak was integrated to represent the abundance of features. The retention time was adjusted based on the peak groups

presented in most samples. To enable feature annotation to metabolites, the exact mass and MS2 fragmentation pattern of the measured features were compared to the records in HMDB (Wishart et al, 2018) and the public MS/MS spectra in MSIAL (Tsugawa et al, 2015), referred to as MS1 and MS2 annotation, respectively. All samples were pooled to generate one reference sample, which was measured seven times throughout the measurements. The reference samples were used to control the drifting effect of the mass spectrometer's signal, using a method adapted from EigenMS (Karpievitch et al, 2014). In detail, we used singular value decomposition of the reference measurements to estimate the variance contributed by the artifact reasons. These were removed from all measurements to get the normalized metabolite intensity matrix. Missing values were imputed with half of the limit of detection (LOD) methods. Paired *t*-test served to compare the features' intensity from T1 to T2. Significant enrichment of the metabolites in the groups was defined as a mean difference (mean diff.) >1.5 with a false-discovery-rate FDR <0.05. When MS2 spectra were acquired in our experiments, our MS2 fragmentation pattern was manually reviewed and compared to records in the public database or previously measured reference standards to evaluate the correctness of the annotation.

For functional analysis of untargeted metabolomics data, the online tool MetaboAnalyst was used as previously described (Pang et al, 2022). Peak intensities were uploaded including retention time and numeric mass (*M/z*) values. Features with a constant or single value across samples were deleted. Missing values were replaced by 1/5 of min positive values of their corresponding variables. A 25% interquartile range variance filter was applied for data filtering. The Mummichog 2.0 algorithm was used with *p* < 0.05 as a cut-off. The MFN human pathway library served for identification of significantly enriched pathways with at least two entries per pathway. Knowledge-driven integration of metabolomic and proteomic data was performed with the online tool MetaboAnalyst. Compounds and proteins with significant changes have been uploaded and joint pathway analysis has been calculated using all KEGG pathways. Tight integration was applied by combining queries.

Serum proteomics

Proteins from 1 µl sample were digested using the autoSP3 protocol (Müller et al, 2020) on an Agilent Bravo liquid handling system in a 96-well format. Samples were randomized on the plate. Briefly, samples were diluted in 100 mM ammonium bicarbonate including complete protease inhibitor cocktail (Roche). Proteins were denatured, reduced and alkylated by addition of a 4x buffer including TCEP, CAA, and SDS and heated at 95 °C for 10 min. A 1:1 mixture of hydrophobic and hydrophilic magnetic beads was added (Sera-Mag, Cytiva) and acetonitrile (ACN) filled up to a final concentration of 50% (v/v). Proteins were allowed to bind to beads while shaking at room temperature. Beads were washed twice with 80% ethanol (v/v) and once with ACN. Bead pellets were resuspended in 100 mM ammonium bicarbonate and trypsin protease (Sigma-Aldrich) added at an enzyme:protein ratio of 1:50 (w/w). Digestion was performed overnight at 37 °C. Peptides were acidified to a final concentration of 1% TFA (v/v). 0.5% of this solution was loaded on Evotips (Evosep). As quality control, four pooled serum samples (Pat1_T2, Pat2_T1, Pat3_T3, Pat7_T2, Pat8_T1, Pat9_T3, Pat13_T2, Pat14_T1, Pat15_T3, Pat19_T1, and Pat20_T2) were distributed on different positions on the plate.

LC-MS analysis was performed on a Evosep One LC (Evosep) using the 30 spd method with a 15 cm column (150 µm ID, 1.5 µm, PepSep). Eluted peptides were electrosprayed into an Exploris 480 mass spectrometer (Thermo Fisher Scientific). The mass spectrometer was operated in data-independent acquisition mode with a full scan with resolution *R* = 120,000 at *m/z* 200, *m/z* range between 380 and 980, 300% AGC target, 100 ms max. injection time; followed by 30 DIA windows at 20 *m/z* with an overlap of 1 *m/z* at resolution *R* = 30000 at *m/z* 200, 3000% AGC target, auto IT in centroid mode.

Data was analyzed by Spectronaut (version 18.0.230605.50606) in directDIA+ mode using the database uniprot_sprot_2022-01-07_HUMAN (20375 protein entries) downloaded in the software. Two missed cleavages were chosen, and Carbamidomethyl (C) as fixed modification as well as Acetyl (protein N-term) and Oxidation (M) as variable modifications. Samples were normalized and identified (Qvalue) set for precursor filtering.

mRNA quantification

Quantification of mRNA expression was performed on a Light-Cycler 480 (Roche) as described in detail previously (Möhnle et al, 2018; Hirschberger et al, 2019) in accordance with the MIQUE guidelines. In brief, the miRNeasy RNA Isolation Kit (#217004, Qiagen, Hilden, Germany) with on-column DNA digestion served for RNA isolation, which was performed as per the manufacturer's instructions. Quantity and purity of RNA were assessed on a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific). First-strand cDNA was synthesized with equal amounts of RNA using a Superscript III reverse transcriptase (Invitrogen), oligo (dT) primers and random hexamers (Qiagen). Ribosomal Protein L13a (RPL13A) and TATA box binding protein (TBP) served as reference genes in all experiments (Ledderose et al, 2011). Primers, probes and identifiers of multiplexing assays are given in Table EV1. Duplicate values were used for all analysis. The second derivative maximum method was applied to define quantification cycles by the LightCycler software. A Quantification cycle (Cq) cut-off was set for Cq 35; values beyond this cut-off were considered as unspecific.

Immunoblot

Cells were lysed in cell lysis buffer (#9803, Cell Signaling Technology) supplemented with a protease and phosphatase inhibitor (#5871 | #5870, Cell Signaling Technology). Equivalent protein concentrations were loaded onto polyacrylamide stacking gels (Mini-PROTEAN TGX 4-20%, #456-1094, Bio-Rad). After transfer, protein was blotted with 1:400 Total OXPHOS Human Antibody Cocktail (#ab110411, Abcam), 1:500 pDRP1, 1:500 DRP1, 1:1000 OPA1, and 1:1000 MFN2 antibodies (#3455S, #5391, #11925S, #67589, Cell Signaling Technology). As a loading control, 1:2000 beta-actin antibody (#5125, Cell Signaling Technology) was used. For mitochondrial isolates, 1:800 HSP60 (#4870, Cell Signaling Technology) served as a loading control.

