Glycolytic imprinting of monocytes impairs HIF1α-driven migration of dendritic cells in tuberculosis

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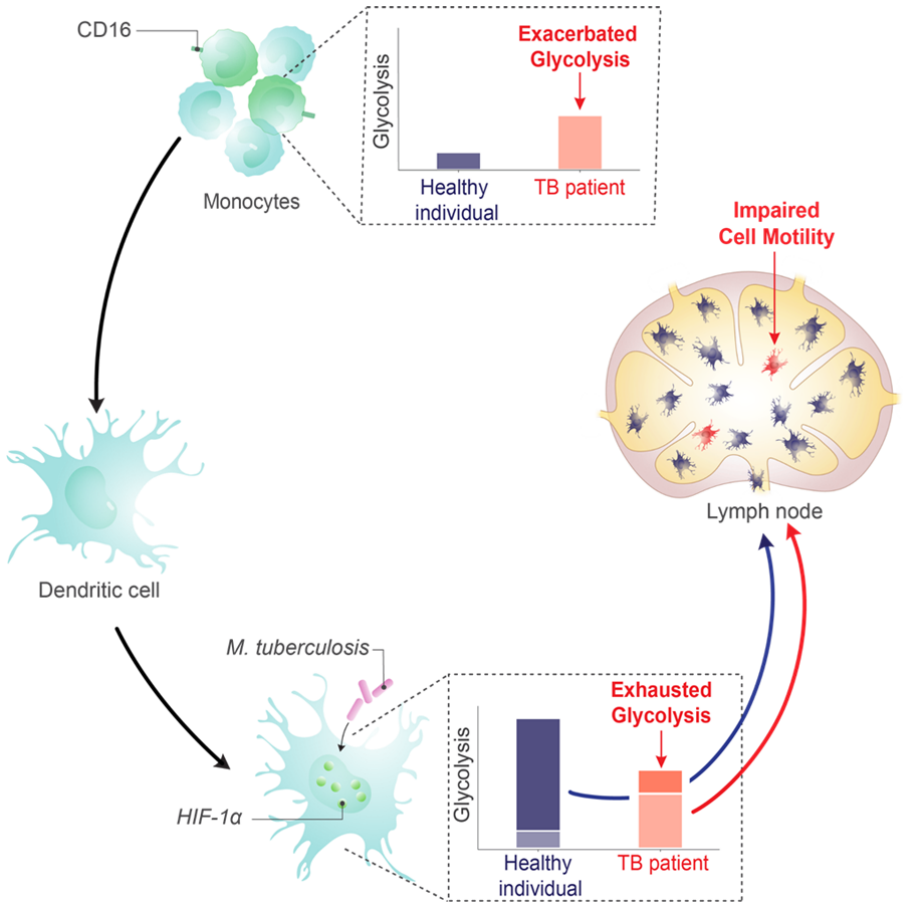
Graphical Abstract



Summary

Dendritic cells (DCs) are a key player in the host response to the tuberculosis (TB) agent, Mycobacterium tuberculosis (Mtb), which interfere with DC functions. However, how metabolic pathways influence DC function and activation in TB is unknown. We found that Mtb triggers both glycolysis and to a lesser extent mitochondrial respiration in human monocyte-derived DCs. This was accompanied by an increase in expression of the transcription factor HIF-1α and several glycolytic genes, dependent on TLR2 engagement by Mtb. Inhibition of either HIF-1α or glycolysis resulted in a strong alteration of the DCs migration capacities. In addition, DCs derived from monocytes of TB patients are deficient in inducing glycolysis and cell migration in response to Mtb, rescuable by induction of HIF-1α activity. Indeed, monocytes from TB patients are already highly glycolytic, differentiating into poorly migratory DCs. The data uncover a crucial role for glycolysis in DC migration and suggest that its modulation may be a promising avenue to better control TB.

Introduction

Tuberculosis (TB) remains a major global health problem, responsible for approximately 1.5 million deaths annually. The causative agent of TB, *Mycobacterium tuberculosis* (Mtb), is a highly successful pathogen that has evolved several strategies to weaken the host immune response. Although reliable immune correlates of protective immunity against Mtb are still not well-defined, it is widely accepted that Th1 cells contribute to protection by secreting IFN-γ and promoting antimycobacterial activity in macrophages[1]. Importantly, the induction of a strong Th1 immune response relies on the generation of immunogenic dendritic cells (DCs) with strong migratory properties[2–5]. Mtb has been shown to interfere with several DC functions, thus impairing the induction and development of adaptive immunity[6–8]. For instance, we and others previously reported that Mtb-exposed DCs have low capacity for mycobacterial antigen presentation and stimulation of Mtb-specific $CD4^+$ T cells[9–13]. Additionally, Mtb-infected DCs were reported to have impaired ability to migrate to lymph nodes *in vitro*[14,15] and in murine models[3,5]; however, the underlying molecular mechanisms of these phenotypes and their relevance to the migratory activity of monocyte-derived DCs in TB patients remain unknown.

Rapid, directed migration of DCs towards secondary lymphoid organs requires essential changes at the cellular and molecular levels[16]. Relatedly, the metabolic state of DCs is complex and varies according to cell origin, differentiation and maturation state as well as local microenvironment, among other factors[17–20]. Studies have reported that upon pathogen sensing, the transcription factor hypoxia-inducible factor-1α (HIF-1α) increases glycolysis, which promotes immunogenic functions of DCs, such as IL-12 production, costimulatory marker expression[21], and cell migration[22–24]. By contrast, it was shown that HIF-1α represses the proinflammatory output of LPS-stimulated DCs and can inhibit DC-induced T-cell responses in other settings[25]. To reconcile these disparate roles for HIF-1α, it has been proposed that the impact of metabolic pathway activation on DC function depends on DC subset[18]. To this point,

most prior studies have been conducted using murine conventional DCs and plasmacytoid DCs[19]. Recently, with the implementation of high-dimensional techniques, it was demonstrated that distinct metabolic wiring is associated with individual differentiation and maturation stages of DCs[26], highlighting the importance of defining the metabolic profile of specific subsets of DCs under particular physiological or pathological conditions[20]. Given the key role of DCs in the host response to TB, it is thus crucial to investigate DC metabolism in the context of Mtb infection[27].

We previously demonstrated that the TB-associated microenvironment, as conferred by the acellular fraction of TB patient pleural effusions, inhibits HIF-1α activity leading to a reduction in glycolytic and microbicidal phenotypes in macrophages[28]. Moreover, activation of HIF-1α enhances Mtb control at early times post-infection in mouse models[29], and this effect was associated with a metabolic switch of alveolar macrophages towards an M1-like profile[28]. Given that HIF-1α activation promotes protection at early stages of Mtb infection and given its role as a key regulator of DC migration and inflammation[30], we hypothesized that HIF-1α could affect the functionality of DCs in regulating the initiation and orchestration of the adaptive immune response to Mtb, a process known to be delayed upon Mtb infection[5,6]. Here, we show that HIF-1α-mediated glycolysis promotes DC activation and migration in the context of TB. Importantly, we report pathologically active glycolysis in monocytes from TB patients, which leads to poor glycolytic induction and migratory capacities of monocyte-derived DCs.

Results

Mtb impacts metabolism in human monocyte-derived DCs

To determine the impact of Mtb on the metabolism of human monocyte-derived DCs (Mo-DCs), we assessed metabolic parameters associated either with glycolysis or oxidative phosphorylation (OXPHOS) upon Mtb stimulation. Cells undergoing aerobic glycolysis are characterized by increased consumption of glucose and the production and release of lactate. We measured lactate release and glucose consumption in Mo-DCs stimulated for 24h with equivalent doses of either irradiated (iMtb) or viable Mtb. DCs treated with either iMtb or viable Mtb released increased levels of lactate and consumed more glucose than untreated DCs (**Figure 1A-B**). Consistently, both iMtb treatment and Mtb infection resulted in an increase in expression of the key glycolysis-activating regulator HIF-1α at both mRNA and protein levels (**Figure 1C-D**). Expression of the gene encoding the glycolytic enzyme lactate dehydrogenase A (*LDHA*), which catalyzes the conversion of lactate to pyruvate, was also increased in iMtb-treated or Mtb-infected DCs (**Figure 1E**). In agreement with their enhanced glycolysis profile, DCs stimulated with iMtb had increased expression of the glucose transporter GLUT1 (SLC2A1)[36] (**Figure 1F)**. Of note, *LDHA* and *GLUT1* are HIF-1α target genes, and their upregulation correlated with the increase in HIF-1α expression upon Mtb stimulation. Because irradiated and viable Mtb induced comparable activation of glycolysis, we subsequently performed all our assays with irradiated Mtb only in the rest of the study due to biosafety reasons. To assess changes in the mitochondria, we measured mitochondrial mass and morphology upon iMtb stimulation. We found a higher mitochondrial mass as well as larger individual mitochondria in iMtb-stimulated DCs compared to untreated DCs (**Figure 1G-H**). Thus, our data indicate that Mtb triggers both glycolysis and OXPHOS in Mo-DCs.

Mtb exposure shifts DCs to a glycolytic profile over OXPHOS

To further characterize the metabolic profile of iMtb-stimulated DCs, we next assessed the intracellular rates of glycolytic *vs.* mitochondrial ATP production using Seahorse technology. Bioenergetic profiles revealed that iMtb increased the rate of protons extruded over time, or proton efflux rate (PER) as well as the basal oxygen consumption rate (OCR) in Mo-DCs (**Figure 2A**). The measurements of basal extracellular acidification rate (ECAR) and OCR were used to calculate ATP production rate from glycolysis (GlycoATP) and mitochondrial oxidative phosphorylation (MitoATP). In line with our earlier findings, the ATP production rates from both glycolysis and mitochondrial respiration were augmented upon iMtb-stimulation (**Figure 2B**). Of note, the relative contribution of GlycoATP to overall ATP production was increased, while MitoATP contribution was decreased in iMtb-treated cells compared to untreated cells (**Figure 2C**). To further check the heterogeneity, we also evaluated the metabolism of DCs at single cell level using the SCENITH technology[35]. This method is based on the fact that a decrease in ATP levels is tightly coupled with a decrease in protein synthesis and display similar kinetics[35]. By treating the cells with glucose or mitochondrial respiration inhibitors and measuring their impact on protein synthesis by puromycin incorporation via flow cytometry, glucose and mitochondrial dependences can be quantified. Two additional derived parameters, glycolytic capacity and fatty acids and amino acids oxidation (FAO & AAO) capacity were also calculated. Similarly to Seahorse results, SCENITH technology revealed a lower reliance on OXPHOS in parallel with an increase in the glycolytic capacity of Mtb-stimulated DCs (**Figure 2D-E**). No differences were observed for glucose dependence and FAO & AAO capacity (**Figure 2D-E**). These results confirmed the reconfiguration of DC metabolism by iMtb treatment, with an increase in the relative glycolytic contribution to overall metabolism at the expense of the OXPHOS pathway. Together, metabolic profiling indicates that a metabolic switch toward aerobic glycolysis occurs in Mo-DCs exposed to Mtb.

Mtb triggers the glycolytic pathway through TLR2 ligation

Since Mtb is sensed by Toll-like receptors (TLR) 2 and 4[37], we investigated the contribution of these receptors to glycolysis activation in Mo-DCs upon Mtb stimulation. Using specific neutralizing antibodies for these receptors, we found that TLR2 ligation, but not that of TLR4, was required to trigger the glycolytic pathway, as reflected by a decrease in lactate release, glucose consumption and HIF-1α expression in iMtb-stimulated DCs treated with an anti-TLR2 antibody (**Figure 3A-C**). As a control, and as expected given the reliance on TLR4 for LPS sensing[38], lactate release and glucose consumption were abolished in LPS-stimulated DCs in the presence of neutralizing antibodies against TLR4 but not TLR2 (**Figure S1A-B**). Moreover, blockade of TLR2 also diminished glycolytic ATP production in iMtb-stimulated DCs without altering OXPHOS-associated ATP production (**Figure 3D**) or the size and morphology of mitochondria (**Figure S1C**), suggesting that TLR2 engagement by iMtb is required for the induction of glycolysis but not mitochondrial respiration. Interestingly, TLR2 ligation was also necessary for lactate release triggered by viable Mtb, (**Fig S1D**). To further confirm the involvement of TLR2 in the induction of glycolysis, we tested the effect of synthetic (Pam_3CSK_4) or mycobacterial (peptidoglycans, PTG) TLR2 agonists[39,40] and found that both ligands induced lactate release and glucose consumption in DCs (**Figure 3E-F**), without affecting cell viability (**Figure S1E**). Thus, our data indicate that Mtb induce glycolysis in Mo-DCs through TLR2 engagement.

