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# Toll-like Receptor 4-Induced Glycolytic Burst in Human Monocyte-Derived Dendritic Cells Results from p38-Dependent Stabilization of HIF-1 $\alpha$ and Increased Hexokinase II Expression

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1 **Toll Like Receptor 4-induced glycolytic burst in human monocyte-derived DCs**  
2 **results from p38-dependent stabilization of HIF-1 $\alpha$  and increased hexokinase**  
3 **II expression.**

4

5 **Running Title**

6 **TLR4 glycolytic burst involves a p38-MAPK-HIF-1 $\alpha$  axis**

7

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**Abstract**

Cell metabolism now appears as an essential regulator of immune cells activation. In particular, TLR stimulation triggers metabolic reprogramming of dendritic cells (DCs) with an increased glycolytic flux, whereas inhibition of glycolysis alters their functional activation. The molecular mechanisms involved in the control of glycolysis upon TLR stimulation are poorly understood for human DCs. TLR4 activation of human monocyte-derived DCs (MoDCs) stimulated glycolysis with an increased glucose consumption and lactate production. Global hexokinase (HK) activity, controlling the initial rate-limiting step of glycolysis, was also increased. TLR4-induced glycolytic burst correlated with a differential modulation of hexokinase isoenzymes. LPS strongly enhanced the expression of HK2, whereas HK3 was reduced, HK1 remained unchanged and HK4 was not expressed. Expression of the other rate-limiting glycolytic enzymes was not significantly increased. Exploring the signaling pathways involved in LPS-induced glycolysis with various specific inhibitors, we observed that only the inhibitors of p38-MAPK (SB203580) and of HIF-1 $\alpha$  DNA binding (Echinomycin) reduced both the glycolytic activity and production of cytokines triggered by TLR4 stimulation. In addition, LPS-induced HK2 expression required p38-MAPK-dependent HIF-1 $\alpha$  accumulation and transcriptional activity. TLR1/2 and TLR2/6 stimulation increased glucose consumption by MoDCs through alternate mechanisms that are independent of p38-MAPK activation. TBK1 contributed to glycolysis regulation when DCs were stimulated via TLR2/6. Therefore, our results indicate that TLR4-dependent upregulation of glycolysis in human MoDCs involves a p38-MAPK-dependent HIF-1 $\alpha$

- 1 accumulation, leading to an increased hexokinase activity supported by enhanced HK2
- 2 expression.
- 3

## 1 **Introduction**

2

3 TLRs are common sensors expressed by dendritic cells (DCs) recognizing pathogen-  
4 associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs),  
5 initiating an appropriate immune response to fight infection (1). TLR signaling triggers  
6 human DC maturation, promoting the secretion of pro-inflammatory cytokines, inducing the  
7 expression of co-stimulatory molecules so that DCs acquire the ability to stimulate naïve T  
8 cells (2). TLR4 is a receptor for Gram-negative bacteria LPS (3). This receptor can be also  
9 activated by other microbial ligands such as viral proteins (4-6) and by DAMPs, such as  
10 HMGB1 (high-mobility group box 1) (7) or oxidized phospholipids (8). TLR4 is also involved in  
11 the sensing of nutrients and metabolic stress. Indeed, saturated free fatty acids (FFA) can  
12 activate TLR4 signaling via their binding to the hepatokine Fetuin A which is a TLR4 ligand (9).  
13 Its increased production in steatotic and inflamed liver contributes to the secretion of  
14 inflammatory cytokines by monocytes and adipose tissue (10). By regulating the expression  
15 of many genes involved in innate immunity and metabolic reprogramming (11), TLR4 is at  
16 the crossroads of innate immunity and metabolic inflammation (12) and is involved in the  
17 pathogenesis of metabolic diseases such as obesity and type-2 diabetes (13).

18 Glucose is a major nutrient for cellular bioenergetics production. Glycolysis converts glucose  
19 to pyruvate by a series of enzymatic reaction steps, generating 2 moles of ATP per mole of  
20 glucose. Intermediary metabolites of glycolysis are precursors also fueling the pentose-  
21 phosphates, lipids and amino-acids pathways (11). Three rate-limiting enzymes are  
22 controlling the glycolytic flux. The first one, the hexokinase (HK), controls the conversion of  
23 glucose to glucose-6-phosphate. There are 4 isoenzymes of HK (HK-I, II, III, IV) encoded by 4  
24 different genes (HK 1, 2, 3, 4). HK1 and HK3 have a large spectrum of tissue expression while

1 HK2 and HK4 have an expression profile that is more restricted to metabolically relevant  
2 tissues. HK2 is overexpressed in virtually all cancer cells. Other rate-limiting enzymes of  
3 glycolysis are the phosphofructokinase (PFK), and pyruvate kinase. PFK is encoded by 3  
4 different genes (PFKL, PFKP and PFKM) whose expression is tissue dependent. Pyruvate  
5 kinase is encoded by the PKM gene in immune cells, generating 2 isoforms (PKM1 and  
6 PKM2). The pyruvate produced by glycolysis can be converted to lactate that is excreted by  
7 specific monocarboxylate transporters or to oxaloacetate or acetyl-CoA to fuel the TCA cycle,  
8 coupling glycolysis to oxidative phosphorylation, for the generation of high amount of ATP in  
9 the presence of oxygen. During hypoxia, oxidative phosphorylation is reduced and glycolysis  
10 is increased to face energetic needs (14). However, activation of glycolysis can also occur  
11 under aerobic conditions and Otto Warburg first discovered that tumor cells have a high rate  
12 of glycolysis and that most pyruvate is converted to lactate, even when oxygen is available  
13 (15). Both innate and adaptive immune cells can shift to aerobic glycolysis upon stimulation.  
14 Cell metabolism is now appreciated as a key regulator of T cell differentiation, modulating  
15 effector and memory functions (16). The transcriptional control of glycolysis in T cells mainly  
16 rely on the expression of hypoxia-inducible factor 1 (HIF-1) and the proto-oncogene MYC  
17 (17). Accumulation of HIF-1 $\alpha$  in normoxic conditions has been observed in several cell types  
18 upon LPS stimulation (14, 18, 19). Several molecular mechanisms may result in this  
19 accumulation, including activation of the mammalian target of rapamycin complex 1  
20 (mTORC1) (20). In primary human plasmacytoid DCs, increased glycolysis was found to play a  
21 key role in the regulation of anti-viral functions, including IFN- $\alpha$  production (21). Tolerogenic  
22 DCs also show a metabolic signature characterized by high mitochondrial respiration and  
23 glycolytic capacity (22). TLR4 stimulation of DCs or macrophages results in increased  
24 glycolytic activity, an essential process for their proper activation (23-29). Indeed, a

1 metabolic reprogramming to aerobic glycolysis was described to be associated to a  
2 proinflammatory M1 phenotype of murine macrophages, whereas glycolytic activity was  
3 unchanged in alternate activated M2 macrophages (11, 30). The molecular mechanisms  
4 involved in glycolysis upregulation upon TLR4 stimulation remain to be clarified and may  
5 differ according to cell types and functions.