Statistical analyses

Statistical analysis was performed using GraphPad Prism 10 (GraphPad Software, Inc., USA). Paired *t*-test or Wilcoxon matched-pairs signed-rank test, as appropriate, served for comparisons. Normal distribution was tested using the D'Agostino & Pearson and Shapiro-Wilk test. If not stated otherwise, data are

The paper explained**Problem**

Postoperative complications such as infections and wound healing disorders are common after major surgery. Up to 35% of patients suffer from nosocomial infections that delay recovery, and increase both mortality and overall healthcare costs. This is primarily caused by suppression of the adaptive immune system, particularly affecting cytotoxic T cells (CTL). The mechanism underlying this immunosuppression is unclear, and no therapeutic options are available.

Results

After major surgery, CTL exhibit a strongly reduced cytotoxic capability and their cytokine secretion is almost disrupted due to a blockade of cytolytic granules exocytosis. We found that this dysfunction is driven by systemic oxidative stress in the blood of surgical patients. We identified transiently emerging MDSC as the predominant source of this oxidative stress through exacerbated production of reactive oxygen species (ROS). These ROS overwhelm CTL antioxidant defenses and substantially damage CTL mitochondria. This results in impaired mitochondrial function with cell-intrinsic ROS production, leading to compromised ATP production and diminished CTL effector functions. Importantly, treatment with a mitochondria-targeted antioxidant (MitoTempo) improved mitochondrial integrity and CTL function in this model.

Impact

We uncovered a new mechanism of postoperative immune suppression, where MDSC-derived oxidative stress induces mitochondrial damage in CTL, creating an immunometabolic paralysis of CTL. Targeting oxidative stress with specific antioxidants may help restore CTL immunometabolism in surgical patients. These findings open up new therapeutic strategies that could lower the risk of infections, improve recovery, and reduce healthcare costs of patients after major surgery.

depicted as mean $\pm$ SEM or as box plots with mean, median, twenty-fifth and seventy-fifth percentiles and range, with dots indicating individual values. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Biological replicates and statistical parameters are reported in the figure legends.

Data availability

The datasets produced in this study are available in the following databases: Transcriptomics: Array Express, [E-MTAB-14974](https://www.ebi.ac.uk/biostudies/ArrayExpress/studies/E-MTAB-14974?query=E-MTAB-14974), <https://www.ebi.ac.uk/biostudies/ArrayExpress/studies/E-MTAB-14974?query=E-MTAB-14974>. Proteomics: ProteomeXchange Consortium via the PRIDE partner repository, PXS060723, <https://www.ebi.ac.uk/pride/archive/projects/PXD060723/>. Metabolomics: MassIVE Repository, MSV000097035, <https://massive.ucsd.edu/ProteoSAFe/private-dataset.jsp?task=b04359ba2b2c4278a344d855d5d1a85b>. Microscopy: BioImage Archive, S-BIAD2197, <https://www.ebi.ac.uk/biostudies/bioimages/studies/S-BIAD2197>.

The source data of this paper are collected in the following database record: [biostudies:S-SCDT-10_1038-S44321-025-00324-1](https://www.ebi.ac.uk/biostudies/S-SCDT-10_1038-S44321-025-00324-1).

Expanded view data, supplementary information, appendices are available for this paper at <https://doi.org/10.1038/s44321-025-00324-1>.

Peer review information

A peer review file is available at <https://doi.org/10.1038/s44321-025-00324-1>

References

- Albertsmeier M, Quaiser D, von Dossow-Hanfstringl V, Winter H, Faist E, Angele MK (2015) Major surgical trauma differentially affects T-cells and APC. *Innate Immun* 21:55–64
- Ananth AA, Tai L-H, Lansdell C, Alkayyal AA, Baxter KE, Angka L, Zhang J, Tanese de Souza C, Stephenson KB, Parato K et al (2016) Surgical stress abrogates pre-existing protective T cell mediated anti-tumor immunity leading to postoperative cancer recurrence. *PLoS One* 11:e0155947
- Bain CR, Myles PS, Corcoran T, Dieleman JM (2023) Postoperative systemic inflammatory dysregulation and corticosteroids: a narrative review. *Anaesthesia* 78:356–370
- Baixaui F, Martín-Cófreces NB, Morlino G, Carrasco YR, Calabia-Linares C, Veiga E, Serrador JM, Sánchez-Madrid F (2011) The mitochondrial fission factor dynamin-related protein 1 modulates T-cell receptor signalling at the immune synapse. *EMBO J* 30:1238–1250
- Beury DW, Carter KA, Nelson C, Sinha P, Hanson E, Nyandjo M, Fitzgerald PJ, Majeed A, Wali N, Ostrand-Rosenberg S (2016) Myeloid-derived suppressor cell survival and function are regulated by the transcription factor Nrf2. *J Immunol* 196:3470–3478
- Boomer JS, To K, Chang KC, Takasu O, Osborne DF, Walton AH, Bricker TL, Jarman 2nd SD, Kreisel D, Krupnick AS et al (2011) Immunosuppression in patients who die of sepsis and multiple organ failure. *JAMA* 306:2594–2605
- Botha AJ, Moore FA, Moore EE, Kim FJ, Banerjee A, Peterson VM (1995) Postinjury neutrophil priming and activation: an early vulnerable window. *Surgery* 118:358–364
- Buck MD, O'Sullivan D, Klein Geltink RI, Curtis JD, Chang C-H, Sanin DE, Qiu J, Kretz O, Braas D, van der Windt GJW et al (2016) Mitochondrial dynamics controls T cell fate through metabolic programming. *Cell* 166:63–76
- Chaudhry A, Shi R, Luciani DS (2020) A pipeline for multidimensional confocal analysis of mitochondrial morphology, function, and dynamics in pancreatic β -cells. *Am J Physiol Endocrinol Metab* 318:E87–E101
- Cornell TT, Sun L, Hall MW, Gurney JG, Ashbrook MJ, Ohye RG, Shanley TP (2012) Clinical implications and molecular mechanisms of immunoparalysis after cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 143:1160–1166.e1
- Dale DC, Boxer L, Liles WC (2008) The phagocytes: neutrophils and monocytes. *Blood* 112:935–945
- Das R, Chakrabarti O (2020) Mitochondrial hyperfusion: a friend or a foe. *Biochem Soc Trans* 48:631–644
- de Saint Basile G, Ménasché G, Fischer A (2010) Molecular mechanisms of biogenesis and exocytosis of cytotoxic granules. *Nat Rev Immunol* 10:568–579
- Desdín-Micó G, Soto-Herederó G, Mittelbrunn M (2018) Mitochondrial activity in T cells. *Mitochondrion* 41:51–57
- Dobson GP (2020) Trauma of major surgery: a global problem that is not going away. *Int J Surg* 81:47–54
- El-Benna J, Hurtado-Nedelec M, Marzaioli V, Marie J-C, Gougerot-Pocidallo M-A, Dang PM-C (2016) Priming of the neutrophil respiratory burst: role in host defense and inflammation. *Immunol Rev* 273:180–193
- Franke A, Lante W, Kurig E, Zöller LG, Weinhold C, Markewitz A (2006) Hyporesponsiveness of T cell subsets after cardiac surgery: a product of altered cell function or merely a result of absolute cell count changes in peripheral blood? *Eur J Cardiothorac Surg* 30:64–71