HIF-1α is required for DC maturation upon iMtb stimulation but not for $CD4^+$ T lymphocyte polarization

To determine the impact of glycolysis on DC maturation and the capacity to activate T cells, we inhibited HIF-1α activity in iMtb-stimulated DCs. Treatment with the HIF-1α inhibitor PX-478 (PX) diminished lactate release and glucose consumption in iMtb-stimulated DCs without affecting cell viability at the indicated concentration (**Figure S2A-C**). These results were confirmed using echinomycin (Ech), another HIF-1α inhibitor (**Figure S2D-F**). HIF-1α inhibition by PX significantly abolished ATP

production associated with glycolysis without affecting absolute levels of OXPHOS-derived ATP production in iMtb-stimulated DCs (**Figure 4A and Figure S2G**). Although HIF-1α inhibitors did not affect the uptake of iMtb by DCs (**Figure S2H**), we observed a reduction in the expression of activation markers CD83 and CD86, but not in the inhibitory molecule PD-L1, upon treatment with PX (**Figure 4B**) or Ech (**Figure S3**). We then measured cytokine production in iMtb-stimulated DCs after HIF-1α inhibition and noted a reduction in TNF-α and an increase in IL-10 production by PX-treated cells (**Figure 4C**). To assess the capacity of DCs to activate T cells in response to mycobacterial antigens, we co-cultured DCs and autologous $CD4^+$ T cells from PPD^+ donors in the presence or absence of HIF-1α inhibitors and measured the overall IFN-γ and IL-17 production in culture supernatants as well as cell surface expression of T cell markers. We found no significant differences in the activation profile of autologous $CD4^+$ T cells in coculture with iMtb-stimulated DCs treated or not with PX (**Figure 4D-E and Figure S4**). We conclude that while HIF-1α is important for the maturation of iMtb-stimulated Mo-DCs, it does not influence their capacity to activate $CD4^+$ T cells, at least *in vitro*.

HIF-1α-mediated glycolysis triggers the motility of DCs upon iMtb stimulation

Since DC migration to lymph nodes is essential to initiate an adaptive immune response and glycolytic activity has been reported to control DC migration upon stimulation[22,23], we evaluated the migratory properties of iMtb-stimulated DCs in the presence of inhibitors of HIF-1α and glycolysis. First, we confirmed that PX and oxamate (OX), a well-established glycolysis inhibitor, diminished the glycolytic activity of iMtb-stimulated human Mo-DCs, as demonstrated by reduced lactate release (**Figure S2A and 5A**). Next, using a transwell migration assay, we found that PX and OX treatment significantly diminished the chemotactic activity of iMtb-stimulated human Mo-DCs in response to CCL21 (**Figure 5B**), a CCR7 ligand responsible for the migration of DCs into lymphoid organs. We also assessed the 3 dimentional (3D)

migration capacity of iMtb-stimulated DCs through a collagen matrix in which DCs use an amoeboid migration mode[41] and found that 3D migration was significantly impaired upon HIF-1α or glycolysis inhibition (**Figure 5C**). The role of glycolysis in the migration of iMtb-stimulated Mo-DCs was further confirmed using an additional glycolysis inhibitor, GSK2837808A, which reduced both the release of lactate by iMtb-stimulated Mo-DCs and their migration in response to CCL21 (**Figure S5A-B**). To further investigate the effects of glycolysis on cell migration, we turned to an *in vivo* model. Murine bone marrow-derived DCs (BMDCs) isolated and stimulated with iMtb in the presence or absence of PX or OX were labeled with CFSE and transferred into naïve mice (**Figure 5D**). Similarly to human Mo-DCs, iMtb stimulation increased glycolysis in BMDCs, which was inhibited by PX and OX treatment *in vitro* (**Figure S5C**). Three hours after the transfer of BMDCs into recipient mice, nearby lymph nodes were collected for DC quantification (**Figure 5D**). A higher number of adoptively transferred DCs (CFSE-labeled $CD11c^+$ cells) were detected in lymph nodes from mice that received iMtb-stimulated BMDCs compared to mice that received untreated BMDCs or iMtb-BMDCs treated with either PX or OX (**Figure 5E and Figure S5D**). Of note, we verified that CCR7 expression on iMtb-stimulated BMDCs was not affected by OX or PX treatment, so the effect could not be ascribed to downregulation of the chemokine receptor (**Figure S5E**). Therefore, we conclude that HIF-1α-mediated glycolysis is required for the successful migration of iMtb-stimulated DCs into lymph nodes.

Stabilization of HIF-1α promotes migration of tolerogenic DCs and DCs derived from TB patient monocytes

Since DC differentiation is skewed, at least partially, towards a tolerogenic phenotype during TB[42–44], we investigated whether tolerogenic DCs can be reprogrammed into immunogenic DCs by modulating their glycolytic pathway after iMtb stimulation. To this end, we generated tolerogenic Mo-DCs by adding dexamethasone (Dx) before stimulation with iMtb in the presence or absence of dimethyloxalylglycine (DMOG),

which stabilizes the expression of HIF-1α. HIF-1α expression is tightly regulated by prolyl hydroxylase domain containing proteins which facilitate the recruitment of the von Hippel-Lindau (VHL) protein, leading to ubiquitination and degradation of HIF-1α by the proteasomes[45]. DMOG inhibits the prolyl hydroxylase domain-containing proteins. Acquisition of the tolerogenic phenotype was confirmed by the lack of upregulation of costimulatory markers CD83 and CD86, as well as by increased PD-L1 expression in iMtb-DCs treated with Dx compared to control iMtb-DCs (**Figure S6A**). Moreover, Dx-treated DCs did not exhibit an increase in lactate release, consumption of glucose or induction of HIF-1α expression in response to iMtb, showing a high consumption of levels of glucose under basal conditions (**Figure 6A-B**). Of note, HIF-1α stabilization using DMOG restored the HIF-1α expression and lactate production in response to iMtb in Dx-treated DCs and increased the consumption of glucose (**Figure 6A-B**). Activation of HIF-1α also improved 3D amoeboid migration, as well as 2D migration capacity of DCs towards CCL21 of iMtb-stimulated Dx-treated DCs (**Figure 6C-D and Figure S6B**). Confirming the relevance of these findings to human TB patients, we found that iMtb-stimulated Mo-DCs from TB patients were deficient in their capacity to migrate towards CCL21 (**Figure 6E**) and in glycolytic activity, compared to Mo-DCs from healthy subjects (**Figure 6F-G**). Strikingly, stabilizing HIF-1α expression using DMOG in Mo-DCs from TB patients restored their chemotactic activity in response to iMtb (**Figure 6H**). These data indicate that the impaired migratory capacity of iMtb-stimulated tolerogenic DCs or TB patient-derived DCs can be restored via HIF-1α stabilization; thus, glycolysis is critical for DC function during TB in both murine and human contexts.

Glycolytic activation in CD16^{+} monocytes from TB patients leads to DCs with poor migration capacity

Since we observed differences in the metabolic activity of DCs derived from monocytes of TB patients when compared to healthy donors, we next focused on evaluating the

release of lactate by DC precursors from both subject groups during the first hours of DC differentiation with IL-4/GM-CSF. We found a high release of lactate by monocytes from TB patients compared to healthy donors after 1 h of differentiation (**Figure 7A**). Lactate accumulation increased in both subject groups after 24h with IL-4/GM-CSF (**Figure 7A**). Based on these differential glycolytic activities displayed by DC precursors from both subject groups at very early stages of the differentiation process, we decided to evaluate the *ex vivo* metabolic profile of monocytes using SCENITH. To this end, we assessed the baseline glycolytic capacity of the three main populations of monocytes: classical ($CD14^{+}$, $CD16^{-}$), intermediate ($CD14^{+}$, $CD16^{+}$), and non-classical ($CD14^{dim}$, $CD16^{+}$) monocytes. We found that both populations of CD16+ monocytes from TB patients had a higher glycolytic capacity than monocytes from healthy donors (**Figure 7B**). As we have previously demonstrated that $CD16^{+}$ monocytes from TB patients generate aberrant DCs[42], we hypothesized that the different metabolic profile of this monocyte subset could yield DCs with exhausted glycolytic activity and thus lower migration capacity upon Mtb exposure. To test this hypothesis, we treated with DMOG to increase the activity of HIF-1α during the first 24h of monocyte differentiation from healthy donors, leading to an exacerbated increase in lactate release at early stages of the differentiation (**Figure 7C**). Such early addition of DMOG to healthy monocytes resulted in the generation of DCs (6 days with IL-4/GM-CSF) characterized by equivalent levels of CD1a as control DCs, with a significant decrease in the expression of DC-SIGN (**Figure 7D**). In terms of activation marker expression, DCs differentiated from DMOG-pretreated cells responded to iMtb by upregulating CD86 at higher levels compared to control cells, with an accompanying trend towards reduced upregulation of CD83 (**Figure 7E**). We also observed that DCs from DMOG pretreated-cells exhibited a lower migratory capacity in response to iMtb (**Figure 7F**), reminiscent of the 2D migration capacities of Mo-DCs from TB patients. Altogether, our data suggest that the activated glycolytic status of pathological monocytes from TB patients leads to the generation of DCs with low motility in response to Mtb.

Discussion

In this study, we provide evidence for the role of HIF-1α-mediated glycolysis in promoting the migratory capacity of DCs upon encounter with Mtb. Our approach to quantify the *ex vivo* metabolism of monocytes shows that $CD16^+$ monocytes from TB patients display a pathological glycolytic activity that may result in the generation of DCs with poor migratory capacities in response to Mtb. Our results suggest that under extensive chronic inflammatory conditions, such as those found in TB patients, circulating monocytes may be metabolically preconditioned to differentiate into DCs with low immunogenic potential.

Upon Mtb infection of naive mice, initial accumulation of activated $CD4^+$ T cells in the lung is delayed, occurring between 2-3 weeks postinfection[3,46]. The absence of sterilizing immunity induced by TB vaccines (such as BCG) has been proposed to result from delayed activation of DCs and the resulting delay in antigen presentation and activation of vaccine-induced $CD4^+$ T-cell responses[47]. In this context, it was demonstrated that Mtb-infected Mo-DCs recruited to the site of infection exhibit low CCR7 expression and impaired migration to lymph nodes compared to uninfected Mo-DCs[48]. Additionally, Mo-DCs have been found to play a key role in transporting Mtb antigens from the lung to the draining lymph node, where conventional DCs present antigens to naive T cells[49]. The migratory capacity of responding DCs is thus of paramount importance to the host response to Mtb infection.

In this study, we found that Mtb exposure triggers glycolysis in Mo-DCs, which promotes their migration capacity in a HIF-1α-dependent manner. Recently, it was shown that glycolysis was required for CCR7-triggered murine DC migration in response to LPS[22–24]. Glycolysis was also reported to be required for the migration of other immune cells such as macrophages[50] and regulatory T cells[51]. Consistently, we show that inhibition of HIF-1α-dependent glycolysis impairs human Mo-DC migration

upon Mtb stimulation. The linkage between cellular metabolism and migratory behavior are supported by studies that have elucidated how glycolysis can be mechanically regulated by changes in the architecture of the cytoskeleton, ultimately impacting the activity of glycolytic enzymes[52,53]. In addition, interesting links between cellular mechanics and metabolism have been previously described for DCs, highlighting the potential to alter DC mechanics to control DC trafficking and consequently T cell priming[16]. However, studies focused on the molecular mechanisms by which metabolic pathways impact the machinery responsible for cell movement in the context of TB infection will be required to more fully understand potential handles for therapeutic manipulation.

We demonstrated that tolerogenic DCs induced by dexamethasone as well as DCs derived from TB patient monocytes exhibit lower lactate release and impaired trafficking toward CCL21 upon Mtb stimulation; both of these phenotypes could be rescued by stabilization of HIF-1α expression. To our knowledge, this is the first study addressing the metabolic status and migratory activity of Mo-DCs from TB patients. With respect to the metabolism of tolerogenic DCs broadly, our results are consistent with reported data showing that DC tolerance can be induced by drugs promoting OXPHOS, such as vitamin D and dexamethasone[54–56]. It was interesting to note that, although migration of tolerogenic DCs did not increase upon Mtb stimulation, it was increased under basal conditions, which is in agreement with previous data showing a high steady-state migration capacity of putatively tolerogenic DCs[57].