6 In murine bone marrow-derived DCs (BM-DCs), two different molecular pathways have been  
7 depicted for glycolytic reprogramming upon TLR4 stimulation. Activation of TBK1-IKK $\epsilon$  and  
8 AKT kinases was found to control the early increase of glycolysis, favoring mitochondrial  
9 translocation of HK-II, fueling the TCA cycle and fatty acids synthesis (24). In these DCs, the  
10 late increase in glycolytic metabolism was proposed to be a survival response to maintain  
11 ATP production despite the inhibition of oxidative phosphorylation by NO, produced by the  
12 inducible NO synthase (25). This pathway is unlikely to be involved in human DCs since this  
13 enzyme is not expressed (31) and NO production could not be detected in these cells (27).  
14 Studies exploring the molecular mechanisms involved in the regulation of glycolysis in  
15 primary human DCs are lacking for a better understanding of the reciprocal interactions  
16 between cellular metabolic activity and the functional DC activation state.

17 Therefore, we explored the pathways involved in the control of glycolytic activity upon TLR4  
18 stimulation of human monocyte-derived DCs (MoDCs). In contrast to murine DCs, increased  
19 glycolysis did not rely on the TBK1- or AKT-dependent pathways, but on the engagement of a  
20 TLR4-p38-MAPK axis stabilizing HIF-1 $\alpha$  and upregulating HK2 expression. Interestingly, this  
21 pathway does not appear to be involved in the activation of glycolysis triggered by TLR1/2 or  
22 TLR2/6 stimulation.

## 1 **Materials and methods**

### 2 **Reagents**

3 Ultrapure TLR4 ligand LPS from *E. coli* O111:B4, TLR2/6 ligand peptidoglycan from *S. aureus*  
4 (PGN), synthetic TLR1/2 ligand Pam<sub>3</sub>CSK<sub>4</sub> (Pam)), all from InvivoGen (Toulouse, France) were  
5 dissolved in sterile PBS. FP7 was synthesized from commercially available D-glucose by  
6 multistep organic synthesis according to published procedures (32). The purity of the  
7 molecule was assessed by nuclear magnetic resonance and mass spectrometry analysis. A 5  
8 mM stock solution of FP7 was prepared in ethanol/ DMSO 1:1 and conserved at -20°C.

9 The inhibitors SB203580, SP600125, PD98059, BX795, and LY294002 purchased from  
10 InvivoGen, Rapamycin, provided by Calbiochem (San Diego, CA, USA) and HIF-1 $\alpha$  DNA-  
11 binding inhibitor, Echinomycin, provided by Cayman Chemical (Ann Arbor, MI, USA), were  
12 dissolved in sterile culture grade DMSO and stored at -20°C. AZD5363 and SC75741 were  
13 purchased from Selleckchem (Houston, TX, USA) prepared in DMSO and stored at -80°C.  
14 Pepinh MYD, pepinh TRIF and pepinh control (Ctl) purchased from Invivogen were dissolved  
15 in PBS and stored at -20°C.

16 Glucose uptake was measured by incorporation of 2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-  
17 4-yl)amino]-D-glucose (2-NBDG, Sigma Aldrich, Saint-Quentin Fallavier, France) in culture  
18 medium.

19

### 20 **DC generation and treatment**

21 Monocytes were purified from human peripheral blood of healthy donors (33), obtained  
22 from the Etablissement Français du Sang. All experiments were performed in accordance  
23 with the guidelines of the World Medical Association's Declaration of Helsinki. Experimental  
24 procedures were approved by the local institutional review committee. Briefly, PBMCs were



1 isolated by standard density gradient centrifugation on Ficoll-Hypaque (Eurobio,  
2 Courtaboeuf, France). Mononuclear cells were separated from PBLs by centrifugation on a  
3 50% Percoll solution (GE Healthcare, Velizy, France). Monocytes were purified by  
4 immunomagnetic depletion using pan-mouse IgG Dynabeads (ThermoFisher Scientific,  
5 Villebon sur Yvette, France) with a cocktail of mAbs anti-CD19 (4G7 hybridoma), anti-CD3  
6 (OKT3 hybridoma, ATCC, Manassas, VA, USA) and anti-CD56 (NKH1, Beckman Coulter,  
7 Villepinte, France). Monocyte purity was >90% as assessed by CD14 labeling without CD3<sup>+</sup>,  
8 CD19<sup>+</sup> and CD56<sup>+</sup> contaminating cells (data not shown).

9 DCs were differentiated from monocytes cultured at 37°C under 5% CO<sub>2</sub> atmosphere for 6  
10 days at 10<sup>6</sup> cells/ml in RPMI 1640 medium with GlutaMAX (ThermoFisher Scientific)  
11 supplemented with 10% FCS (PAN-Biotech, Aidenbach, Germany), 40 µg/ml gentamycin,  
12 human recombinant GM-CSF and IL-4 (Peprotech, Neuilly-Sur-Seine, France). Six days later,  
13 DCs were harvested, washed twice, and resuspended for treatment at 10<sup>6</sup> cells/ml of fresh  
14 RPMI 1640 medium supplemented with 40 µg/ml gentamycin and 10% FCS (complete  
15 medium). DCs were >95% pure as assessed by CD14 and CD1a labeling.

16 DCs were treated with inhibitors or control solvent for 30 min before addition of TLR ligands  
17 or the same volume of PBS as control. Pretreatment of DCs with inhibitory peptides, pepinh  
18 MYD, TRIF or control was carried out 6h before LPS addition. For quantitative PCR analysis  
19 cells were collected after 6h or 24h incubation. Supernatants and cells were collected after  
20 24h for glucose assay, cytokines assay, phenotyping or cell lysate preparation for western  
21 blot and HK assay.