- Ghosh A, Khanam A, Ray K, Mathur P, Subramanian A, Poonia B, Kotttilil S (2023) CD38: an ecto-enzyme with functional diversity in T cells. *Front Immunol* 14:1146791
- Groth C, Hu X, Weber R, Fleming V, Altevogt P, Utikal J, Umansky V (2019) Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumour progression. *Br J Cancer* 120:16–25
- Guo C, Sun L, Chen X, Zhang D (2013) Oxidative stress, mitochondrial damage and neurodegenerative diseases. *Neural Regen Res* 8:2003–2014
- Hadaya J, Downey P, Tran Z, Sanaiha Y, Verma A, Shemin RJ, Benharash P (2021) Impact of postoperative infections on readmission and resource use in elective cardiac surgery. *Ann Thorac Surg* 113:774–782
- Hadaya J, Verma A, Marzban M, Sanaiha Y, Shemin RJ, Benharash P (2022) Impact of pulmonary complications on outcomes and resource use following elective cardiac surgery. *Ann Surg* 278:e661–e666
- Hirschberger S, Hübner M, Strauß G, Effinger D, Bauer M, Weis S, Giamarellos-Bourboulis EJ, Kreth S (2019) Identification of suitable controls for miRNA quantification in T-cells and whole blood cells in sepsis. *Sci Rep* 9:15735
- Hirschberger S, Strauß G, Effinger D, Marstaller X, Ferstl A, Müller MB, Wu T, Hübner M, Rahmel T, Mascolo H et al (2021) Very-low-carbohydrate diet enhances human T-cell immunity through immunometabolic reprogramming. *EMBO Mol Med* 13:e14323
- Hotchkiss RS, Monneret G, Payen D (2013) Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat Rev Immunol* 13:862–874
- Huang J, Zhao Y, Zhao K, Yin K, Wang S (2023) Function of reactive oxygen species in myeloid-derived suppressor cells. *Front Immunol* 14:1226443
- Huang S, Liu D, Sun J, Zhang H, Zhang J, Wang Q, Gan L, Qu G, Qiu J, Deng J et al (2022) Tim-3 regulates sepsis-induced immunosuppression by inhibiting the NF- κ B signaling pathway in CD4 T cells. *Mol Ther* 30:1227–1238
- Hübner M, Tomasi R, Effinger D, Wu T, Klein G, Bender M, Kilger E, Juchem G, Schwedhelm E, Kreth S (2019) Myeloid-derived suppressor cells mediate immunosuppression after cardiopulmonary bypass. *Crit Care Med* 47:e700–e709
- Huppa JB, Davis MM (2003) T-cell-antigen recognition and the immunological synapse. *Nat Rev Immunol* 3:973–983
- Jannasch O, Kelch B, Adolf D, Tammer I, Lodes U, Weiss G, Lippert H, Mroczkowski P (2015) Nosocomial infections and microbiologic spectrum after major elective surgery of the pancreas, liver, stomach, and esophagus. *Surg Infect* 16:338–345
- Jensen IJ, Sjaastad FV, Griffith TS, Badovinac VP (2018) Sepsis-induced T cell immunoparalysis: the ins and outs of impaired T cell immunity. *J Immunol* 200:1543–1553
- Karpievitch YV, Nikolic SB, Wilson R, Sharman JE, Edwards LM (2014) Metabolomics data normalization with EigenMS. *PLoS ONE* 9:e116221
- Kessner D, Chambers M, Burke R, Agus D, Mallick P (2008) ProteoWizard: open source software for rapid proteomics tools development. *Bioinformatics* 24:2534–2536
- Leadsham JE, Sanders G, Giannaki S, Bastow EL, Hutton R, Naeimi WR, Breitenbach M, Gourlay CW (2013) Loss of cytochrome c oxidase promotes RAS-dependent ROS production from the ER resident NADPH oxidase, Yno1p, in yeast. *Cell Metab* 18:279–286
- Ledderose C, Heyn J, Limbeck E, Kreth S (2011) Selection of reliable reference genes for quantitative real-time PCR in human T cells and neutrophils. *BMC Res Notes* 4:427
- Lisci M, Griffiths GM (2023) Arming a killer: mitochondrial regulation of CD8 T cell cytotoxicity. *Trends Cell Biol* 33:138–147
- Lumsdaine W, Easton RM, Lott NJ, White A, Malmanche TL, de, Lemmert K, Weber DG, Balogh ZJ (2014) Neutrophil oxidative burst capacity for peri-operative immune monitoring in trauma patients. *Injury* 45:1144–1148
- Margraf A, Ludwig N, Zarbock A, Rossaint J (2020) Systemic inflammatory response syndrome after surgery: mechanisms and protection. *Anesth Analg* 131:1693–1707
- Markewitz A, Faist E, Lang S, Endres S, Fuchs D, Reichart B (1993) Successful restoration of cell-mediated immune response after cardiopulmonary bypass by immunomodulation. *J Thorac Cardiovasc Surg* 105:15–24
- Markewitz A, Faist E, Lang S, Hültner L, Weinhold C, Reichart B (1996) An imbalance in T-helper cell subsets alters immune response after cardiac surgery. *Eur J Cardiothorac Surg* 10:61–67
- Martínez-Reyes I, Cuezva JM (2014) The H(+)-ATP synthase: a gate to ROS-mediated cell death or cell survival. *Biochim Biophys Acta* 1837:1099–1112
- Menges P, Kessler W, Kloecker C, Feuerherd M, Gaubert S, Diedrich S, van der Linde J, Hegenbart A, Busemann A, Traeger T et al (2012) Surgical trauma and postoperative immune dysfunction. *Eur Surg Res* 48:180–186
- Möhnlé P, Hirschberger S, Hinske LC, Briegel J, Hübner M, Weis S, Dimopoulos G, Bauer M, Giamarellos-Bourboulis EJ, Kreth S (2018) MicroRNAs 143 and 150 in whole blood enable detection of T-cell immunoparalysis in sepsis. *Mol Med* 24:54
- Monteseirín J, Bonilla I, Camacho J, Conde J, Sobrino F (2001) Elevated secretion of myeloperoxidase by neutrophils from asthmatic patients: the effect of immunotherapy. *J Allergy Clin Immunol* 107:623–626
- Müller T, Kalxdorf M, Longuespée R, Kazdal DN, Stenzinger A, Krijgsveld J (2020) Automated sample preparation with SP3 for low-input clinical proteomics. *Mol Syst Biol* 16:e9111
- Murphy MP (2009) How mitochondria produce reactive oxygen species. *Biochem J* 417:1–13
- Ohl K, Tenbrock K (2018) Reactive oxygen species as regulators of MDSC-mediated immune suppression. *Front Immunol* 9:2499
- Pang Z, Zhou G, Ewald J, Chang L, Hacariz O, Basu N, Xia J (2022) Using MetaboAnalyst 5.0 for LC-HRMS spectra processing, multi-omics integration and covariate adjustment of global metabolomics data. *Nat Protoc* 17:1735–1761
- Papadopoulos P, Pistiki A, Theodorakopoulou M, Christodouloupoulou T, Damoraki G, Goukos D, Briassoulis E, Dimopoulos I, Armaganidis A, Nanas S et al (2017) Immunoparalysis: clinical and immunological associations in SIRS and severe sepsis patients. *Cytokine* 92:83–92
- Peoples JN, Saraf A, Ghazal N, Pham TT, Kwong JQ (2019) Mitochondrial dysfunction and oxidative stress in heart disease. *Exp Mol Med* 51:1–13
- Pérez-Granda MJ, Barrio JM, Cuerpo G, Valerio M, Muñoz P, Hortal J, Pinto AG, Bouza E, Cardiovascular Infection Study Group (2024) Infectious complications following major heart surgery from the day of the surgery to hospital discharge. *BMC Infect Dis* 24:73
- Picard M, Shirihai OS (2022) Mitochondrial signal transduction. *Cell Metab* 34:1620–1653
- Quintana A, Schwindling C, Wenning AS, Becherer U, Rettig J, Schwarz EC, Hoth M (2007) T cell activation requires mitochondrial translocation to the immunological synapse. *Proc Natl Acad Sci USA* 104:14418–14423
- Rankin JS, Oguntolu O, Binford RS, Trochtenberg DS, Muhlbaier LH, Stratton CW (2011) Management of immune dysfunction after adult cardiac surgery. *J Thorac Cardiovasc Surg* 142:575–580
- Relja B, Land WG (2020) Damage-associated molecular patterns in trauma. *Eur J Trauma Emerg Surg* 46:751–775
- Smith CA, Want EJ, O'Maille G, Abagyan R, Siuzdak G (2006) XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. *Anal Chem* 78:779–787
- Stinchcombe JC, Majorovits E, Bossi G, Fuller S, Griffiths GM (2006) Centrosome polarization delivers secretory granules to the immunological synapse. *Nature* 443:462–465