It has been widely demonstrated that immune cells have the ability to switch to glycolysis following engagement of TLRs[58]. Our work showed that TLR2 ligation by either viable or irradiated Mtb was necessary to trigger glycolysis in DCs, at least at early times post-stimulation. It remains to be elucidated whether persistent interaction between DCs and Mtb might lead to an attenuation in glycolysis over time, as has been

reported for macrophages[59]. In this regard, our data demonstrates that chronic Mtb infection leads to monocytes bearing an exacerbated glycolytic status which results in DCs with exhausted glycolytic capacity. Although DCs stimulated with iMtb in the presence of HIF-1α inhibitor exhibited differences in activation markers and cytokine profile, we found that they were still able to activate $CD4^+$ T cells from PPD+ donors in response to iMtb. These findings complement previous evidence showing that LPS-induced mature DCs inhibit T-cell responses through HIF-1α activation in the presence of glucose, leading to greater T cell activation capacity in low glucose contexts such as at the synapsis between DCs and T cells[25]. In this work, we did not detect an impact on T cell activation upon HIF-1α inhibition in DCs, but we observed a clear reduction in their migration capacity that may limit or delay DC encounters with T cells *in vivo*, leading to poor T cell activation in the lymph nodes. In this regard, mouse studies have shown that DC migration directly correlates with T cell proliferation[60]. However, we cannot rule out the possibility that other $CD4^+$ T cell subsets (such as regulatory T cells), CD1-restricted T cells, and/or $CD8^+$ T cell subsets could be differentially activated by iMtb-stimulated DCs lacking HIF-1α activity.

Three different populations of human monocytes have been identified: classical ($CD14^+$, $CD16^-$), intermediate ($CD14^+$, $CD16^+$), and non-classical ($CD14^{dim}$, $CD16^+$) monocytes[61]. These monocyte subsets are phenotypically and functionally distinct. Classical monocytes readily extravasate into tissues in response to inflammation, where they can differentiate into macrophage-like or DC-like cells[62]; intermediate monocytes are well-suited for antigen presentation, cytokine secretion, and differentiation; and non-classical monocytes are involved in complement and Fc gamma-mediated phagocytosis and their main function is cell adhesion[63,64]. Unlike non-classical monocytes, the two $CD14^+$ monocyte populations are known to extravasate into tissues and thus are likely to act as precursors capable at giving rise to Mo-DCs in inflamed tissues. However, the DC differentiation capacity of the intermediate

population is still not well defined. We previously demonstrated that monocytes from TB patients generate aberrant DCs, and that CD16$^+$ monocytes generate aberrant DCs upon treatment with GM-CSF and IL-4[42]. Here, we demonstrated that glycolysis seems to play a dual role during DC differentiation from monocytes, on the one side, being required for fully differentiated-DC migration to lymph nodes in response to Mtb and, on the other side, leading to DCs with poor iMtb-responsive migratory capacity if activated during the onset of DC differentiation. Similarly, we found that CD16$^+$ cells from TB patients display a pathologically activated glycolytic status. Additionally, we showed that monocytes from TB patients are not only enriched in CD16$^+$ cells, but also display an altered chemokine receptor expression profile[65], demonstrating that both phenotype and function of a given monocyte subset may differ under pathological conditions. While it is difficult to determine whether the heightened glycolytic profile of monocytes may limit their differentiation into DCs *in vivo*, we provided evidence that an increase in HIF1α-mediated glycolysis in precursors leads to the generation of cells with poor ability to migrate in response to CCL21 *in vitro.* In line with this observation, a recent paper revealed a significant increase in the glycolytic capacity occurs during the first 24h of monocyte differentiation towards a tolerogenic DC phenotype, as induced by vitamin D3[26], highlighting the detrimental role of an early activated inflammatory profile in DC precursors. A possible explanation for these effects may be found in lactate accumulation in monocytes during DC differentiation. Lactate signaling in immune cells leads to metabolic alterations in DCs that program them to a regulatory state[66], and lactate has also been shown to suppress DC differentiation and maturation[18]; thus, excessive precursor glycolytic activity may result in DCs biased toward regulatory functions.

Taken together, our data provide novel insights into the immunometabolic pathways involved in the trafficking of DCs to the lymph nodes, which may be targetable to generate sterilizing vaccine-induced immunity against TB. To this end, we propose that

the development of tools aiming to improve the migratory capacity of DCs by fostering their HIF-1α-mediated glycolytic activity may increase the effectiveness of preventive strategies for TB, especially given that the number of DCs to reach the lymph node was shown to be a critical parameter for the outcome of DC-based vaccination[60]. Understanding how Mtb rewires the innate immune response at the early stages is crucial for the design of preventive and therapeutic vaccines, as well as novel therapies and disease biomarkers.

Methods and Protocols

Chemical Reagents

LPS from Escherichia coli O111:B4 was obtained from Sigma-Aldrich (St. Louis, MO, USA). Dexamethasone (Dx) was from Sidus (Buenos Aires, Argentina). PX-478 2HCL was purchased from Selleck Chemicals (Houston, USA) and DMOG from Santa Cruz, Biotechnology (Palo Alto, CA, USA). Additionally, GSK2837808A was purchased from Cayman Chemical (Michigan, USA) together with echinomycin and sodium oxamate.

Bacterial strain and antigens

Mtb H37Rv strain was grown at 37°C in Middlebrook 7H9 medium supplemented with 10% albumin-dextrose-catalase (both from Becton Dickinson, New Jersey, USA) and 0.05% Tween-80 (Sigma-Aldrich). The Mtb γ-irradiated H37Rv strain (NR-49098) was obtained from BEI Resource (NIAID, NIH, USA).

Preparation of monocyte-derived DCs

Buffy coats from healthy donors were prepared at Centro Regional de Hemoterapia Garrahan (Buenos Aires, Argentina) according to institutional guidelines (resolution number CEIANM-664/07). Informed consent was obtained from each donor before blood collection. Monocytes were purified by centrifugation on a discontinuous Percoll gradient (Amersham, Little Chalfont, UK) as previously described[31]. Then, monocytes were allowed to adhere to 24-well plates at $5x10^5$ cells/well for 1h at 37°C in warm RPMI-1640 medium (ThermoFisher Scientific, Waltham, MA). The mean purity of adherent monocytes was 85 % (range: 80-92%). The medium was then supplemented to a final concentration of 10% Fetal Bovine Serum (FBS, Sigma-Aldrich), human recombinant Granulocyte-Macrophage Colony-Stimulating Factor (10 ng/ml, GM-CSF, Peprotech, New Jersey, USA) and IL-4 (20 ng/ml, Biolegend, San Diego, USA). Cells were allowed to differentiate for 5-7 days (DC-SIGN$^+$ cells in the culture > 90%).

DC stimulation

DCs were stimulated with either irradiated Mtb (iMtb) or viable Mtb at equivalent OD_{600} doses for 24h at 37 ºC. The cells were washed three times, and their phenotype and functionality were evaluated together with survival of activated cells; cell number and viability were determined by either trypan blue exclusion assays or MTT. Infections were performed in the biosafety level 3 (BSL-3) laboratory at the Unidad Operativa Centro de Contención Biológica (UOCCB), ANLIS-MALBRAN (Buenos Aires), according to the biosafety institutional guidelines.

DC treatments

When indicated, neutralizing monoclonal antibodies (mAb), or their corresponding isotype antibodies as mock controls, were added 30 min prior to DC stimulation to inhibit TLR2 (309717, Biolegend) or TLR4 (312813, Biolegend). In addition, DCs were incubated with PX-478 (20μM) or Echinomycin (1nM) with the purpose of inhibiting HIF-1α activity, DMOG (50μM) to stabilize HIF-1α, and oxamate (20mM) or GSK2837808A (20μM) to inhibit glycolysis. DC stimulation with iMtb occurred 30 min after treatment without drug washout.

In figure 6 and S6, dexamethasone-induced tolerogenic dendritic cells (Dx-DC) were generated by incubating DCs with 0.1μM of dexamethasone for 1h. Thereafter, cells were washed, and complete medium was added. Tolerogenic Dx-DCs were then stimulated or not with iMtb in the presence or not of DMOG (50μM).

Determination of metabolite concentrations

Lactate production and glucose concentrations in the culture medium was measured using the spectrophotometric assays Lactate Kit and Glicemia Enzimática AA Kit both from Wiener (Argentina), which are based on the oxidation of lactate or glucose, respectively, and the subsequent production of hydrogen peroxide[32]. The consumption

of glucose was determined by assessing the reduction in glucose levels in culture supernatants in comparison with RPMI 10% FBS. The absorbance was read using a Biochrom Asys UVM 340 Microplate Reader microplate reader and software.

Quantitative RT-PCR

Total RNA was extracted with Trizol reagent (Thermo Fisher Scientific) and cDNA was reverse transcribed using the Moloney murine leukemia virus reverse transcriptase and random hexamer oligonucleotides for priming (Life Technologies, CA, USA). The expression of the genes *HIF-1α* and *LDH-A* was determined using PCR SYBR Green sequence detection system (Eurogentec, Seraing, Belgium) and the CFX Connect Real-Time PCR Detection System (Bio-Rad, CA, USA). Gene transcript numbers were standardized and adjusted relative to eukaryotic translation elongation factor 1 alpha 1 (*EeF1A1*) transcripts. Gene expression was quantified using the ΔΔCt method. Primers used for RT-PCR were as follows: EeF1A1 Fwd: 5'- TCGGGCAAGTCCACCACTAC -3' and Rev: 5'-CCAAGACCCAGGCATACTTGA-3'; HIF-1α Fwd: 5'-ACTAGCCGAGGAAGAACTATGAA-3' and Rev: 5'-TACCCACACTGAGGTTGGTTA-3'; and LDH-A Fwd: 5'-TGGGAGTTCACCCATTAAGC-3' and Rev: 5'-AGCACTCTCAACCACCTGCT-3'.

Immunofluorescence analysis

FITC-, PE- or PerCP.Cy5.5-labelled mAbs were used for phenotypic analysis of the following cell-surface receptor repertoires: FITC-anti-CD1a (clone HI149, eBioscience), PE-anti-DC-SIGN (clone 120507, RD System), PerCP.Cy5.5-anti-CD86 (clone 374216, Biolegend), FITC-anti-CD83 (clone HB15e, eBioscience), PE-anti-PD-L1 (clone MIH1, BD Pharmingen) and in parallel, with the corresponding isotype control antibody. Approximately $5x10^5$ cells were seeded into tubes and washed once with PBS. Cells were stained for 30 min at 4ºC and washed twice. Additionally, cells were stained for 40 min at 4°C with fluorophore-conjugated antibodies PE-anti-Glut1 (clone 202915

R&D Systems, Minnesota, USA) and in parallel, with the corresponding isotype control antibody. For HIF-1α determination, DCs were permeabilized with methanol and incubated with PE-anti-HIF-1α (clone 546-16, Biolegend). Stained populations were gated according to forward scatter (FSC) and side scatter (SSC) analyzed on FACScan (Becton Dickinson). Isotype matched controls were used to determine auto-fluorescence and non-specific staining. Analysis was performed using the FCS Express (De Novo Software) and results were expressed as median fluorescence intensity (MFI) or percentage of positive cells.

Soluble cytokines determinations

Supernatants from DC populations or DC-T cell cocultures were harvested and assessment of TNF-α, IL-10, IL-17A or IFN-γ production was measured by ELISA, according to manufacturers' instructions (eBioscience). The detection limit was 3 pg/ml for TNF-α and IL-17A, 6 pg/ml for IFN-γ, and 8 pg/ml for IL-10.

$CD4^+$ T cell activation assay

Specific lymphocyte activation (recall) assays were carried out in cells from tuberculin purified protein derivate-positive skin test (PPD^+) healthy donors by culturing DC populations and autologous T cells at a ratio of 10 T cells to 1 DC in round bottom 96-well culture plates for 5 days as detailed previously[9]. The numbers of DCs were adjusted to live cells before the start of the co-cultures. After 5 days, $CD4^+$ T cell subsets were identified by immunolabeling according to the differential expression of CCR4, CXCR3, and CCR6 as previously reported[33]: $CXCR3^+CCR4^-CCR6^-$ (Th1), $CXCR3^-CCR4^+CCR6^-$ (Th2), $CXCR3^-CCR4^+CCR6^+$ (Th17), and $CXCR3^+CCR4^-CCR6^+$ (Th1* or Th1/Th17). The fluorochrome-conjugated antibodies used for flow cytometry analysis were CD4-FITC (clone A161A1, Biolegend), CXCR3-PE-Cy7 (clone G025H7, Biolegend), CCR4-PerCPCy5.5 (clone 1G1, BD Bioscience),

CCR6-APC (clone 11A9, BD Bioscience), and CD3-APC-Cy7 (clone HIT3a, Biolegend). A viability dye, Zombie Violet (Biolegend), was used to exclude dead cells. Fluorescence Minus One (FMO) control was used to set proper gating for CXCR3-PE-Cy7, CCR4-PerCPCy5.5, and CCR6-APC detection. Cells were analyzed by fluorescence-activated cell sorting (FACS), using the BD FACSCANTO cytometer and FlowJo™ Software (BD Life Sciences).