22

23 **Phenotype analysis**

1 Monocyte and DC phenotypes were determined after labeling with FITC-labeled anti-CD14, -  
2 CD19, -HLA-DR, -CD80, -CD54, and PE-labeled anti-CD3, -CD56, -CD1a, -CD86, -CD83 and -  
3 CD40 (Beckman Coulter). Flow cytometry analysis was carried out using a FACSCanto II (BD  
4 Biosciences, Le Pont de Claix, France). For each double labeling, at least 5000 events in the  
5 live cell gate were acquired. Viability of cells was monitored using propidium iodide staining  
6 (BD Biosciences) of dead cells.

7

### 8 **Cytokine assays**

9 Clarified culture supernatants were collected 24h after treatment and stored at -20°C. IL-8,  
10 MIP-1 $\beta$ , IL-6, IL-10, IL-12p40, TNF $\alpha$  were assayed using quantitative cytokine specific  
11 Cytometric Bead Array Flex Sets (BD Biosciences).

12

### 13 **Glucose consumption and lactate production**

14 Metabolites were quantified in freshly clarified cell supernatants and control medium using  
15 glucose (HK) and lactate assay kits (Sigma-Aldrich) according to the manufacturers'  
16 instructions. Quantifications were normalized to DCs cell number at day 6.

17

### 18 **Quantitative real-time PCR (qRT-PCR)**

19 For total RNA isolation, 6h- or 24h-treated DCs were collected and washed twice with PBS.  
20 RNA extraction was performed using the NucleoSpinRNA Mini kit from Macherey Nagel  
21 according to the manufacturers' instructions. cDNA were synthesized using RNA-to-cDNA kit  
22 (Applied Biosystems, Villebon sur Yvette, France) before quantitative PCR experiment using  
23 the following primers: for HK1 DNA forward 5'-gcctctatttgaagggcgg-3', reverse 5'-  
24 gacacagtcacatcgagc-3', for HK2 DNA forward 5'-tcccctgccaccagacta-3', reverse 5'-

1 tggacttgaatcccttggtc-3', for HK3 DNA forward 5'-CATCGTGGACTTCCAGCAGAAG-3', reverse  
2 5'-CTTGGTCCAGTTCAGGAGGATG, for HK4 DNA forward 5'-CAGAAGGCTCAGAAGTCGGG-3'  
3 reverse 5'-TGGTGTGGTCTTCACGCT-3', for PKM1 DNA forward 5'-  
4 CGAGCCTCAAGTCACTCCAC-3', reverse 5'-GTGAGCAGACCTGCCAGACT-3', for PKM2 DNA  
5 forward 5'-ATTATTTGAGGAACTCCGCCGCCT-3', reverse 5'-ATTCCGGGTCACAGCAATGATGG-  
6 3'(34), for PFKL DNA forward 5'-AAGAAGTAGGCTGGCACGACGT-3', reverse 5'-  
7 GCGGATGTTCTCCACAATGGAC-3', for PFKP DNA forward 5'-AGGCAGTCATCGCCTTGCTAGA-3',  
8 reverse 5'-ATCGCCTTCTGCACATCCTGAG-3', for PFKM DNA forward 5'-  
9 GCTTCTAGCTCATGTCAGACCC-3', reverse 5'-CCAATCCTCACAGTGGAGCGAA-3', for TBP DNA  
10 forward 5'-CCACGAACCACGGCACTGATTT-3', reverse 5'-CAGTCTGGACTGTTCTTCACTCTT-3'  
11 and SYBRgreen master mix on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad,  
12 Marnes-la-Coquette, France). Levels of mRNA for specific genes are reported as relative gene  
13 expression normalized to the housekeeping gene TBP.

14

### 15 **Hexokinase activity assay**

16 The method used for extracting HK from DCs was adapted from Kuang et al. (35) and the  
17 assay was a modification of that described by Ramière et al. (36). -80°C frozen pelleted cells  
18 were homogenized (100 µl / 2 x 10<sup>6</sup> cells) in pre-cooled homogenization buffer (50 mM Tris–  
19 HCl, 250 mM sucrose, 5 mM EDTA (pH 7.4), 5 mM 2-mercaptoethanol, 10 mM glucose, 0.2%  
20 Triton X-100). After a 20 min incubation on ice, homogenates were pulse-sonicated 5 sec at  
21 half power of the device (EpiShear Probe Sonicator, Active Motif, La Hulpe, BE).  
22 Homogenates were then centrifuged at 500 g for 20 min at 4°C. Supernatants were  
23 immediately used for determination of HK activity. HK activity was measured  
24 spectrophotometrically through NADP<sup>+</sup> reduction in the glucose-6-phosphate

1 dehydrogenase-coupled reaction. HK activity was assayed in medium containing 50 mM  
2 triethanolamine (pH=7.6), 550 mM D-Glucose, 100 mM MgCl<sub>2</sub>, 14 mM NADP<sup>+</sup>, 125 U/mL  
3 glucose-6-phosphate dehydrogenase (*Saccharomyces cerevisiae*), equilibrated to 37 °C. The  
4 reaction was started by addition of ATP (final concentration 19 mM) and absorbance was  
5 continuously recorded for 30 min at 340 nm (TECAN Infinite M200). Dilutions of purified  
6 recombinant hexokinase (Sigma-Aldrich) were used as standards and results were expressed  
7 as IU/mg of proteins.

8

### 9 **Phosphoprotein detection**

10 Day 6 DCs were pre-treated in water bath at 37°C with inhibitors for 30 min before TLR  
11 ligands addition for 15 min. TLR stimulation was stopped on ice before two cold PBS-washes.  
12 Then cells were fixed with cytofix solution (BD Biosciences) for 10 min at 37°C before  
13 permeabilization with methanol 90% for 30 min on ice. Phosphoprotein staining was  
14 performed by incubation one hour at room temperature with the following anti-human  
15 antibodies: Purified anti-phospho-p38 MAPK ((Thr180/Tyr182), Cell signaling, Leiden, The  
16 Netherlands), PE-conjugated anti-phospho-NF-κB ((Ser536), Cell signaling), purified anti-  
17 phospho-AKT ((Ser473), Cell signaling), PE-conjugated anti-phospho-SAPK/JNK  
18 ((Thr183/Tyr185), Cell signaling). When non-labeled primary antibody was used, cells were  
19 further incubated one hour at room temperature with secondary Alexa-488-conjugated anti-  
20 rabbit antibody (ThermoFisher Scientific). Cells were analyzed using a FACSCanto II (BD  
21 Biosciences).