- Sun Z, Mao A, Wang Y, Zhao Y, Chen J, Xu P, Miao C (2017) Treatment with anti-programmed cell death 1 (PD-1) antibody restored postoperative CD8⁺ T cell dysfunction by surgical stress. *Biomed Pharmacother* 89:1235–1241
- Taylor NJ, Nishtala A, Manakkat Vijay GK, Abeles RD, Auzinger G, Bernal W, Ma Y, Wendon JA, Shawcross DL (2013) Circulating neutrophil dysfunction in acute liver failure. *Hepatology* 57:1142–1152
- Tondera D, Grandemange S, Jourdain A, Karbowski M, Mattenberger Y, Herzig S, Da Cruz S, Clerc P, Raschke I, Merkwirth C et al (2009) SLP-2 is required for stress-induced mitochondrial hyperfusion. *EMBO J* 28:1589–1600
- Torrance HDT, Longbottom ER, Vivian ME, Lalabekyan B, Abbott TEF, Ackland GL, Hinds CJ, Pearse RM, O'Dwyer MJ (2018) Post-operative immune suppression is mediated via reversible, Interleukin-10 dependent pathways in circulating monocytes following major abdominal surgery. *PLoS ONE* 13:e0203795
- Torrance HDT, Pearse RM, O'Dwyer MJ (2016) Does major surgery induce immune suppression and increase the risk of postoperative infection? *Curr Opin Anaesthesiol* 29:376–383
- Triolo M, Wade S, Baker N, Khacho M (2023) Evaluating mitochondrial length, volume, and cristae ultrastructure in rare mouse adult stem cell populations. *STAR Protoc* 4:102107
- Tsugawa H, Cajka T, Kind T, Ma Y, Higgins B, Ikeda K, Kanazawa M, VanderGheynst J, Fiehn O, Arita M (2015) MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nat Methods* 12:523–526
- Vardhana SA, Hwee MA, Berisa M, Wells DK, Yost KE, King B, Smith M, Herrera PS, Chang HY, Satpathy AT et al (2020) Impaired mitochondrial oxidative phosphorylation limits the self-renewal of T cells exposed to persistent antigen. *Nat Immunol* 21:1022–1033
- Veglia F, Sanseviero E, Gabrilovich DI (2021) Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. *Nat Rev Immunol* 21:485–498
- Vourc'h M, Roquilly A, Asehnoune K (2018) Trauma-induced damage-associated molecular patterns-mediated remote organ injury and immunosuppression in the acutely ill patient. *Front Immunol* 9:1330
- Ward NS, Casserly B, Ayala A (2008) The compensatory anti-inflammatory response syndrome (CARS) in critically ill patients. *Clin Chest Med* 29:617–625
- Winterbourn CC, Kettle AJ, Hampton MB (2016) Reactive oxygen species and neutrophil function. *Annu Rev Biochem* 85:765–792
- Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, Vázquez-Fresno R, Sajed T, Johnson D, Li C, Karu N et al (2018) HMDB 4.0: the human metabolome database for 2018. *Nucleic Acids Res* 46:D608–D617
- Xiao W, Mindrinos MN, Seok J, Cuschieri J, Cuenca AG, Gao H, Hayden DL, Hennessy L, Moore EE, Minei JP et al (2011) A genomic storm in critically injured humans. *J Exp Med* 208:2581–2590
- Youle RJ, van der Bliek AM (2012) Mitochondrial fission, fusion, and stress. *Science* 337:1062–1065
- Zorov DB, Juhaszova M, Sollott SJ (2014) Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev* 94:909–950