Chemotactic activity of DCs

Each DC population (4 x 10^5 cells in 75 µl) was placed on the upper chamber of a transwell insert (5 µm pore size, 96-well plate; Corning), and 230 µL of media (RPMI with 0.5 % FCS) with human recombinant CCL21 (200ng/ml) (Peprotech) was placed in the lower chamber. After 3 h, cells that had migrated to the lower chamber were removed and analyzed. The relative number of cells migrating was determined on a flow cytometer using Calibrite beads (BD Biosciences), where a fixed number of beads was included in each sample and the number of cells per 1,000 beads was evaluated. Data were normalized to the number of initial cells.

***In vivo* migration assay**

DCs were differentiated from bone marrow precursors obtained from naive BALB/c mice in the presence of murine GM-CSF (10ng/ml) and IL-4 (10ng/ml) both from Biolegend for 7 days. After differentiation, DCs were treated with oxamate (20mM) or PX-478 (10uM) and stimulated with iMtb. After 24h, DCs were stained with CFSE (5µM) and inoculated intradermically in the inguinal zone of naïve BALB/c mice. 3h post-injection, inguinal lymph nodes close to the site of inoculation were harvested and cells were stained with fluorophore-conjugated antibody PE-anti-CD11c (clone HL3, BD Pharmingen). Analysis was performed using the FlowJo Software and results were expressed as the percentage of $CFSE^+/CD11c^+$ cells.

3D migration assay

$0.5x10^5$ DCs were seeded on top of fibrillar collagen matrices polymerized from Nutragen 2mg/ml, 10% v/v MEM 10X (MEM invitrogen, Carlsbad, CA), UltraPure distilled water and 4-6% v/v bicarbonate buffer (pH=9) 7.5%. After 24h, cellular migration was quantified by taking images using an inverted microscope (Leica DMIRB, Leica Microsystems, Deerfield, IL) and the software Metamorph, as described previously[34]. Alternatively, in a similar manner, matrices were polymerized using Collagen (Sigma-Aldrich, C9791-10MG) in figure 5C. After 24h of cellular migration, matrices were fixed with PFA 4% during 30 min at room temperature and stained with DAPI (Cell signaling). Collagen was removed and membranes were mounted with DAKO. Images were taken by confocal microscopy (FluoView FV 1000), and cells were counted per field.

Measurement of cell respiration with Seahorse flux analyzer

Bioenergetics were determined using a seahorse XFe24 analyzer. ATP production rates and relative contribution from the glycolysis and the OXPHOS were measured by the Seahorse XF Real-Time ATP Rate Assay kit. DCs (2×10^5 cells/well) were cultured in 3 wells per condition. The assay was performed in XF Assay Modified DMEM. Three consecutive measurements were performed under basal conditions and after the sequential addition of oligomycin and rotenone/antimycin (Agilent, USA). Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were measured. Mitochondrial ATP production rate was determined by the decrease in the OCR after oligomycin addition. On the other hand, the complete inhibition of mitochondrial respiration with rotenone plus antimycin A, allows accounting for mitochondrial-associated acidification, and when combined with proton efflux rate (PER) data, allows calculation of Glycolysis ATP production rate. All OCR and ECAR values were normalized. Briefly, before the assay brightfield imaging was performed. Cellular area

per condition was calculated by ImageJ software and imported into Wave (Agilent) using the normalization function.

SCENITH™ assay

SCENITH experiments were performed as previously described[35] using the SCENITH kit containing all reagents and anti-puromycin antibodies (www.scenith.com). Briefly, DCs or PBMCs were treated for 40 min at 37°C in the presence of the indicated inhibitors of various metabolic pathways and puromycin. After the incubation, puromycin was stained using fluorescently labeled anti-Puromycin monoclonal antibody (clone R4743L-E8) with Alexa Fluor 647 or Alexa Fluor 488 and analyzed by flow cytometry. For metabolic analysis of monocyte subsets, PBMCs were labelled with PE-anti-CD16 (clone 3G8, Biolegend) and PECy7-anti-CD14 (clone HCD14, Biolegend) mAbs. The impact of the various metabolic inhibitors was quantitated as described[35].

Transmission electron microscopy

DCs were fixed in 2.5% glutaraldehyde / 2% paraformaldehyde (EMS, Delta-Microscopies) dissolved in 0.1 M Sorensen buffer (pH 7.2) for 1h at room temperature, and then preserved in 1% paraformaldehyde (PFA) dissolved in Sorensen buffer. Adherent cells were treated for 1h with 1% aqueous uranyl acetate then dehydrated in a graded ethanol series and embedded in Epon. Sections were cut on a Leica Ultracut microtome and ultrathin sections were mounted on 200 mesh onto Formvar carbon coated copper grids. Finally, thin sections were stained with 1% uranyl acetate and lead citrate and examined with a transmission electron microscope (Jeol JEM-1400) at 80 kV. Images were acquired using a digital camera (Gatan Orius).

Changes of mitochondrial mass

Mitochondrial mass was determined in DCs by fixing the cells with PFA 4% and labeling them with the probe MitoSpy Green FM (Biolegend). Green fluorescence was analyzed by flow cytometry (FACScan, BD Biosciences).

Patient blood donors

TB patients were diagnosed at the División Tisioneumonología, Hospital F.J.Muñiz (Buenos Aires, Argentina) by the presence of recent clinical respiratory symptoms, abnormal chest radiography, and positive culture of sputum or positive sputum smear test for acid-fast-bacilli. Written, informed consent was obtained according to Ethics Committee from the Hospital Institutional Ethics Review Committee. Exclusion criteria included HIV positive patients and the presence of concurrent infectious diseases or comorbidities. Blood samples were collected during the first 15 days after commencement of treatment. All tuberculous patients had pulmonary TB (Table I).

Table I. Demographic and clinical characteristics of TB patients

Age, years (range)	35 (19-67)
Gender, %	M, 87% F, 13%
Nationality, %	Argentina, 70% Bolivia, 19% Paraguay, 4% Peru, 7%
TB disease localization, %	Pulmonary, 94% Pulmonary + Extrapulmonary, 6%
AFB* in sputum, %	3+, 21% 2+, 14% 1+, 52% -, 13%
Leukocyte count, mean ± SEM, cell/µL	8433 ± 606

Lymphocyte mean ± SEM, %	17 ± 2
Monocyte mean ± SEM, %	8 ± 0,7

* Acid-fast-bacilli (AFB) in sputum: -, 1+, 2+, 3+ are defined according to the International Union Against Tuberculosis and Lung Disease (IUATLD)/ World Health Organization (WHO) quantification scale

Statistical analysis

All values are presented as the median ± SEM of 3-13 independent experiments. Each independent experiment corresponds to 1 donor. For Seahorse assays, OCR and PER values are shown as mean ± SD. Comparisons between unpaired experimental conditions were made using either ANOVA for parametric data or Friedman test for non-parametric data followed by Dunn's Multiple Comparison Test. Comparisons between paired experimental conditions were made using the two-tailed Wilcoxon Signed Rank Test for non-parametric data or T test for parametric data. A p-value of 0.05 was considered significant.

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AUTHOR CONTRIBUTIONS

Conceptualization & methodology: MM, FF, MFQ, MV, CV and LB. Investigation: MM, MJ, ZV, JB, JLMF, MG, SM, MBV, FF, VGP, MV, CV and LB. Resources: SI, RM, LC, PGM, DP, RA, CV, MdCS and LB. Writing: MM, GL-V, RA, MdCS, ON, CV and LB. Visualization: MM, FF, CV and LB. Funding acquisition: ON, RA, CV and LB. Corresponding authors: LB and CV are responsible for ownership and responsibility that are inherent to aspects of this study.

DECLARATION OF INTERESTS

The authors have declared that no conflict of interest exists.

References

1. Weiss, G. & Schaible, U. E. Macrophage defense mechanisms against intracellular bacteria. *Immunol. Rev.* **264**, 182–203 (2015).

2. Khader, S. A. *et al.* Interleukin 12p40 is required for dendritic cell migration and T cell priming after Mycobacterium tuberculosis infection. *J. Exp. Med.* (2006). doi:10.1084/jem.20052545

3. Wolf, A. J. *et al.* Mycobacterium tuberculosis infects dendritic cells with high frequency and impairs their function in vivo. *J. Immunol.* (2007).

4. Cooper, A. M. Cell-Mediated Immune Responses in Tuberculosis. *https://doi.org/10.1146/annurev.immunol.021908.132703* **27**, 393–422 (2009).

5. Lai, R. *et al.* CD11b+ Dendritic Cell–Mediated Anti–Mycobacterium tuberculosis Th1 Activation Is Counterregulated by CD103+ Dendritic Cells via IL-10. *J. Immunol.* **200**, 1746–1760 (2018).

6. Urdahl, K. B., Shafiani, S. & Ernst, J. D. Initiation and regulation of T-cell responses in tuberculosis. *Mucosal Immunol.* **4**, 288–293 (2011).

7. Chandra, P., Grigsby, S. J. & Philips, J. A. Immune evasion and provocation by Mycobacterium tuberculosis. *Nat. Rev. Microbiol. 2022 2012* **20**, 750–766 (2022).

8. Ernst, J. D. Mechanisms of M. tuberculosis Immune Evasion as Challenges to TB Vaccine Design. *Cell Host Microbe* **24**, 34–42 (2018).

9. Balboa, L. *et al.* Monocyte-derived dendritic cells early exposed to Mycobacterium tuberculosis induce an enhanced T helper 17 response and transfer mycobacterial antigens. *Int. J. Med. Microbiol.* **306**, 541–553 (2016).

10. Wolf, A. J. *et al.* Initiation of the adaptive immune response to Mycobacterium tuberculosis depends on antigen production in the local lymph node, not the lungs . *J. Exp. Med.* (2008). doi:10.1084/jem.20071367

11. Balboa, L. *et al.* Mycobacterium tuberculosis impairs dendritic cell response by altering CD1b, DC-SIGN and MR profile. *Immunol. Cell Biol.* (2010). doi:10.1038/icb.2010.22

12. Harding, C. V. & Boom, W. H. Regulation of antigen presentation by Mycobacterium tuberculosis: a role for Toll-like receptors. *Nat. Rev. Microbiol.* **8**, 296–307 (2010).

13. Srivastava, S., Grace, P. S. & Ernst, J. D. Antigen export reduces antigen presentation and limits T cell control of M. tuberculosis. *Cell Host Microbe* **19**, 44–54 (2016).

14. Roberts, L. L. & Robinson, C. M. Mycobacterium tuberculosis infection of human dendritic cells decreases integrin expression, adhesion and migration to chemokines. *Immunology* (2014). doi:10.1111/imm.12164

15. Rajashree, P., Supriya, P. & Das, S. D. Differential migration of human monocyte-derived dendritic cells after infection with prevalent clinical strains of Mycobacterium tuberculosis. *Immunobiology* **213**, 567–575 (2008).

16. Currivan, E., Finlay, D. & Moreira, D. Dendritic cells metabolism: a strategic path to improve antitumoral DC vaccination. *Clin. Exp. Immunol.* **208**, 193–201 (2022).

17. Basit, F., Mathan, T., Sancho, D. & De Vries, J. M. Human dendritic cell subsets undergo distinct metabolic reprogramming for immune response. *Front. Immunol.* **9**, 2489 (2018).

18. Wculek, S. K., Khouili, S. C., Priego, E., Heras-Murillo, I. & Sancho, D. Metabolic Control of Dendritic Cell Functions: Digesting Information. *Front. Immunol.* **10**, 775 (2019).

19. Du, X., Chapman, N. M. & Chi, H. Emerging Roles of Cellular Metabolism in Regulating Dendritic Cell Subsets and Function. *Front. Cell Dev. Biol.* **6**, (2018).

20. Møller, S. H., Wang, L. & Ho, P. C. Metabolic programming in dendritic cells tailors immune responses and homeostasis. *Cell. Mol. Immunol.* **19**, 370–383 (2022).

21. Everts, B. & Pearce, E. J. Metabolic control of dendritic cell activation and function: Recent advances and clinical implications. *Frontiers in Immunology* (2014). doi:10.3389/fimmu.2014.00203

22. Guak, H. *et al.* Glycolytic metabolism is essential for CCR7 oligomerization and dendritic cell migration. *Nat. Commun.* (2018). doi:10.1038/s41467-018-04804-6

23. Liu, J. *et al.* CCR7 Chemokine Receptor-Inducible lnc-Dpf3 Restrains Dendritic Cell Migration by Inhibiting HIF-1α-Mediated Glycolysis. *Immunity* **50**, 600-615.e15 (2019).