22

### 23 **SDS-PAGE and western blotting**

1 DCs were lysed in buffer containing 20 mM Tris-HCl, 180 mM NaCl, 1 mM EDTA, 0.5% NP40  
2 and 1x cocktail of protease inhibitors (Sigma Aldrich). Lysate proteins were separated by  
3 NuPAGE<sup>®</sup> 4-12% Bis Tris-Gel (ThermoFischer Scientific) in MOPS running buffer. Gels were  
4 transferred to nitrocellulose membranes with the Trans-Blot Turbo Transfer system (Bio-  
5 Rad). Membranes were blocked for 1h with PBS buffer containing 0.1% Tween 20 and 5%  
6 non-fat dry skim milk and incubated 1h with the primary antibody against HIF-1 $\alpha$  (Novus  
7 Biologicals, Lille, France) or actin (clone AC-40, Sigma Aldrich). Membranes were washed and  
8 incubated 1h with horseradish peroxidase-coupled goat polyclonal anti-rabbit IgG or  
9 horseradish peroxidase-coupled goat polyclonal anti-mouse IgG (Jackson ImmunoResearch  
10 laboratories, Suffolk, UK). Western blotting experiments were developed using SuperSignal  
11 West Femto or Pico chemiluminescent substrate (ThermoFisher Scientific).  
12 Chemiluminescent signals were acquired and quantified with a LAS4000 Imager (GE  
13 healthcare).

14

#### 15 **Statistical analysis**

16 Data are expressed as mean  $\pm$  SEM. The Student's t test was used for comparisons of two  
17 sample means. Multiple group comparisons were performed by one-way ANOVA followed  
18 by Bonferroni multiple comparisons test and was applied to the analysis of all dose-  
19 dependent experiments. A p-value less than 0.05 was considered statistically significant.

1 **Results**

2

3 **TLR4 stimulation induces glycolytic burst and upregulation of HK2 expression and activity**

4 Monocyte-derived DCs (MoDCs) stimulated by LPS for 24h showed an increased glucose  
5 consumption and lactate production compared to non-stimulated MoDCs (Fig 1A-B),  
6 indicative of an increased glycolytic activity. The amplitude of this glycolytic burst was  
7 dependent on the dose of LPS and was statistically significant from 10 ng/ml to 1000 ng/ml.  
8 We previously characterized a selective TLR4 antagonist, FP7, inhibiting the secretion of pro-  
9 inflammatory cytokines by LPS-stimulated MoDCs with an IC<sub>50</sub> below 1 μM (27). FP7 also  
10 prevented LPS-induced secretion of IFN-β (Suppl Fig 1), suggesting that FP7 blocked both  
11 endosomal TRIF-dependent and surface MyD88-dependent TLR4 signaling (37). Since FP7  
12 competes with LPS for binding to the TLR4 MD-2 subunit (32, 38), and prevents ligand-  
13 mediated TLR4 internalization (F. Facchini et al., manuscript in preparation), these results  
14 suggest that FP7 may not directly inhibit endosomal TLR4 signaling but rather inhibits  
15 upstream activation of TLR4 receptor by LPS. Treatment of cells with 10 μM FP7 prior to LPS  
16 stimulation prevented the increase in glucose consumption and lactate production triggered  
17 by LPS from 0.1 to 100 ng/ml (Fig 1A-B), showing that this glycolytic regulation was TLR4-  
18 dependent. At the highest LPS concentration (1000 ng/ml), FP7 did not totally block the  
19 increase of the glycolytic flux, consistent with previous data (27). Using a fluorescent  
20 deoxyglucose as a non-hydrolysable substrate of HK (2-NBDG), we measured the capacity of  
21 glucose uptake by the cells after 6h and 24h LPS-stimulation. No difference in the uptake  
22 could be detected between control and LPS-stimulated MoDCs (Fig 1C), indicating that  
23 increased glycolytic activity in TLR4-stimulated MoDCs was not due to an increased  
24 availability of intracellular glucose. We thus analyzed the expression of the 3 rate-limiting

1 glycolytic enzymes, HK, PFK and PKM, at the mRNA level. Compared to control cells, HK2 was  
2 strongly induced in LPS-stimulated MoDCs while HK3 was reduced (Fig 1D). HK1 remained  
3 unchanged, whereas HK4 was not expressed in MoDCs. In contrast to PFKM and PKM1, PFKL,  
4 PFKP and PKM2 were highly expressed in MoDCs but not significantly augmented upon LPS  
5 stimulation (Fig 1E-F). The data indicate that the expression profile of HK isoenzymes is  
6 modified upon TLR4 stimulation. This new expression profile correlates with a major  
7 increase in hexokinase activity in total lysates of LPS-stimulated MoDCs compared to  
8 unstimulated cells (Fig 1G).

9

#### 10 **TLR4-induced glycolytic burst involves p38-MAPK activation and HIF-1 $\alpha$ transcriptional** 11 **activity**

12 To investigate the molecular mechanisms involved in the regulation of glycolysis upon TLR4  
13 stimulation, we screened a panel of inhibitors described to target main actors of the TLR4  
14 signaling pathway. A scheme positioning the inhibitors and their target is presented on a  
15 simplified view of the TLR4 signaling pathway (Fig 2). TLR4 signaling in MoDCs results in the  
16 phenotypic and functional maturation of these cells, inducing cytokines secretion and  
17 enhancing the expression of surface maturation markers. MoDCs were treated with these  
18 drugs prior to LPS stimulation for 24h and secretion of pro-inflammatory cytokines,  
19 expression of phenotypic maturation marker and glucose consumption were measured.

20 As expected, p38-MAPK inhibitor SB203580 (SB) inhibited the secretion of IL-6, IL-8 and MIP-  
21 1 $\beta$  (Fig 3A) (39, 40). Echinomycin (Echino) strongly reduced IL-8 secretion and the inhibitor of  
22 TBK1, BX795, inhibited MIP-1 $\beta$  secretion induced by LPS. Surface expression of the MHC  
23 molecule HLA-DR, of co-stimulation molecules CD86, CD40, and of the CD54 adhesion  
24 molecule was analyzed by FACS (Fig 3B-C). SB significantly inhibited the expression of CD86,

1 CD54 and reduced although not significantly CD40 expression compared to LPS-stimulated  
2 MoDCs. SC75741 and AZD5363 mainly reduced CD54 expression. The other inhibitors did not  
3 induce significant changes in MoDCs phenotype.