Acknowledgements

We thank Katja Gieseke, Florian Gosselin, Bärbel Reincke, Gudrun Prangenberg, and Gabriele Gröger for their excellent technical assistance. We thank the Core Facility Bioimaging and the Protein Analysis Unit of the Biomedical Center of the LMU for providing excellent assistance in acquisition of microscopy images and proteomics, respectively. This work has been funded by the Deutsche Forschungsgemeinschaft (#447514737, S.K.; #542908663, M.H.), by the Friedrich-Baur-Stiftung (#22/22, M.H.) and the Else Kröner-Fresenius Stiftung (2021_EKEA.19, M.H.). S.H. ("FöFoLe + ", #024, LMU Munich, DFG) and M.H. (Program in Vascular Medicine, PRIME, funded by the

Deutsche Forschungsgemeinschaft #413635475) have been supported by the Medical and Clinician Scientist Program of the LMU Munich. The TripleTOF 6600 mass spectrometer was funded in part by the German Research Foundation (INST 95/1434-1 FUG).

Author contributions

Simon Hirschberger: Conceptualization; Resources; Data curation; Formal analysis; Supervision; Validation; Investigation; Visualization; Methodology; Writing—original draft; Project administration; Writing—review and editing. **Martin B Müller:** Resources; Formal analysis; Investigation; Methodology. **Hannah Mascolo:** Investigation. **Melissa Seitz:** Investigation. **Stefan Nibler:** Investigation. **David Effinger:** Investigation; Visualization; Methodology. **Kun Lu:** Resources. **Joscha Büch:** Resources. **Martin Bender:** Resources. **Tobias Kammerer:** Investigation. **Sven Peterß:** Resources. **Karin Kleigrew:** Resources; Investigation; Methodology. **Miriam Abele:** Resources; Investigation; Methodology. **Teresa Barth:** Resources; Investigation; Methodology. **Olga Kushnir:** Investigation. **Axel Imhof:** Resources; Investigation; Methodology. **Steffen Dietzel:** Resources; Investigation; Methodology. **Bernd Wegener:** Resources. **Ralf Sowa:** Investigation. **Frank Vogel:** Resources. **Peter Lamm:** Resources. **Roland Tomasi:** Resources. **Kristian Unger:** Investigation. **Markus Sperandio:** Resources. **Erich Kilger:** Resources. **Simone Kreth:** Conceptualization; Resources; Data curation; Supervision; Funding acquisition; Writing—original draft; Project administration; Writing—review and editing. **Max Hübner:** Conceptualization; Resources; Data curation; Formal analysis; Supervision; Funding acquisition; Validation; Investigation; Visualization; Methodology; Writing—original draft; Project administration; Writing—review and editing.

Source data underlying figure panels in this paper may have individual authorship assigned. Where available, figure panel/source data authorship is listed in the following database record: [biostudies:S-SCDT-10_1038-S44321-025-00324-1](https://biostudies.org/studies/S-SCDT-10_1038-S44321-025-00324-1).

Funding

Open Access funding enabled and organized by Projekt DEAL.

Disclosure and competing interests statement

The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. Creative Commons Public Domain Dedication waiver <http://creativecommons.org/publicdomain/zero/1.0/> applies to the data associated with this article, unless otherwise stated in a credit line to the data, but does not extend to the graphical or creative elements of illustrations, charts, or figures. This waiver removes legal barriers to the re-use and mining of research data. According to standard scholarly practice, it is recommended to provide appropriate citation and attribution whenever technically possible.

© The Author(s) 2025

Expanded View Figures

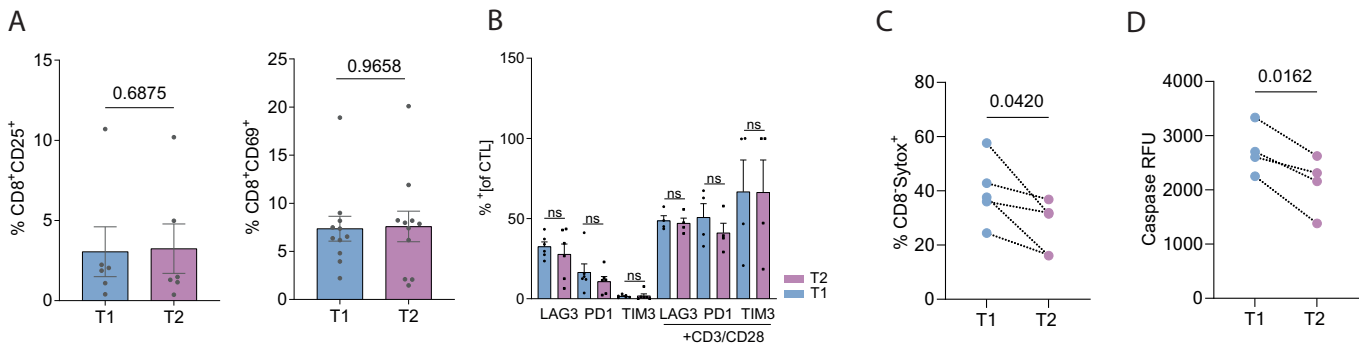


Figure EV1. Post surgical immunosuppression (PITC) of CTL.

(A) Flow cytometric analysis (FCA) of CD25 (left) and CD69 (right) staining and quantification in CTL, $n = 6/11$ individual patients. (B) FCA of lymphocyte-activation gene 3 (LAG3), programmed cell death protein 1 (PD1), and T-cell immunoglobulin and mucin-domain containing-3 (TIM3) staining and quantification as indicated, $n = 6/4$ (not activated/+CD3/CD28) individual patients. P values (left to right): 0.2842, 0.2188, 0.6875, 0.5494, 0.1250, and >0.9999 . (C) Quantification of relative cytotoxicity using a flow cytometric killing assay, $n = 5$ individual patients. (D) Quantification of caspase-3 activity reporter using a CTL-K562 killing assay, $n = 4$ individual patients. Data were represented as mean $\pm$ SEM with dots indicating individual values (A, B) or as individual data points (C, D). P values: as indicated, paired t -test or Wilcoxon matched-pairs signed-rank test, as appropriate.