24. Everts, B. *et al.* TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. *Nat. Immunol.* **15**, 323 (2014).

25. Lawless, S. J. *et al.* Glucose represses dendritic cell-induced T cell responses. *Nat. Commun.* **8**, (2017).

26. Adamik, J. *et al.* Distinct metabolic states guide maturation of inflammatory and tolerogenic dendritic cells. *Nat. Commun. 2022 131* **13**, 1–19 (2022).

27. Kumar, R. *et al.* Immunometabolism of Phagocytes During Mycobacterium tuberculosis Infection. *Front. Mol. Biosci.* **6**, 105 (2019).

28. Marin Franco, J. L. *et al.* Host-derived lipids from tuberculous pleurisy impair macrophage microbicidal-associated metabolic activity. *Cell Rep.* (2020).

29. Baay-Guzman, G. J. *et al.* Dual role of hypoxia-inducible factor 1 α in experimental pulmonary tuberculosis: Its implication as a new therapeutic target. *Future Microbiol.* (2018). doi:10.2217/fmb-2017-0168

30. Liu, J., Zhang, X., Cheng, Y. & Cao, X. Dendritic cell migration in inflammation and immunity. *Cell. Mol. Immunol.* **18**, 2461–2471 (2021).

31. Genoula, M. *et al.* Formation of foamy macrophages by tuberculous pleural effusions is triggered by the interleukin-10/signal transducer and activator of transcription 3 axis through ACAT upregulation. *Front. Immunol.* (2018). doi:10.3389/fimmu.2018.00459

32. Barham, D. & Trinder, P. An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst* **97**, 142–5 (1972).

33. Acosta-Rodriguez, E. V. *et al.* Surface phenotype and antigenic specificity of human interleukin 17–producing T helper memory cells. *Nat. Immunol. 2007 86* **8**, 639–646 (2007).

34. Van Goethem, E., Poincloux, R., Gauffre, F., Maridonneau-Parini, I. & Le Cabec, V. Matrix architecture dictates three-dimensional migration modes of human macrophages: differential involvement of proteases and podosome-like structures. *J. Immunol.* **184**, 1049–1061 (2010).

35. Argüello, R. J. *et al.* SCENITH: A Flow Cytometry-Based Method to Functionally Profile Energy Metabolism with Single-Cell Resolution. *Cell Metab.* (2020). doi:10.1016/j.cmet.2020.11.007

36. Freemerman, A. J. *et al.* Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. *J. Biol. Chem.* **289**, 7884–7896 (2014).

37. Quesniaux, V. *et al.* Toll-like receptor pathways in the immune responses to mycobacteria. *Microbes Infect.* **6**, 946–959 (2004).

38. Chow, J. C., Young, D. W., Golenbock, D. T., Christ, W. J. & Gusovsky, F. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J. Biol. Chem.* **274**, 10689–10692 (1999).

39. Underhill, D. M., Ozinsky, A., Smith, K. D. & Aderem, A. Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 14459–14463 (1999).

40. Schwandner, R., Dziarski, R., Wesche, H., Rothe, M. & Kirschning, C. J. Peptidoglycan- and Lipoteichoic Acid-induced Cell Activation Is Mediated by Toll-like Receptor 2. *J. Biol. Chem.* **274**, 17406–17409 (1999).

41. Cougoule, C. *et al.* Podosomes, But Not the Maturation Status, Determine the Protease-Dependent 3D Migration in Human Dendritic Cells. *Front. Immunol.* **9**, (2018).

42. Balboa, L. *et al.* Impaired dendritic cell differentiation of CD16-positive monocytes in tuberculosis: Role of p38 MAPK. *Eur. J. Immunol.* (2013). doi:10.1002/eji.201242557

43. Parlato, S. *et al.* Impaired IFN-α-mediated signal in dendritic cells differentiates active from latent tuberculosis. *PLoS One* **13**, e0189477 (2018).

44. Sakhno, L. V. *et al.* Impairments of Antigen-Presenting Cells in Pulmonary Tuberculosis. *J. Immunol. Res.* **2015**, (2015).

45. McGettrick, A. F. & O'Neill, L. A. J. The Role of HIF in Immunity and Inflammation. *Cell Metab.* **32**, 524–536 (2020).

46. Reiley, W. W. *et al.* ESAT-6-specific CD4 T cell responses to aerosol Mycobacterium tuberculosis infection are initiated in the mediastinal lymph nodes. *Proc. Natl Acad. Sci. USA* **105**, 10961–10966 (2008).

47. Griffiths, K. L. *et al.* Targeting dendritic cells to accelerate T-cell activation overcomes a bottleneck in tuberculosis vaccine efficacy. *Nat. Commun.* (2016). doi:10.1038/ncomms13894

48. Harding, J. S., Rayasam, A., Schreiber, H. A., Fabry, Z. & Sandor, M. Mycobacterium-Infected Dendritic Cells Disseminate Granulomatous Inflammation. *Sci. Rep.* **5**, (2015).

49. Samstein, M. *et al.* Essential yet limited role for CCR2+ inflammatory monocytes during Mycobacterium tuberculosis-specific T cell priming. *Elife* **2013**, (2013).

50. Semba, H. *et al.* HIF-1α-PDK1 axis-induced active glycolysis plays an essential

role in macrophage migratory capacity. *Nat. Commun. 2016 71* **7**, 1–10 (2016).

51. Kishore, M. *et al.* Regulatory T Cell Migration Is Dependent on Glucokinase-Mediated Glycolysis. *Immunity* **47**, 875-889.e10 (2017).

52. Park, J. S. *et al.* Mechanical regulation of glycolysis via cytoskeleton architecture. *Nature* **578**, 621–626 (2020).

53. Fernie, A. R., Zhang, Y. & Sampathkumar, A. Cytoskeleton Architecture Regulates Glycolysis Coupling Cellular Metabolism to Mechanical Cues. *Trends Biochem. Sci.* **45**, 637–638 (2020).

54. Ferreira, G. B. *et al.* Differential protein pathways in 1,25-dihydroxyvitamin d(3) and dexamethasone modulated tolerogenic human dendritic cells. *J. Proteome Res.* **11**, 941–971 (2012).

55. Ferreira, G. B. *et al.* Proteome analysis demonstrates profound alterations in human dendritic cell nature by TX527, an analogue of vitamin D. *Proteomics* **9**, 3752–3764 (2009).

56. Basit, F. & de Vries, I. J. M. Dendritic Cells Require PINK1-Mediated Phosphorylation of BCKDE1α to Promote Fatty Acid Oxidation for Immune Function. *Front. Immunol.* **10**, 2386 (2019).

57. Ohl, L. *et al.* CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. *Immunity* **21**, 279–288 (2004).

58. Krawczyk, C. M. *et al.* Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* (2010). doi:10.1182/blood-2009-10-249540

59. Hackett, E. E. *et al.* Mycobacterium tuberculosis Limits Host Glycolysis and IL-1β by Restriction of PFK-M via MicroRNA-21. *Cell Rep.* **30**, 124-136.e4 (2020).

60. Martín-Fontecha, A. *et al.* Regulation of Dendritic Cell Migration to the Draining Lymph Node Impact on T Lymphocyte Traffic and Priming. *J. Exp. Med.* **198**, 615–621 (2003).

61. Ziegler-Heitbrock, L. *et al.* Nomenclature of monocytes and dendritic cells in blood. *Blood* **116**, e74–e80 (2010).

62. Ginhoux, F. & Jung, S. Monocytes and macrophages: Developmental pathways and tissue homeostasis. *Nat. Rev. Immunol.* **14**, 392–404 (2014).

63. Wong, K. L. *et al.* Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. *Blood* **118**, e16–e31 (2011).

64. Cros, J. *et al.* Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity* **33**, 375–386 (2010).

65. Balboa, L. *et al.* Paradoxical role of CD16+CCR2+CCR5+ monocytes in tuberculosis: efficient APC in pleural effusion but also mark disease severity in blood. *J. Leukoc. Biol.* (2011). doi:10.1189/jlb.1010577

66. Manoharan, I., Prasad, P. D., Thangaraju, M. & Manicassamy, S. Lactate-Dependent Regulation of Immune Responses by Dendritic Cells and Macrophages. *Front. Immunol.* **12**, 691134–691134 (2021).

Figure legends

Figure 1. Mtb rewires the metabolic network of monocyte-derived DCs (Mo-DCs). Mo-DCs were stimulated with viable or irradiated Mtb (iMtb) at two MOI (1 or 2 Mtb per DC) for 24h. Glycolysis was measured as: **(A)** Lactate release in culture supernatants; **(B)** Glucose uptake measured in culture supernatants; **(C)** Relative expression of *HIF-1α* mRNA normalized to *EeF1A1* control gene. **(D)** Representative histograms of the mean fluorescence intensity (MFI) of HIF-1α as measured by flow cytometry. Quantification shown in graph to the right. **(E)** Relative expression of lactate dehydrogenase A (*LDHA*) mRNA normalized to *EeF1A1* control gene. **(F)** FACS plots show the percentage of Glut1^{+} cells with and without iMtb stimulation in a representative experiment. Quantification of Glut1+ cells plotted to the right. **(G)** MFI of Mitospy probe as a measurement of mitochondrial mass for Mo-DCs with and without iMtb treatment. **(H)** Representative electron microscopy micrographs of control and iMtb-stimulated DCs showing mitochondria colored in cyan (left panels) and quantified morphometric analysis (right panels). Statistical significance was assessed in **(A-E)** using 2-way ANOVA followed by Tukey's multiple comparisons test ($*p < 0.05$; $**p < 0.01$; $****p < 0.0001$), and in **(F-H)** using paired t test ($*p < 0.05$) for iMtb versus controls. All values are expressed as means ± SEM.

Figure 2. Mtb skews DC metabolism toward glycolysis.

Mo-DCs were stimulated with irradiated Mtb (iMtb). **(A)** Kinetic profile of proton efflux rate (PER; left panel) and oxygen consumption rate (OCR; right panel) measurements in control and iMtb-stimulated DCs in response to inhibitor treatments (Oligomycin, O; ROT/AA, Rotenone/Antimycin A), obtained using an Agilent Seahorse XFe24 Analyzer. PER and OCR measurements were normalized to the area covered by cells. **(B)** ATP production rate from mitochondrial oxidative phosphorylation (MitoATP) and glycolysis (glycoATP). MitoATP production rate and glycoATP production rate were calculated from OCR and ECAR measurements in control and iMtb-stimulated DCs. **(C)**

Percentages of MitoATP and GlycoATP relative to overall ATP production. **(D)** Representative histograms showing the translation level after puromycin (Puro) incorporation and staining with a monoclonal anti-Puro (anti-Puro MFI) in response to inhibitor treatment (C, Control; DG, 2-Deoxy-D-Glucose; Oligomycin, O; or combination treatment, DG+O). The bar plots show the values of the anti-Puro MFI from 6 donors. Arrows and numbers inside boxes denote the differences between the MFI of puro in the different treatments that are used to calculate the glucose dependence (1) and fatty acids and amino acids oxidation (FAO & AAO) capacity (4); and the mitochondrial dependency (2) and glycolytic capacity (3). **(E)** Relative contributions of glycolytic and FAO & AAO capacities and glucose and mitochondrial dependences to overall DC metabolism analyzed with SCENITH. Paired t test ($*p < 0.05$; $**p < 0.01$) for iMtb versus controls. Values are expressed as means ± SEM.

Figure 3. Mtb triggers glycolysis through TLR2 ligation in Mo- DCs. Mo-DCs were stimulated with irradiated Mtb (iMtb) in the presence of neutralizing antibodies against either TLR2 (aTLR2), TLR4 (aTLR4), or their respective isotype controls. **(A)** Lactate release as measured in supernatant. **(B)** Glucose uptake as measured in supernatant. **(C)** Mean fluorescence intensity (MFI) of HIF-1α as measured by flow cytometry. **(D)** Kinetic profile of proton efflux rate (PER) and oxygen consumption rate (OCR) measurements (left panels). Metabolic flux analysis showing quantification of mitochondrial ATP production and glycolytic ATP production (right panel). **(E-F)** Mo-DCs were stimulated with Pam3Cys or Mtb peptidoglycan (PTG) at the indicated concentrations. **(E)** Lactate release as measured in supernatant. **(F)** Glucose uptake as measured in supernatant. Statistics in **(A-B, E-F)** are 2-way ANOVA followed by Tukey's multiple comparisons test ($*p < 0.05$; $**p < 0.01$; $****p < 0.0001$). Statistics in **(C-D)** are from paired t test ($*p < 0.05$) for iMtb versus controls. Values are expressed as means ± SEM.