4 Among the tested inhibitors, p38-MAPK inhibitor SB was the most potent inhibitor of the  
5 glycolytic burst triggered by LPS (Fig 4A). Echinomycin, which prevents DNA binding of HIF-  
6  $1\alpha$  to Hypoxia Response Elements, also significantly reduced the action of LPS on glucose  
7 consumption (Fig 4A). The other drugs did not significantly reduce glucose consumption by  
8 LPS-stimulated cells. The inhibitors showed no significant toxicity (Fig 4B). These results  
9 indicate that p38-MAPK and HIF- $1\alpha$  are major actors of the increased glucose consumption  
10 triggered by LPS and suggest an unexpected link between them. By blocking dimerization of  
11 MyD88 or TRIF, we observed that both TLR adaptors were recruited to trigger the glycolytic  
12 burst upon TLR4 stimulation (Suppl Fig 2), pointing to a common downstream pathway that  
13 may involve TRAF6, a key component upstream of p38-MAPK.

14 BX795 altered the secretion of cytokines and/or the expression of phenotypic marker  
15 without affecting LPS-triggered regulation of glycolysis (Fig 3A-C, 4A). In contrast, treatment  
16 by the HIF- $1\alpha$  antagonist Echinomycin resulted in both reduced LPS-induced glycolytic  
17 activity and IL8 secretion (Fig 3A, 4A), indicating that reduced glycolytic burst may contribute  
18 to the reduction of cytokines secretion or maturation markers expression. Accordingly, we  
19 previously reported that inhibition of the glycolytic burst by 2-deoxyglucose (2-DG)  
20 treatment, affected both cytokines secretion and phenotypic markers expression induced by  
21 TLR4 stimulation (27).

22

23 **LPS-induced HK2 expression requires p38-MAPK dependent HIF- $1\alpha$  stabilization**



1 MoDCs stimulation by LPS resulted in a strong increase of HK2 expression that can be  
2 detected as soon as 6h after LPS addition (Fig 5A). At this early time, HK3 expression was  
3 unchanged compared to control cells while it was decreased 24h post stimulation (Fig 1D).  
4 HK1 remained constant (Fig 5A). Using the inhibitors that reduced glucose consumption by  
5 LPS-treated MoDCs, we found that SB and Echino strongly reduced HK2 transcription  
6 induced by LPS (Fig 5A). SB treatment of MoDCs inhibited the phosphorylation of p38-MAPK,  
7 without affecting the phosphorylation of NF- $\kappa$ B and JNK, as expected (Fig 5B). HIF-1 $\alpha$  is an  
8 important transcription factor for the regulation of HK2 expression (41). It is constitutively  
9 expressed and the regulation of its degradation controls its accumulation and activity (42,  
10 43). In total lysates from MoDCs, we observed that LPS stimulation for 24h resulted in an  
11 important accumulation of HIF-1 $\alpha$  that was prevented by SB treatment (Fig 5C-D). Therefore  
12 HIF-1 $\alpha$  accumulation triggered by LPS appeared to be dependent on p38-MAPK activation.

13

#### 14 **p38-MAPK-HIF-1 $\alpha$ axis is not engaged in the glycolytic burst induced by TLR1/2 and TLR2/6** 15 **stimulation**

16 As other bacterial ligands can induce MoDCs functional activation, we tested the effect of  
17 PGN and Pam, the ligands for TLR2/6 and TLR1/2 respectively, on glucose consumption. Both  
18 TLR ligands significantly increased glucose consumption of MoDCs although to a different  
19 level (Fig 6A). PGN, like LPS, induced a strong increase of glucose consumption, while Pam  
20 was less efficient (50% of the LPS increase). Inhibition of glucose consumption by SB reached  
21 70% and 65% when it was induced by LPS-TLR4 and Pam-TLR1/2 respectively, whereas it was  
22 only 35% after PGN-TLR2/6 stimulation. P38-MAPK phosphorylation was detected upon  
23 stimulation of all these TLRs and was inhibited by SB (Fig 6B). The inhibition of this kinase by  
24 SB strongly impacted the secretion of cytokines triggered by TLR4. Cytokines secretion

1 elicited by TLR2/6 or TLR1/2 tended to be reduced by SB but to a lesser extend (Fig 6C-D).  
2 Therefore, the amplitude of glycolytic burst and of its inhibition by SB indicates that  
3 glycolysis regulation by TLRs is unlikely to result from a common mechanism.  
4 Under the same conditions, we analyzed cells content in HIF-1 $\alpha$  and observed that TLR1/2  
5 and TLR2/6 stimulation of MoDCs, by Pam or PGN respectively, resulted in a weak  
6 accumulation of HIF-1 $\alpha$  compared to TLR4 stimulation by LPS (Fig 6E). SB treatment did not  
7 modify the amount of HIF-1 $\alpha$  upon TLR2/6 and TLR1/2 in contrast to TLR4 stimulation. In line  
8 with this observation, the inhibition of HIF-1 $\alpha$  transcriptional activity by Echinomycin did not  
9 significantly impact glucose consumption triggered by PGN and Pam (Fig 7), suggesting that  
10 this pathway does not play a major role in glycolysis regulation by TLR2/6 and TLR1/2. As  
11 expected, glycolysis inhibition by 2-DG, reduced glucose consumption triggered by PGN and  
12 Pam. The screening of other inhibitors showed a weak contribution of TBK1 in the  
13 upregulation of glucose consumption by PGN, whereas neither PI3K nor AKT or mTOR  
14 inhibition significantly altered the glycolytic burst (Fig 7). The screening did not allow the  
15 identification of a pathway involved in the glycolytic regulation upon TLR1/2 stimulation by  
16 Pam.  
17 Therefore, the regulation of HIF-1 $\alpha$  stabilization upon PGN or Pam stimulation did not  
18 depend on p38-MAPK activation. TLR2/6 and TLR1/2 stimulation resulted in a p38-  
19 independent HIF-1 $\alpha$  slight accumulation and increased glucose consumption. In contrast  
20 TLR4 regulation of glycolysis in human MoDCs implicates p38-MAPK-dependent stabilization  
21 of HIF-1 $\alpha$  (Fig 8).