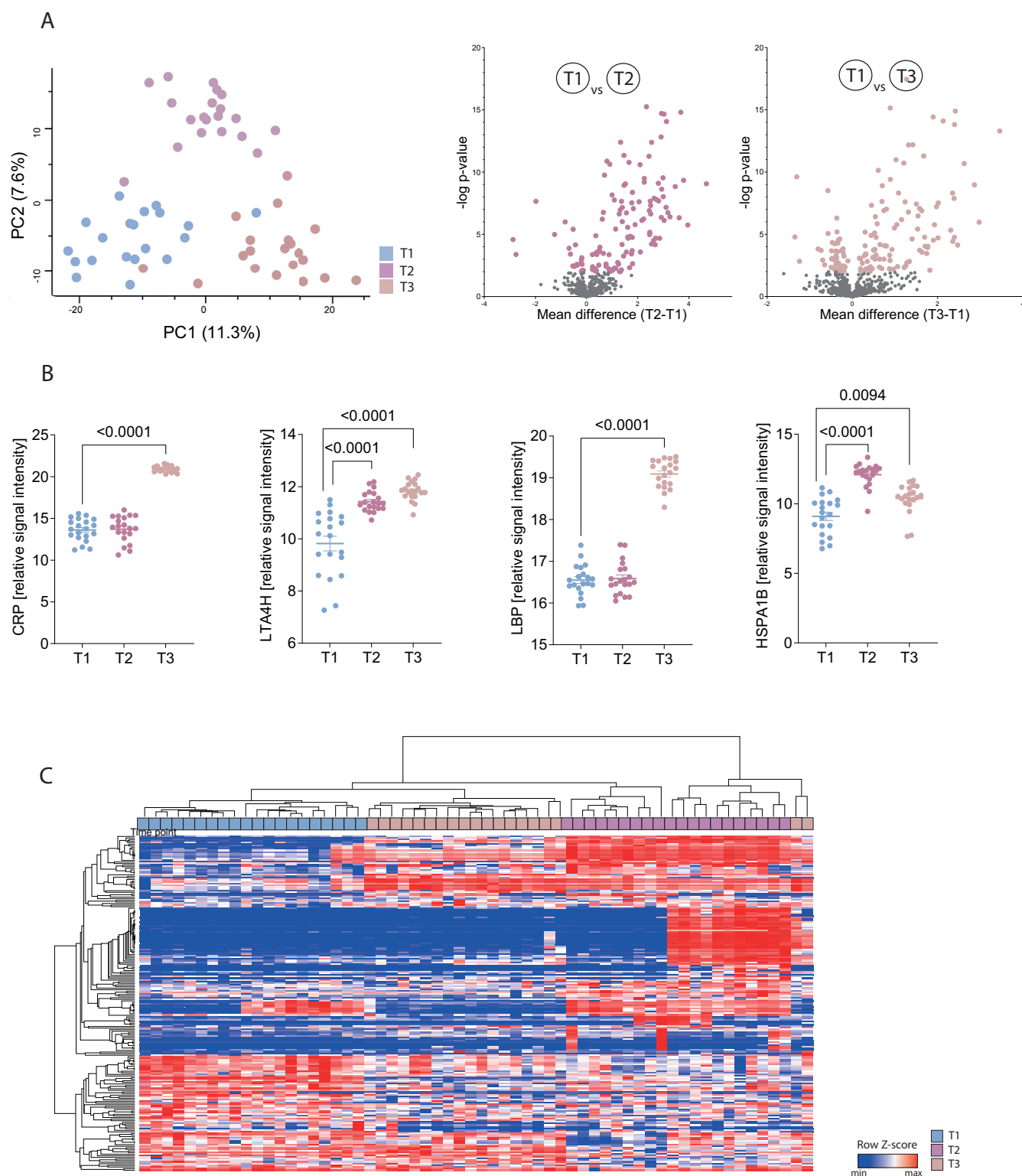


Figure EV2. Serum proteomics and metabolomics reveal an oxidative stress signature after major surgery.

(A) Principal component analysis depicting proteomic profiling results during the course of major surgery, time points as indicated (left) and Volcano plots for protein abundance of comparisons as noted on the top. Proteins with $-\log p$ value > 2 are colored, $n = 20$ individual patients. (B) Relative signal intensity of serum proteins during the course of major surgery as indicated, $n = 20$ individual patients. Mean $\pm$ SEM with dots representing individual values, p values as indicated, paired t -test. (C) Heat map depicting quantity of significantly differentially expressed serum metabolites. Raw z-scores and time points as indicated, $n = 20$ individual patients.