Figure 4. HIF-1α is required for DC maturation upon iMtb stimulation but not for $CD4^+$ T lymphocyte polarization. (A-C) Mo-DCs were stimulated with irradiated Mtb (iMtb) in the presence or absence of the HIF-1α inhibitor PX-478 (PX). **(A)** Metabolic flux analysis showing quantification of mitochondrial ATP production and glycolytic ATP production, as in Figure 2C. **(B)** Mean fluorescence intensity (MFI) of CD83, CD86 and PD-L1 as measured by flow cytometry. **(C)** TNF-α and IL-10 production by Mo-DCs measured by ELISA. **(D-E)** Monocytes from PPD^+ healthy donors were differentiated towards DCs, challenged or not with iMtb in the presence or absence of PX for 24 h, washed, and co-cultured with autologous $CD4^+$ T cells for 5 days. **(D)** Extracellular secretion of IFN-γ and IL-17 as measured by ELISA. **(E)** Absolute abundance of Th1, Th17, Th2 and Th1/Th17 $CD4^+$ T cells after coculture with DCs. When indicated lymphocytes without DCs were cultured (Ly). Statistical significance based on 2-way ANOVA followed by Tukey's multiple comparison test ($*p < 0.05$; $**p < 0.01$).

Figure 5. HIF1α-mediated-glycolysis is required to trigger migratory activity in iMtb-stimulated DCs. Mo-DCs were treated or not with HIF-1a inhibitor PX-478 (PX) or glycolysis inhibitor oxamate (OX) and stimulated with iMtb for 24h. **(A)** Lactate release as measured in supernatants in DCs stimulated or not with iMtb in the presence of OX. **(B)** Percentage of migrated cells towards CCL21 relative to the number of initial cells per condition. **(C)** Three-dimensional amoeboid migration of DCs through a collagen matrix after 24h. Cells within the matrix were fixed and stained with DAPI. Images of the membrane of each insert were taken and the percentage of cells per field were counted. **(D)** Representative schematic of the experimental setup for *in-vivo* migration assays. **(E)** Percentages of migrating BMDCs (CFSE-labeled among $CD11c^+$) recovered from inguinal lymph nodes. Statistical significance assessed by **(A-B)** ANOVA followed by Dunnett's multiple comparisons test ($*p < 0.05$; $**p < 0.01$); **(C)** Nested ANOVA followed by Dunnett's multiple comparisons test ($*p < 0.05$; $**p < 0.01$); **(E)** ANOVA followed by Holm-Sidak's multiple comparisons test ($*p < 0.05$).

Figure 6. Stabilization of HIF-1α promotes migration of tolerogenic DCs and Mo-DCs from TB patients. Tolerogenic Mo-DCs were generated by dexamethasone (Dx) treatment and were stimulated or not with iMtb in the presence or absence of HIF-1α activator DMOG. **(A)** Lactate release and glucose uptake as measured in supernatant. **(B)** Mean fluorescence intensity (MFI) of HIF-1α. Representative histograms and quantification are shown. **(C)** Three-dimensional amoeboid migration of DCs through a collagen matrix. After 24h of migration, images of stacks within the matrix were taken every 30 μm. Percentage of migrating cells were defined as cells in the stacks within the matrix relative to total number of cells. **(D)** Chemotactic activity towards CCL21 *in vitro*. **(E-H)** Mo-DCs were generated from healthy subjects (HS) or TB patients, and DCs were stimulated or not with iMtb. **(E)** Chemotaxis index towards CCL21 (relative to unstimulated DCs). **(F)** Lactate production ratio relative to unstimulated DCs. **(G)** Glycolytic capacity assessed by SCENITH. **(H)** Chemotactic activity towards CCL21 of Mo-DCs from TB patients stimulated with iMtb and treated or not with DMOG. Statistical significance assessed by **(A-D)** 2-way ANOVA followed by Tukey's multiple comparisons test ($*p < 0.05$; $**p < 0.01$); **(E-G)** Unpaired T test ($*p < 0.05$); **(H)** Paired T test ($*p < 0.05$).

Figure 7. Exacerbation of glycolysis in CD16$^+$ monocytes from TB patients leads to DCs with poor migration capacity. (A) Monocytes from TB patients or healthy subjects (HS) were isolated and cultured with IL-4 and GM-CSF for 24h. Accumulation of lactate in culture supernatants were measured at 1 and 24h of differentiation. (**B**) Glycolytic capacity measured by SCENITH of monocyte subsets as defined by their CD14 and CD16 expression from HS and TB patients. **(C-F)** Monocytes from HS were treated with DMOG during the first 24h of differentiation with IL-4/GM-CSF (earlyDMOG) and removed afterwards. On day 6 of differentiation, cells were stimulated or not with iMtb. **(C)** Monocyte lactate release after 24h of DMOG addition.

(D) Percentage of cells expressing CD1a and DC-SIGN after full 6 days of differentiation **(E)** MFI of CD83 and CD86 on day 6 differentiated cells stimulated or not with iMtb for further 24h. **(F)** Chemotactic activity towards CCL21 of DCs. Statistical significance was assessed by **(A)** Paired T test for 0 *vs.* 24h ($*p < 0.05$) and 2-way ANOVA for HS *vs.* TB at each time ($**p < 0.01$); **(B)** unpaired T test ($*p < 0.05$; $**p < 0.01$); **(C-D)** paired T test ($**p < 0.01$); **(E-F)** 2-way ANOVA followed by Tukey's multiple comparisons test ($*p < 0.05$; $**p < 0.01$).

Figure 1

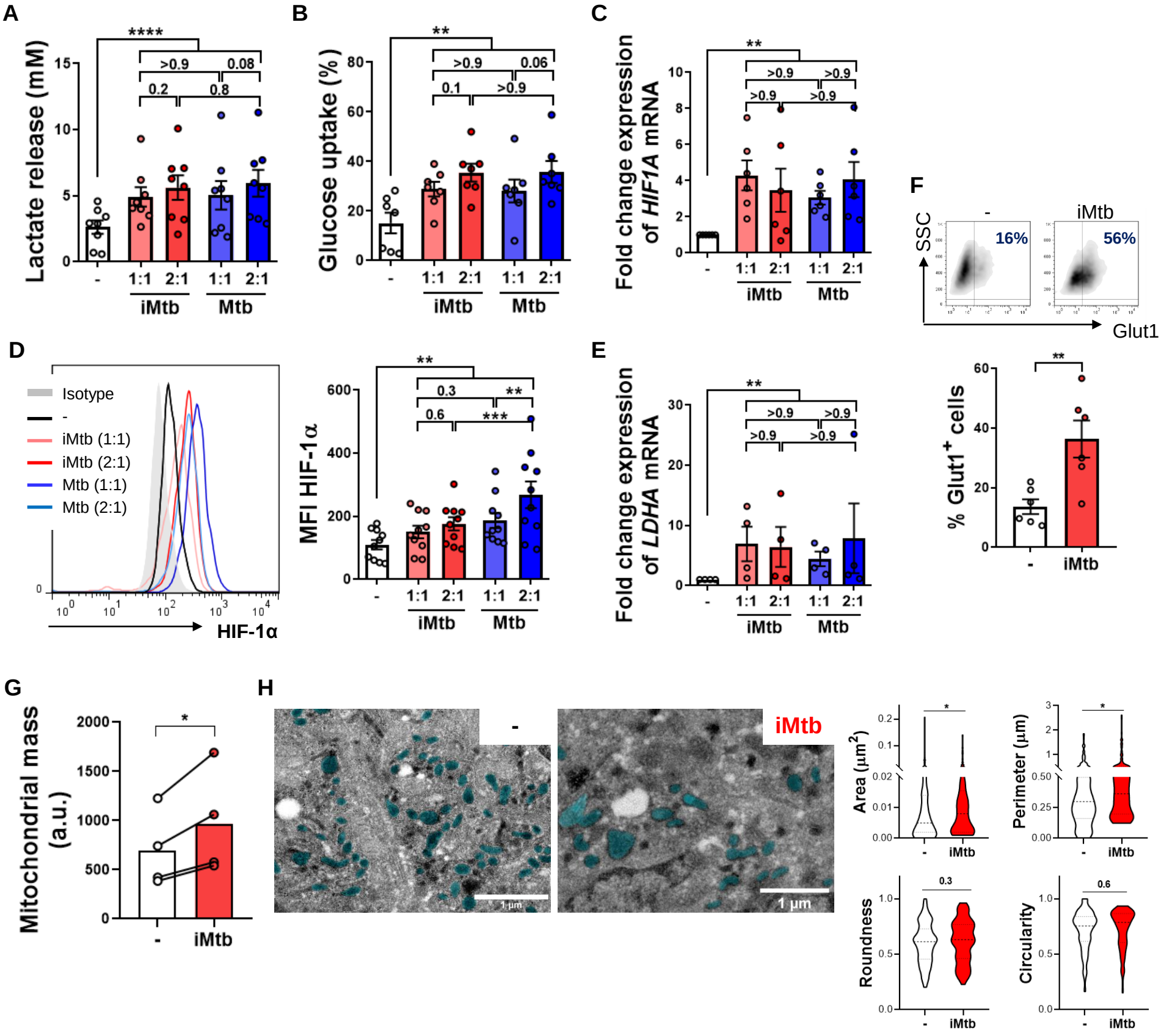

A
Lactate release (mM)
****
>0.9
0.08
0.2
0.8
-
1:1
2:1
1:1
2:1
iMtb
Mtb
B
Glucose uptake (%)
**
>0.9
0.06
0.1
>0.9
C
Fold change expression of HIF1A mRNA
**
>0.9
>0.9
>0.9
>0.9
F
SSC
Glut1
iMtb
16%
56%
D
Isotype
-
iMtb (1:1)
iMtb (2:1)
Mtb (1:1)
Mtb (2:1)
HIF-1α
MFI HIF-1α
0.3
0.6
***
E
Fold change expression of LDHA mRNA
% Glut1+ cells
G
Mitochondrial mass (a.u.)
*
H
iMtb
1 µm
Area (µm2)
Perimeter (µm)
Roundness
Circularity
0.3
0.6

Figure 2

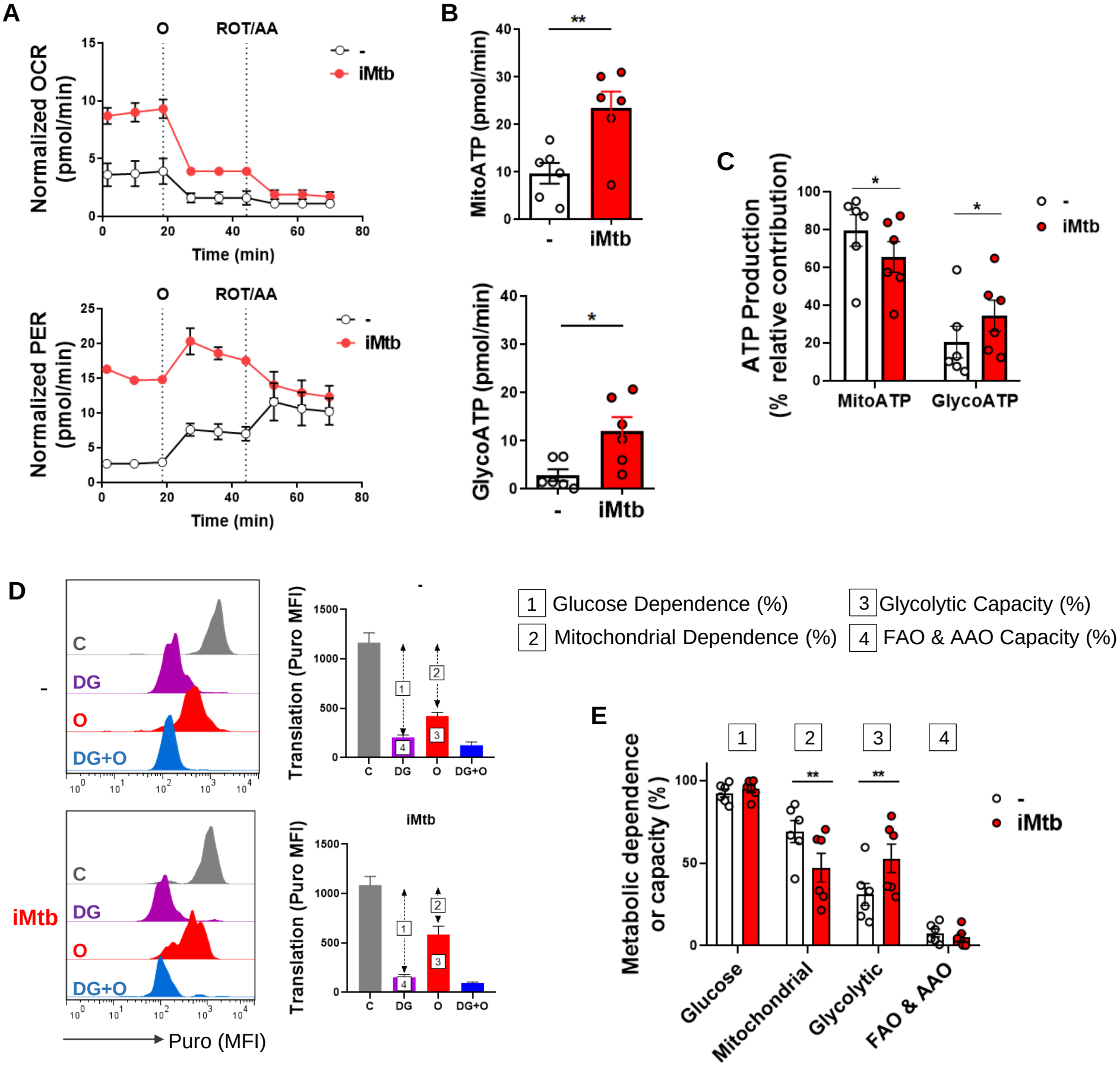

A
O
ROT/AA
Normalized OCR (pmol/min)
Time (min)
-
iMtb
Normalized PER (pmol/min)
B
MitoATP (pmol/min)
GlycoATP (pmol/min)
C
ATP Production (% relative contribution)
MitoATP
GlycoATP
D
C
DG
O
DG+O
Translation (Puro MFI)
iMtb
Puro (MFI)
1 Glucose Dependence (%)
2 Mitochondrial Dependence (%)
3 Glycolytic Capacity (%)
4 FAO & AAO Capacity (%)
E
Metabolic dependence or capacity (%)
Glucose
Mitochondrial
Glycolytic
FAO & AAO