22

## 1 **Discussion**

2 The present study aimed at characterizing the molecular mechanisms involved in the  
3 metabolic reprogramming of human MoDCs triggered by TLR4 stimulation. We showed that  
4 TLR4-dependent glycolysis activation in these cells was correlated to an increased global HK  
5 activity and an increased expression of HK2. HIF-1 $\alpha$ , a master transcriptional regulator of  
6 glycolytic enzymes, accumulated in TLR4-stimulated MoDCs. An inhibitor of the  
7 transcriptional activity of HIF-1 $\alpha$  inhibited both TLR4-induced HK2 expression and glucose  
8 consumption. Data are highlighting the role of p38-MAPK activation in HIF-1 $\alpha$  stabilization  
9 and glycolysis regulation upon TLR4 stimulation. Our study has analyzed in detail the  
10 molecular pathways of glycolysis regulation in this model of immunostimulatory DCs (44,  
11 45). Malinarich et al. compared the metabolic profile of tolerogenic DCs and  
12 immunostimulatory MoDCs and found that tolerogenic DCs had the highest glycolytic  
13 capacity upon ATP synthase inhibition, whereas they had a similar glycolytic rate to that of  
14 LPS-stimulated MoDCs (22). Similarly to us, they found that LPS stimulation enhanced lactate  
15 secretion and glycolytic rate of MoDCs without affecting glucose uptake.

16 Previous results with mouse DCs showed that activation of TBK1-IKK $\epsilon$  and AKT kinases  
17 controlled the early increase in glycolysis triggered by TLR4 (24). In our experimental  
18 conditions, TBK1 and AKT inhibitors did not impact the glycolytic activity of LPS-stimulated  
19 MoDCs (Fig 4A). Moreover, no phosphorylation of AKT could be detected after LPS  
20 stimulation of MoDCs (cf. Suppl Fig S3). Pam induced AKT phosphorylation (cf. Suppl Fig S3),  
21 however inhibiting PI3K or AKT kinases did not significantly alter the glycolytic  
22 reprogramming of MoDCs upon TLR1/2 or TLR2/6 stimulation (Fig 7). TLR2/6 stimulation by  
23 PGN induced an important increase of glucose consumption by MoDCs, which was  
24 significantly reduced by TBK1 inhibition (Fig 7). This is consistent with recent data showing

1 that TBK1 is involved in TLR2 signaling (46). Our results indicate that TBK1 contributed to this  
2 metabolic regulation, by mechanisms different from those described for murine BM-DCs (24)  
3 since AKT was not involved. Thus, glycolysis activation by TLR4, TLR2/6 and TLR1/2 appears  
4 to engage distinct signaling pathways in human MoDCs.

5 Major differences in the regulation of glycolytic activity have been reported between mouse  
6 and human DCs. mTOR was shown to be an important regulator of LPS-induced murine DC  
7 activation and glucose consumption, but not in human myeloid DCs (47). In agreement with  
8 these results, we observed that the mTORC1 inhibitor Rapamycin did not impact the  
9 upregulation of glycolysis upon LPS stimulation in MoDCs (Fig 4). Moreover, human MoDCs  
10 and macrophages do not produce any NO (27, 31), an inhibitor of the oxidative  
11 phosphorylation that was shown to boost the glycolytic pathway in mouse DCs (25).  
12 Therefore, results obtained from murine BM-DCs or macrophages cannot be readily  
13 extrapolated to human DCs.

14 Previous works indicated that LPS stimulation of mouse BM-DCs and human MoDCs resulted  
15 in HIF-1 $\alpha$  accumulation (14, 18, 19). In normoxia, HIF-1 $\alpha$  degradation is regulated by  
16 hydroxylation of proline and asparagine residues by prolyl-hydroxylase domain enzymes  
17 (PHDs). The interaction of the von Hippel–Lindau (VHL) factor with these hydroxylated  
18 residues, recruits an E3 ubiquitin–ligase that targets HIF-1 $\alpha$  to the proteasome for  
19 degradation (48). Hypoxia induces HIF-1 $\alpha$  accumulation via PHD inhibition due to the lack of  
20 its oxygen cosubstrate (18). However, data describing the molecular mechanisms controlling  
21 HIF-1 $\alpha$  accumulation in primary cells, upon inflammation in normoxic conditions are sparse.  
22 In murine macrophages, LPS-induced HIF-1 $\alpha$  accumulation seems to require NF- $\kappa$ B- and ERK-  
23 dependent transcriptional events (49, 50). In other studies, ROS production and succinate  
24 accumulation upon LPS stimulation inhibit PHD activity thus increasing HIF-1 $\alpha$  stability (51,

1 52). In murine BM-DCs, sequestration of iron, a PHD co-factor, stabilizes HIF-1 $\alpha$  upon LPS  
2 stimulation (18). The AKT-mTOR-HIF-1 $\alpha$  pathway was found to upregulate glycolysis in  
3 murine monocytes (20). In human MoDCs, our results point to a different molecular  
4 mechanism involving a p38-MAPK dependent HIF-1 $\alpha$  stabilization. This is crucial to the  
5 glycolytic regulation upon TLR4 activation but p38 activity is not involved in the stabilization  
6 of HIF-1 $\alpha$  upon TLR2/6 stimulation. Although, p38-MAPK phosphorylation is induced by  
7 TLR4, TLR1/2 and TLR2/6 (Fig 6B), the consequence on HIF-1 $\alpha$  accumulation varies. This  
8 differential regulation points to an indirect control of HIF-1 $\alpha$  stability by p38-MAPK. The  
9 molecular mechanism explaining the differential regulation of HIF-1 $\alpha$  accumulation in  
10 MoDCs according to TLR stimulation remains to be discovered. In tumor cells, previous  
11 studies showed that p38-MAPK could contribute to HIF-1 $\alpha$  stabilization (53, 54). One  
12 possible mechanism proposed by Khurana et al. (55) in mouse embryonic fibroblasts, is that  
13 the Ring Finger ubiquitin ligase Siah2 can be phosphorylated by p38-MAPK, increasing the  
14 degradation of PHD3, therefore reducing HIF-1 $\alpha$  degradation.

15 In both mouse and human models, HIF-1 $\alpha$  stabilization alone was not sufficient to induce DC  
16 maturation, but it positively contributes to their functionality upon TLR stimulation (14, 19).

17 In mouse BM-DCs, HIF-1 $\alpha$  induction by the combined effects of LPS and hypoxia plays a key  
18 role in the regulation of glucose consumption, DC maturation and their ability to stimulate  
19 allogeneic T cells (14). In human MoDCs, HIF-1 $\alpha$  stabilization could synergize with TLR  
20 stimulation to favor maturation (19).