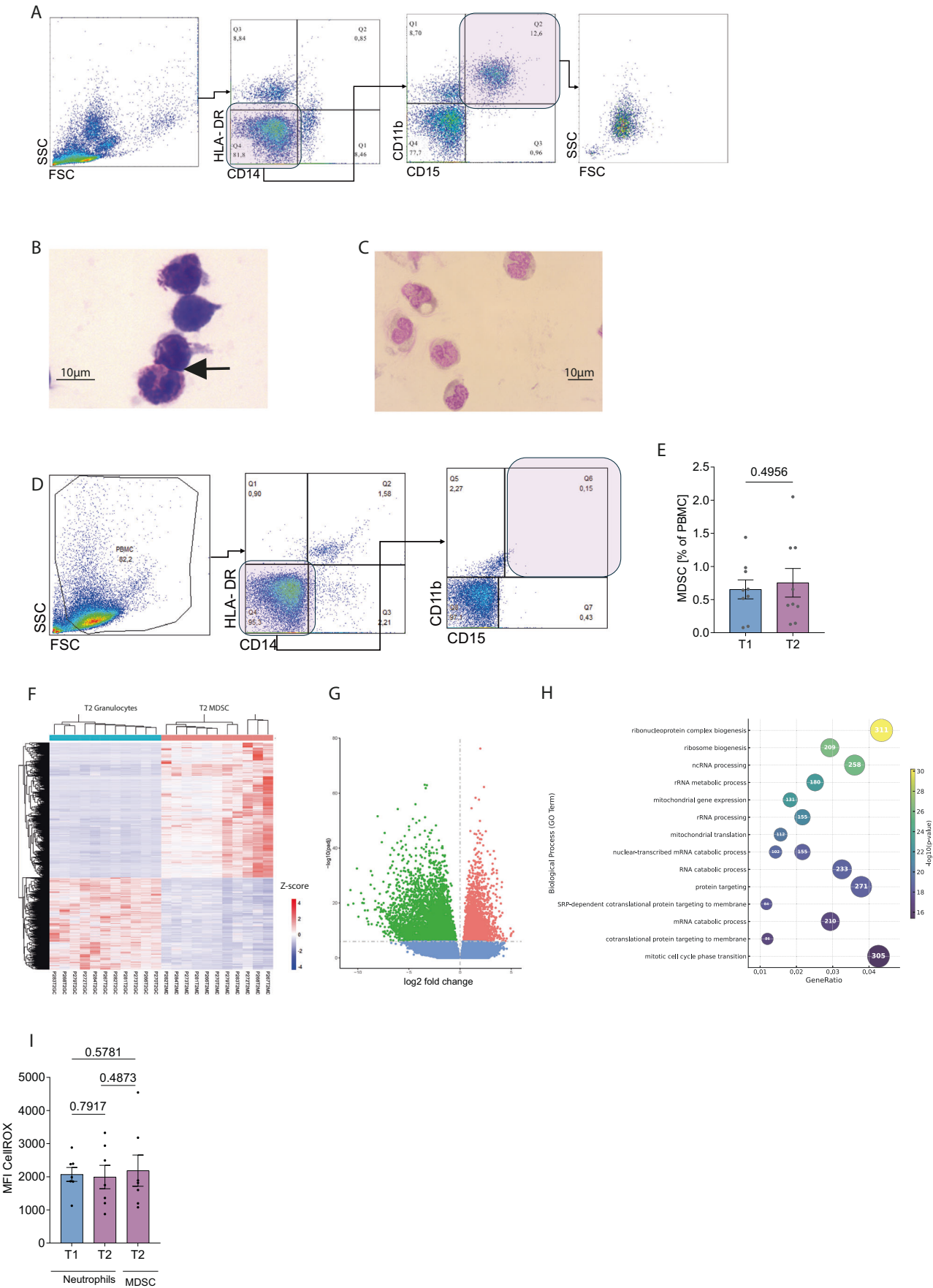


Figure EV3. Transiently emerging myeloid-derived suppressor cells.

(A) Flow cytometric gating strategy for immune phenotyping of CD14⁺HLA-DR⁺CD11b⁺CD15⁺ MDSC derived from patients during the course of major surgery. Representative scatter plots from a postoperative (T2) sample, as depicted in Fig. 3C. (B) Representative May-Grünwald-Giemsa staining of PBMC after major surgery. Transiently present cells depicting a granulocytic morphology (arrows). (C) Representative May-Grünwald-Giemsa staining of isolated MDSC, extracted from PBMC after major surgery using CD15 microbeads. (D) Flow cytometric analysis and immune phenotyping of PBMC from patients with minor surgery with gating strategy for detection of CD14⁺HLA-DR⁺CD11b⁺CD15⁺ MDSC. (E) Quantification of CD14⁺HLA-DR⁺CD11b⁺CD15⁺ MDSC as % of PBMC, derived from patients pre (T1) and post (T2) minor surgery, $n = 10$. (F) Heat map depicting quantity of all significantly differentially expressed transcripts of T2 MDSC vs T2 Granulocytes ($p < 0.0001$). Each pair of MDSC/granulocytes derived from the same patient. Red color indicates an upregulation, and downregulated genes are indicated by blue color. (G) Volcano plots visualizing differential gene expression T2 MDSC/ T2 granulocytes. Log2 fold-changes (x-axis) and $-\log_{10}$ adjusted p values (y-axis) are shown for each gene. The dashed horizontal line depicts the adj. p value threshold of $p < 0.0001$, significantly regulated genes in red (upregulated in MDSC) and green (downregulated in MDSC). (H) Gene Ontology (GO) analysis of differentially expressed pathways in T2 MDSC/ T2 Granulocytes. The gene counts are expressed by the numbers within the bubbles. (I) Flow cytometric analysis and quantification of intracellular ROS as indicated by CellROX green staining in sorted cell populations, time points as stated, $n = 6$ individual patients. Data were represented as mean $\pm$ SEM with dots representing individual values. P values as indicated, paired t -test.

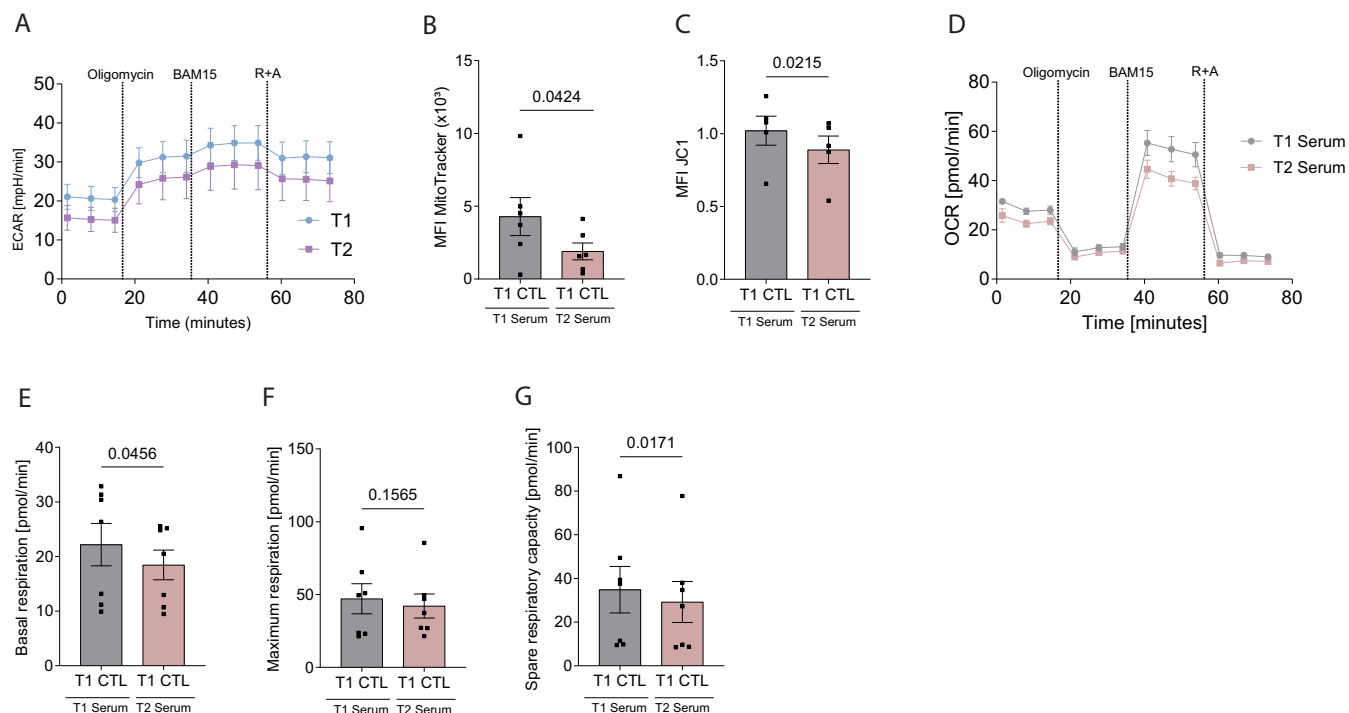


Figure EV4. T1 CTL cultivated with T2 MDSC or T2 serum mimic phenotype of T2 CTL mitochondrial damage.