Figure 3

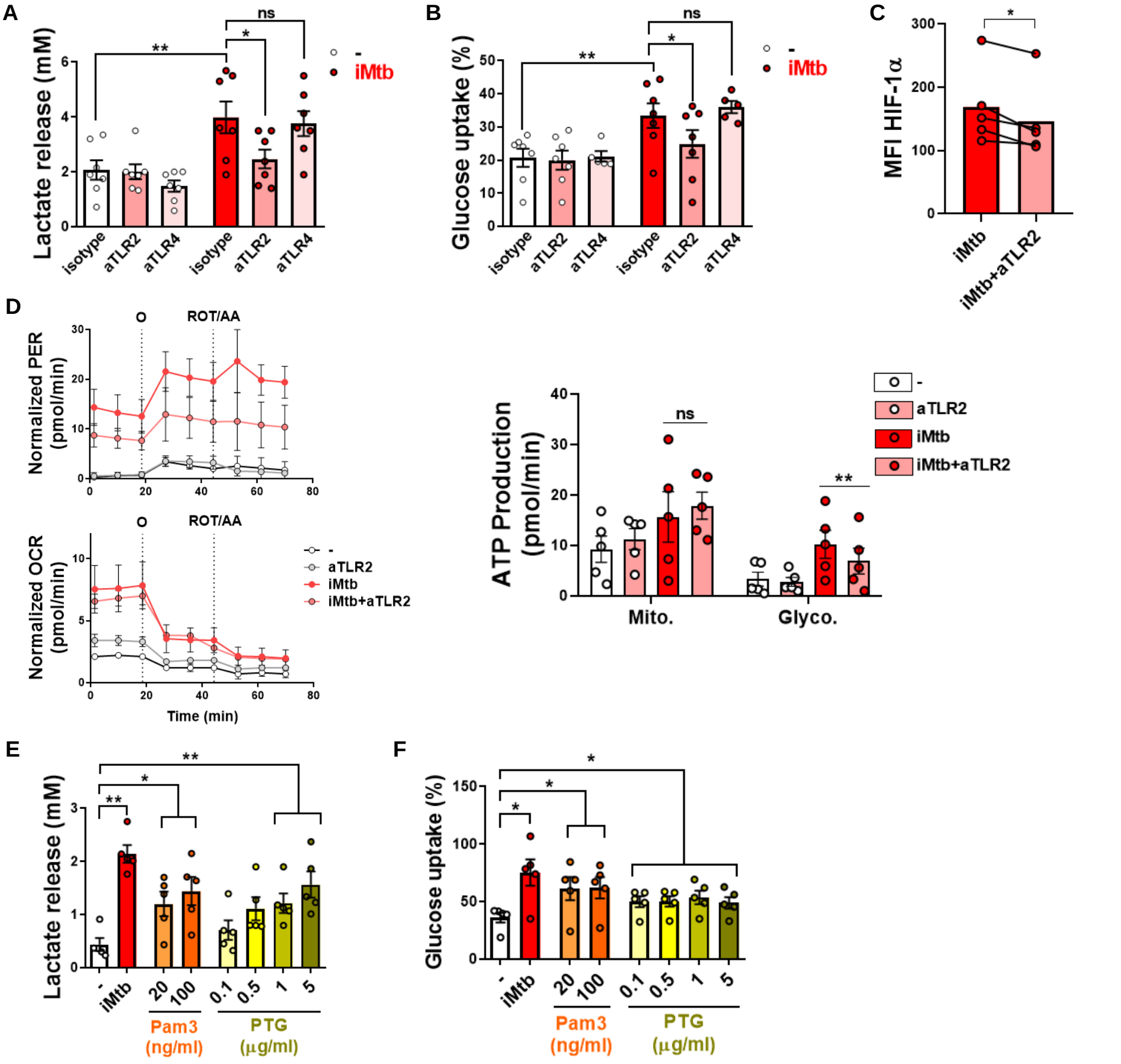

A
Lactate release (mM)
ns
*
**
-
iMtb
isotype
aTLR2
aTLR4
B
Glucose uptake (%)
C
MFI HIF-1α
iMtb
iMtb+aTLR2
D
ROT/AA
Normalized PER (pmol/min)
Normalized OCR (pmol/min)
Time (min)
aTLR2
ATP Production (pmol/min)
Mito.
Glyco.
E
F
Pam3 (ng/ml)
PTG (μg/ml)

Figure 4

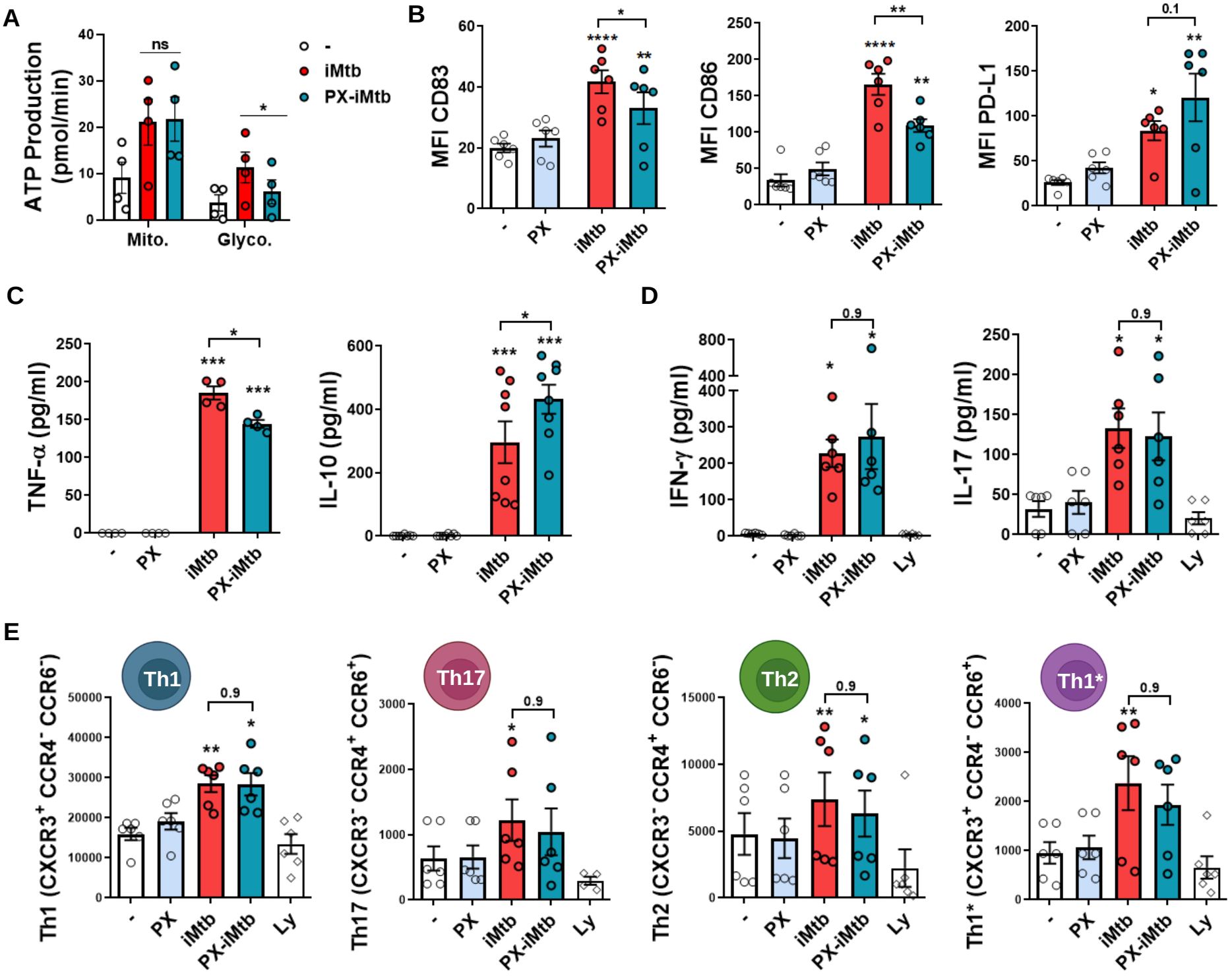
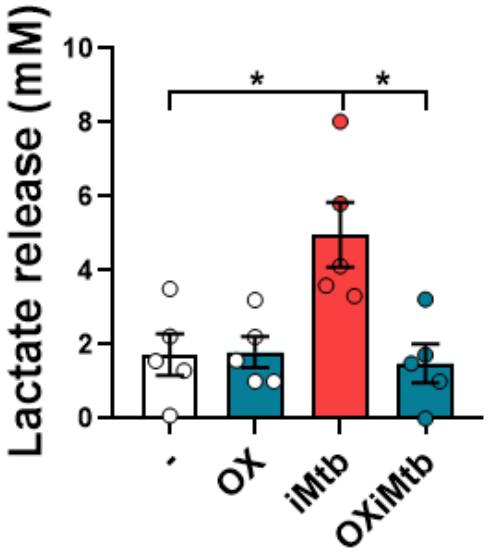

A
ATP Production (pmol/min)
ns
*
Mito.
Glyco.
-
iMtb
PX-iMtb
B
MFI CD83
MFI CD86
MFI PD-L1
0.1
C
TNF-α (pg/ml)
IL-10 (pg/ml)
D
IFN-γ (pg/ml)
IL-17 (pg/ml)
0.9
E
Th1 (CXCR3+ CCR4- CCR6-)
Th17 (CXCR3- CCR4+ CCR6+)
Th2 (CXCR3- CCR4+ CCR6-)
Th1* (CXCR3+ CCR4- CCR6+)
Th1
Th17
Th2
Th1*
PX
iMtb
PX-iMtb
Ly