21 The HIF-1 $\alpha$  DNA-binding inhibitor, Echinomycin, reduced the secretion of IL-8 by LPS-  
22 stimulated DCs (Fig 3A). IL-8 secretion highly depends on enhanced gene transcription and  
23 IL-8 promoter mainly recruits NF- $\kappa$ B, AP-1 and NRF (56) but does not contain HIF response  
24 elements. The reduction of IL-8 secretion by Echinomycin treatment may thus result from

1 the limitation of the glycolytic burst of MoDCs. Indeed, previous results indicate that  
2 hexokinase inhibition by 2-DG suppressed increased glycolysis and reduced the secretion of  
3 many cytokines and especially IL-8 upon LPS-stimulation (27, 29). By reducing the glycolytic  
4 burst triggered by LPS, Echinomycin may impede optimal maturation and especially impact  
5 maturation processes that require active gene transcription, such as cytokine neosynthesis.  
6 Reduced expression of phenotypic maturation markers of MoDCs was observed with 2-DG  
7 which strongly inhibits glycolysis (27), but not with Echinomycin which only partially reduces  
8 LPS-induced glycolytic burst (Fig 3B-C). Glycolysis inhibition by 2-DG strongly reduced the  
9 secretion of inflammatory cytokines and altered the expression of some phenotypic  
10 maturation markers, triggered by PGN and Pam (Suppl Fig 4), reinforcing previous results  
11 highlighting the importance of the glycolytic burst for TLR-induced DC maturation (27, 29).  
12 Reduction by Echinomycin of both the glycolytic burst (Fig 7) and the secretion of pro-  
13 inflammatory cytokines (Suppl Fig 4) triggered by PGN and Pam was not statistically  
14 significant. Even though we cannot totally exclude a contribution of HIF-1 $\alpha$ , this pathway did  
15 not appear to play a major role in glycolysis regulation by TLR2/6 and TLR1/2.  
16 Increasing evidence indicates that metabolic pathways play important roles in the fine  
17 tuning of immune cell functions. Detailed studies have been conducted on T cell  
18 differentiation and mouse macrophage functional polarization according to their metabolic  
19 state (11). The signaling of many receptors can result in the regulation of metabolic  
20 pathways, by controlling the activation of transcription factors and the expression of  
21 metabolic enzymes. Some transcription factors such as HIF-1 $\alpha$  regulate the expression of  
22 both metabolic enzymes and proinflammatory cytokines such as IL-1 $\beta$  in mouse M1  
23 macrophages (52) and TNF $\alpha$  in human monocytes (20). The concentration of metabolites  
24 varying according to the metabolic state of the cells can also impact their immune function.

1 These metabolic signals can be detected by various sensors and are important regulators of  
2 inflammation (57).

3 Our results also show that the increased hexokinase activity after 24h stimulation by TLR4 of  
4 human MoDCs is correlated to increased HK2 gene expression, whereas HK3 was  
5 downregulated, HK1 did not change and HK4 was not expressed. These results differ from  
6 those obtained in murine bone-marrow derived macrophages, where HK1 but not HK2 was  
7 upregulated via mTORC1 activation upon LPS and ATP stimulation (58). This difference  
8 however is in agreement with the lack of effect of the mTORC1 inhibitor Rapamycin on  
9 glycolysis upregulation in MoDCs. Hexokinases catalyze the first step of glucose metabolism,  
10 producing glucose-6-phosphate which is a precursor for glycolysis but also for the pentose-  
11 phosphate and hexosamine biosynthetic pathways. Thus, HK plays a key role in glycolysis  
12 and biosynthetic pathways. HK-II is the predominant isoenzyme in insulin-sensitive tissues  
13 such as heart, adipose tissue and skeletal muscle and it is also upregulated in many types of  
14 tumors associated with enhanced aerobic glycolysis (Warburg effect), suggesting that it is a  
15 major regulator of the glycolytic rate in these cells (59). HK-II activity is upregulated by its  
16 binding to the outer mitochondrial membrane protein voltage-dependent anion channel 1  
17 (VDAC1). Our results indicate that HK2 upregulation also plays a key role in metabolic  
18 regulation of human primary DCs. Although the molecular mechanisms triggered by TLR4 to  
19 stimulate glycolysis are different in humans MoDCs and mouse BM-DCs, they can both  
20 involve HK-II protein modulation (24). In the future, HK-II intracellular localization should be  
21 investigated in MoDCs in response to TLR4 stimulation. Moreover, the contribution of other  
22 non-enzymatic functions of HK-II should also be analyzed. Indeed, overexpression and  
23 mitochondrial association of HK-II confer protection to apoptotic or necrotic stimuli in  
24 different cell types by several mechanisms (59). Increased expression of HK2 upon LPS

1 stimulation may contribute to pro-survival effects of LPS stimulation of MoDCs. HK-II, but not  
2 other HKs, bind and inhibit mTORC1 in the absence of glucose, facilitating autophagy in  
3 response to glucose starvation. Thus HK-II can protect cells from cellular damage and  
4 provide energy by recycling intracellular constituents (60).

5 Metabolomics of immune cells recently established that TLR4 induces glycolytic  
6 reprogramming and a drastic rewiring of TCA cycle to generate inflammatory intermediates  
7 that contribute to macrophages and DCs activation. Dysregulated TLR4-triggered  
8 inflammatory response can be involved in pathologies such as sepsis (61), neuropathic pain  
9 (62), amyotrophic lateral sclerosis (63), some autoimmune diseases such as rheumatoid  
10 arthritis (64), or obesity-associated type II diabetes (65). Our results unravel a novel  
11 molecular mechanism linking TLR4 signaling and glycolysis activation and together with  
12 previous reports reinforce the idea that a better understanding of mechanism underlying  
13 TLR4-induced glycolytic reprogramming should offer innovative therapeutic windows.

14  
15



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1

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6

7 **Abbreviations used in this article:** BM-DC, bone marrow-derived DC; DC, dendritic cell;  
8 Echino, echinomycin; HIF, hypoxia-inducible factor; HK, hexokinase; MoDC, monocyte-  
9 derived DC; mTORC, mammalian target of rapamycin complex; Pam, Pam<sub>3</sub>CSK<sub>4</sub>; PFK,  
10 phosphofructokinase; PGN, peptidoglycan; PHD, prolyl-hydroxylase domain enzymes; PKM,  
11 pyruvate kinase M; SB, SB203580; TCA, tricarboxylic acid;