(A) Pooled seahorse ECAR plot, mean $\pm$ SEM from five individual patients. (B-G) CTL of patients before major surgery (T1) co-cultivated with 20% serum from patients before (T1) or after (T2) major surgery. (B) FCA of MitoTracker green staining and quantification, $n = 6$ individual patients. (C) FCA of JC1 staining and quantification of red/green fluorescence ratio, $n = 5$ individual patients. (D) Representative seahorse OCR plot. (E-G) Seahorse quantification of (E) basal OCR, (F) maximal OCR, and (G) spare respiratory capacity in CTL, $n = 7$ from three individual patients. Data were represented as mean $\pm$ SEM with dots representing individual values. P values as indicated, paired t -test or Wilcoxon matched-pairs signed-rank test, as appropriate.

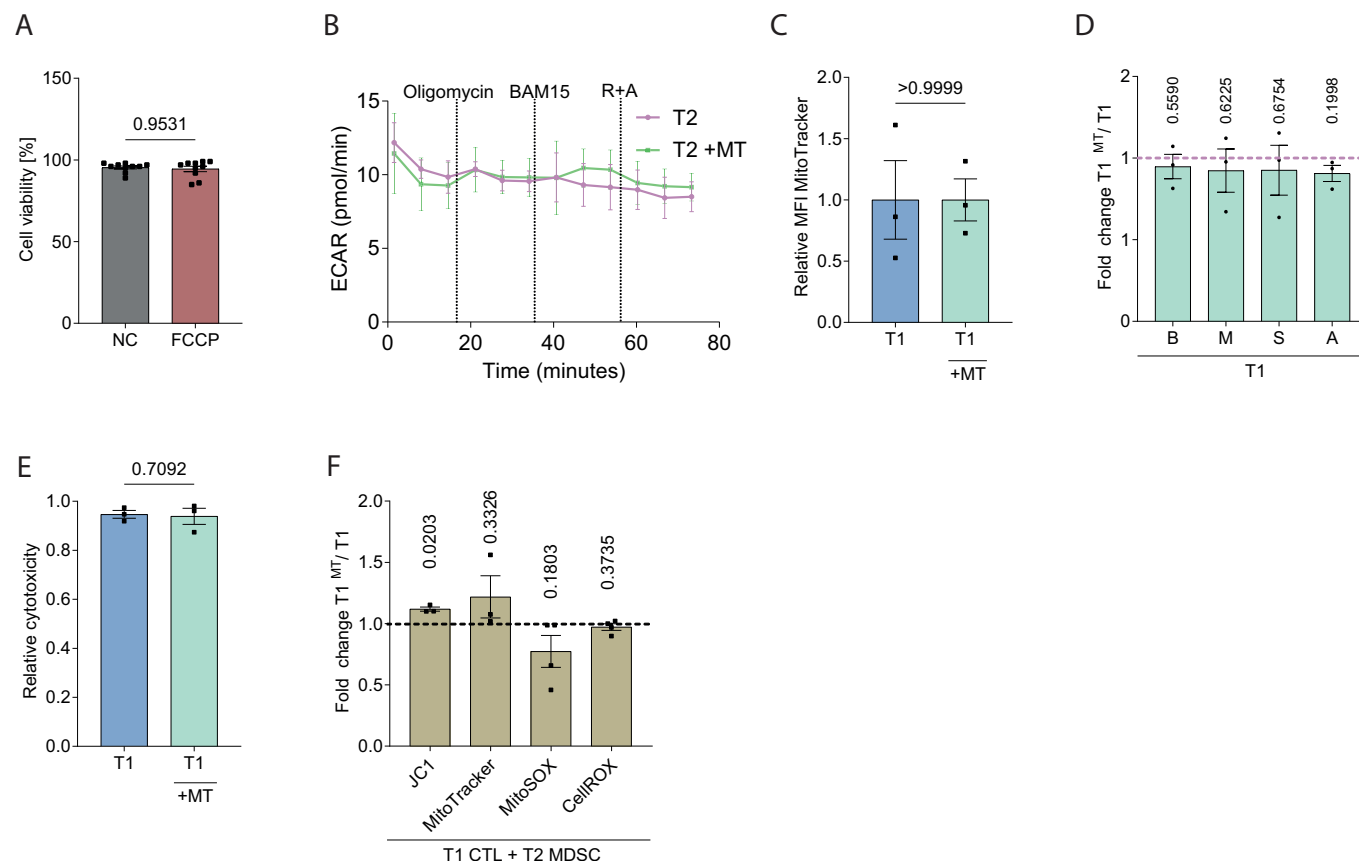


Figure EV5. No impact of FCCP on cell viability and no impact of mitochondrial antioxidative treatment using MitoTEMPO (MT) on T1 CTL immunometabolism.

(A) Cell viability of CTL treated with low-dose FCCP, $n = 10$, p value as indicated. (B) Pooled seahorse ECAR plot, mean $\pm$ SEM from six individual patients. (C) FCA of MitoTracker green and quantification in CTL, $n = 3$ individual patients. (D) Fold change of basal OCR (B), maximum OCR (M), spare respiratory capacity (S), and ATP production (A), $n = 3$ individual patients. (E) Quantification of relative CTL cytotoxicity using a flow cytometric killing assay, $n = 3$ individual patients. (F) Fold change of mitochondrial parameters as indicated comparing MT treated T1 CTL co-cultivated with MDSC versus untreated T1 CTL co-cultivated with MDSC, $n = 4/3/3/4$. Data were represented as mean $\pm$ SEM. P values as indicated, one-sample t -test (D) paired t -test or Wilcoxon matched-pairs signed-rank test, as appropriate.