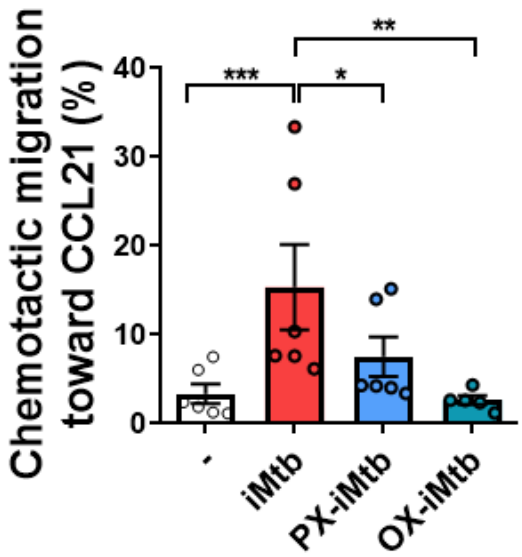
Figure 5

Human Mo-DCs

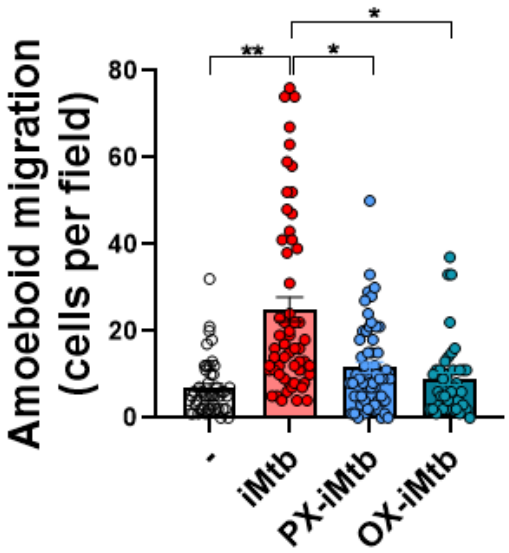
A



B

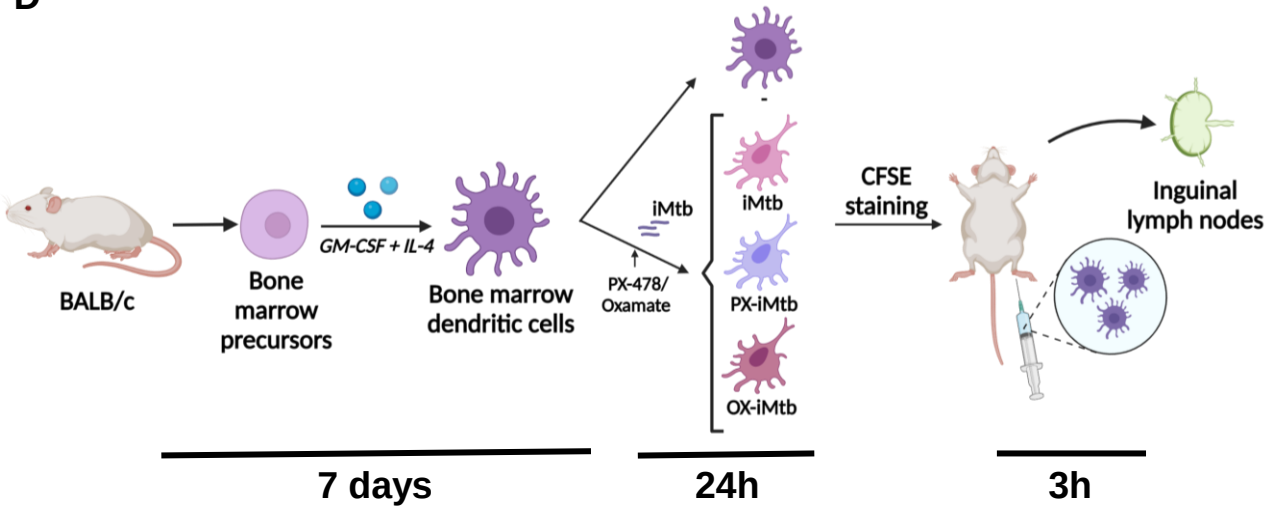


C



Murine BMDCs

D



E

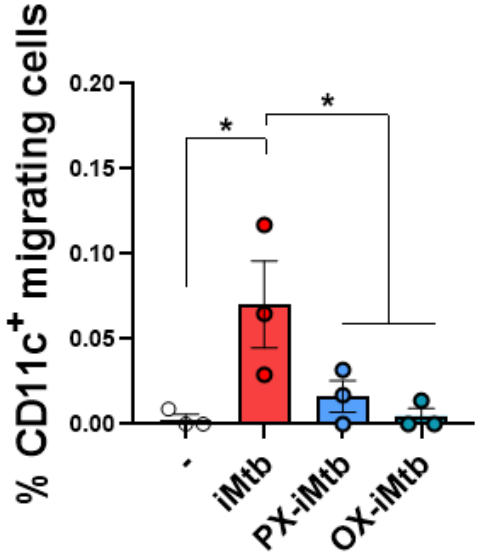


Figure 6

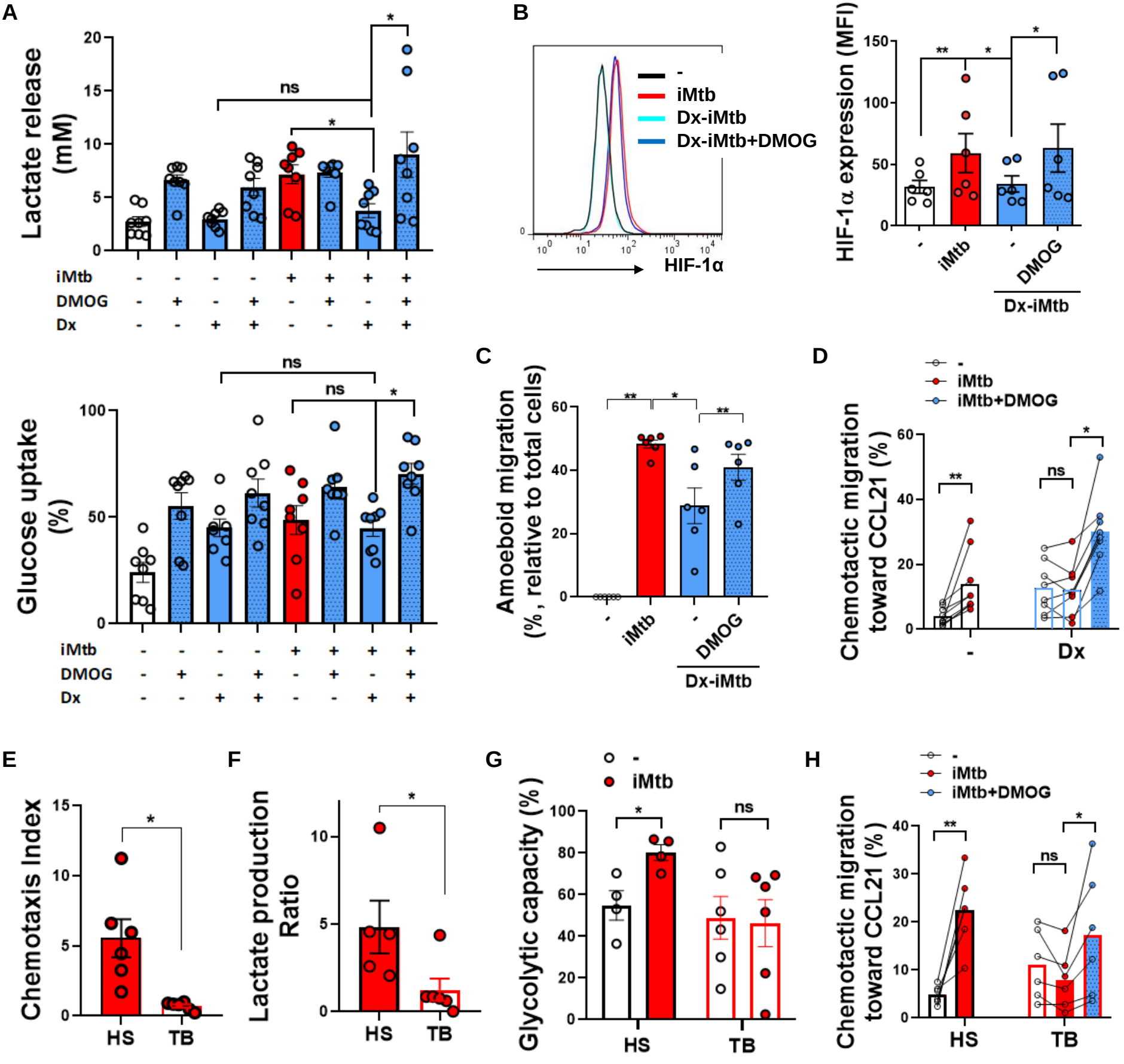
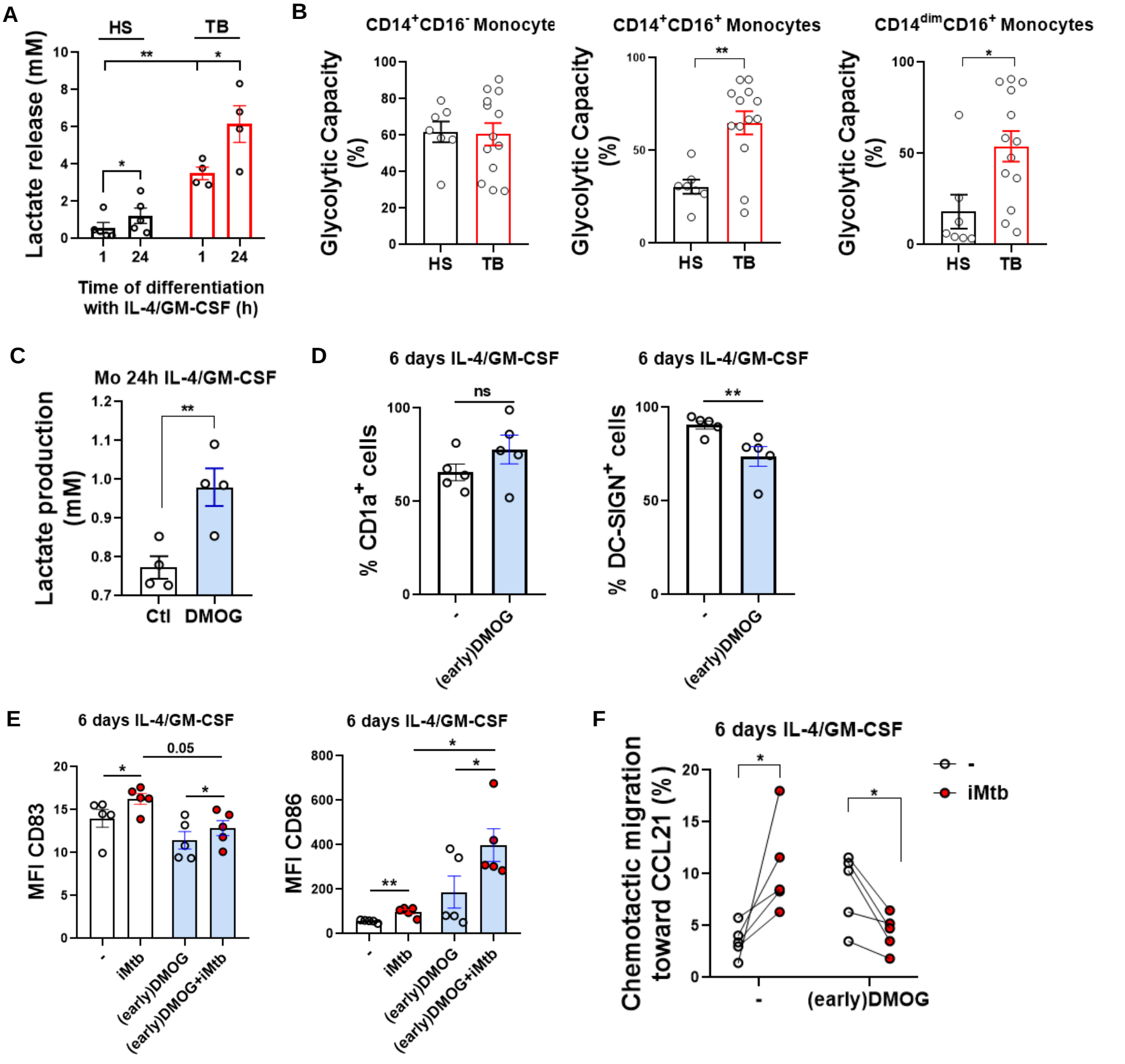

A
Lactate release (mM)
ns
*
iMtb
DMOG
Dx
B
-
iMtb
Dx-iMtb
Dx-iMtb+DMOG
HIF-1α
HIF-1α expression (MFI)
**
*
DMOG
Dx-iMtb
Glucose uptake (%)
ns
ns
*
C
Amoeboid migration (%, relative to total cells)
**
*
**
D
iMtb+DMOG
Chemotactic migration toward CCL21 (%)
**
ns
*
Dx
E
Chemotaxis Index
HS
TB
F
Lactate production Ratio
G
Glycolytic capacity (%)
H

Figure 7


A
HS
TB
Lactate release (mM)
Time of differentiation with IL-4/GM-CSF (h)
B
CD14+CD16- Monocyte
CD14+CD16+ Monocytes
CD14dimCD16+ Monocytes
Glycolytic Capacity (%)
HS
TB
C
Mo 24h IL-4/GM-CSF
Lactate production (mM)
Ctl
DMOG
D
6 days IL-4/GM-CSF
% CD1a+ cells
% DC-SIGN+ cells
ns
(early)DMOG
E
6 days IL-4/GM-CSF
MFI CD83
MFI CD86
0.05
iMtb
(early)DMOG+iMtb
F
6 days IL-4/GM-CSF
Chemotactic migration toward CCL21 (%)
iMtb