12

1 **Figure Legends**

2 **Fig. 1: TLR4 stimulation of glucose consumption and lactate production correlates with**  
3 **enhanced expression of HK2 and increased hexokinase activity.** Dendritic cells (DCs) were  
4 differentiated from human peripheral blood monocytes for 6 days. DCs were seeded at  $1 \times$   
5  $10^6$  cells/ml in fresh culture medium before treatment. (A-B) DCs were treated at 37°C with  
6 10  $\mu$ M FP7 (+FP7) or control solvent (-FP7) 30 min prior to stimulation with increasing  
7 amounts of LPS for 24h. Glucose consumption and lactate production were monitored using  
8 enzymatic detection kits. Means  $\pm$  SEM from 11 (A) or 5 (B) independent experiments are  
9 shown. (C) DCs were stimulated with 10 ng/ml LPS for 6h or 24h. Uptake of 2-NBDG was  
10 allowed for 15 min at 37°C. Internalization of the fluorescent probe was analyzed by flow  
11 cytometry. (D-F) DCs were stimulated or not with 10 ng/ml LPS for 24h. Gene expression was  
12 analyzed by quantitative RT-PCR and normalized to housekeeping gene TBP expression. (G)  
13 DCs, stimulated or not with 10 ng/ml LPS for 24h, were washed and lysed in homogenization  
14 buffer for hexokinase activity assay. Results are expressed as U/mg of proteins

15

16 **Fig. 2: Schematic representation of TLR4 signaling pathways.** Drug targets are indicated by T  
17 marks on a simplified view of TLR4 signaling pathways.

18

19 **Fig. 3: Drugs effect on DC phenotypic and functional activation by LPS.** DCs were seeded at  
20  $1 \times 10^6$  cells/ml in fresh culture medium, treated at 37°C for 30 min with 20  $\mu$ M SB203580,  
21 10  $\mu$ M SP600125, 40  $\mu$ M PD98059, 5  $\mu$ M SC75741, 100 nM Rapamycin, 5  $\mu$ M BX795, 10  $\mu$ M  
22 LY294002, 10  $\mu$ M AZD5363, 10 nM Echinomycin or control solvent (solv) prior to stimulation  
23 with 10 ng/ml LPS for 24h. Control cells (Ctl) received PBS instead of LPS. Cells and  
24 supernatants were collected. (A) Cytokine secretion was assayed in DC supernatants by

1 Cytometric Bead Array. Data represents mean cytokine secretion from at least 3  
2 independent experiments. (B-C) Surface expression of DC maturation markers was  
3 monitored by flow cytometry and means  $\pm$  SEM of fluorescence intensity from at least 3  
4 independent experiments are shown.

5

6 **Fig. 4: LPS-triggered increase in glucose consumption involves p38-MAPK activation and**  
7 **HIF-1 $\alpha$  transcriptional activity.** DCs were seeded at  $1 \times 10^6$  cells/ml in fresh culture medium.  
8 Cells were treated at 37°C for 30 min with 20  $\mu$ M SB203580, 10  $\mu$ M SP600125, 40  $\mu$ M  
9 PD98059, 5  $\mu$ M SC75741, 100 nM Rapamycin, 5  $\mu$ M BX795, 10  $\mu$ M LY294002, 10  $\mu$ M  
10 AZD5363, 10 nM Echinomycin or control solvent prior to stimulation with 10 ng/ml LPS for  
11 24h. (A) Glucose consumption was monitored using enzymatic detection kits. To measure  
12 the effect of inhibitors, results were normalized to control LPS-treated cells from the same  
13 experiment. Means  $\pm$  SEM from 3 to 11 independent experiments are shown. (B) Cells were  
14 collected and analyzed by flow cytometry for propidium iodide labeling.

15

16 **Fig. 5: LPS-induced HK2 expression requires p38-dependent HIF-1 $\alpha$  stabilization.**

17 DCs were seeded at  $1 \times 10^6$  cells/ml in fresh culture medium. Cells were treated at 37°C for  
18 30 min with 20  $\mu$ M SB203580 or 10 nM Echinomycin prior to stimulation with 10 ng/ml LPS  
19 for 6h (A), 15 min (B), or 24h (C-D). (A) Gene expression was analyzed by quantitative RT-PCR  
20 and normalized to housekeeping gene TBP expression. Results are presented as fold  
21 induction to control (Ctl) cells that received solvent. (B) After fixation and permeabilization,  
22 cells were labeled for phospho-p38 MAPK (pp38), phospho-NF- $\kappa$ B (pNF- $\kappa$ B) or phospho-JNK  
23 (pJNK) and analyzed by flow cytometry. (C-D) Cell lysates were analyzed by western blot to



1 detect HIF-1 $\alpha$  and actin. Chemiluminescent signals from 3 independent experiments were  
2 quantified and means  $\pm$  SEM are shown.

3

4 **Fig. 6: p38 MAPK-dependent HIF-1 $\alpha$  stabilization is not engaged by TLR2/6 or TLR1/2**  
5 **stimulation.**

6 DCs were seeded at  $1 \times 10^6$  cells/ml in fresh culture medium. Cells were treated at 37°C with  
7 20  $\mu$ M SB203580 (SB) or control solvent (Ctl) 30 min prior to stimulation with 10 ng/ml LPS,  
8 10  $\mu$ g/ml PGN or Pam or PBS for 15min (B) or 24h (A, C-E). (A) Glucose consumption was  
9 monitored using enzymatic detection kits. Means  $\pm$  SEM from 7 independent experiments  
10 are shown. (B) After fixation and permeabilization, cells were labeled for phospho-p38 MAPK  
11 (pp38) and analyzed by flow cytometry. (C-D) Cytokine secretion was assayed in DC  
12 supernatants by Cytometric Bead Array. Data represents mean cytokine secretion from at  
13 least 5 independent experiments. (E) Cell lysates were analyzed by western blot to detect  
14 HIF-1 $\alpha$  and actin. Spliced image is shown from a single experiment and a single exposition.  
15 Chemiluminescent signals from 3 independent experiments were quantified and means  $\pm$   
16 SEM are shown.

17

18 **Fig. 7: Screening of inhibitors effect on glucose consumption upon TLR2/6 (PGN) or TLR1/2**  
19 **(Pam) ligands.** DCs were seeded at  $1 \times 10^6$  cells/ml in fresh culture medium, treated at 37°C  
20 for 30 min with 40 $\mu$ M PD68059, 10  $\mu$ M SP600125, 5  $\mu$ M SC75741, 100 nM Rapamycin, 5  $\mu$ M  
21 BX795, 10  $\mu$ M LY294002, 10  $\mu$ M AZD5363, 10 nM Echinomycin, 5mM 2DG or control solvent  
22 (solv) prior to stimulation with 10  $\mu$ g/ml PGN or Pam for 24h. Glucose consumption was  
23 monitored using enzymatic detection kits. Results were normalized to control ligand-

1 stimulated cells from the same experiment. Means  $\pm$  SD from 2-5 independent experiments  
2 are shown. \* $p < 0.05$  compared to solvent

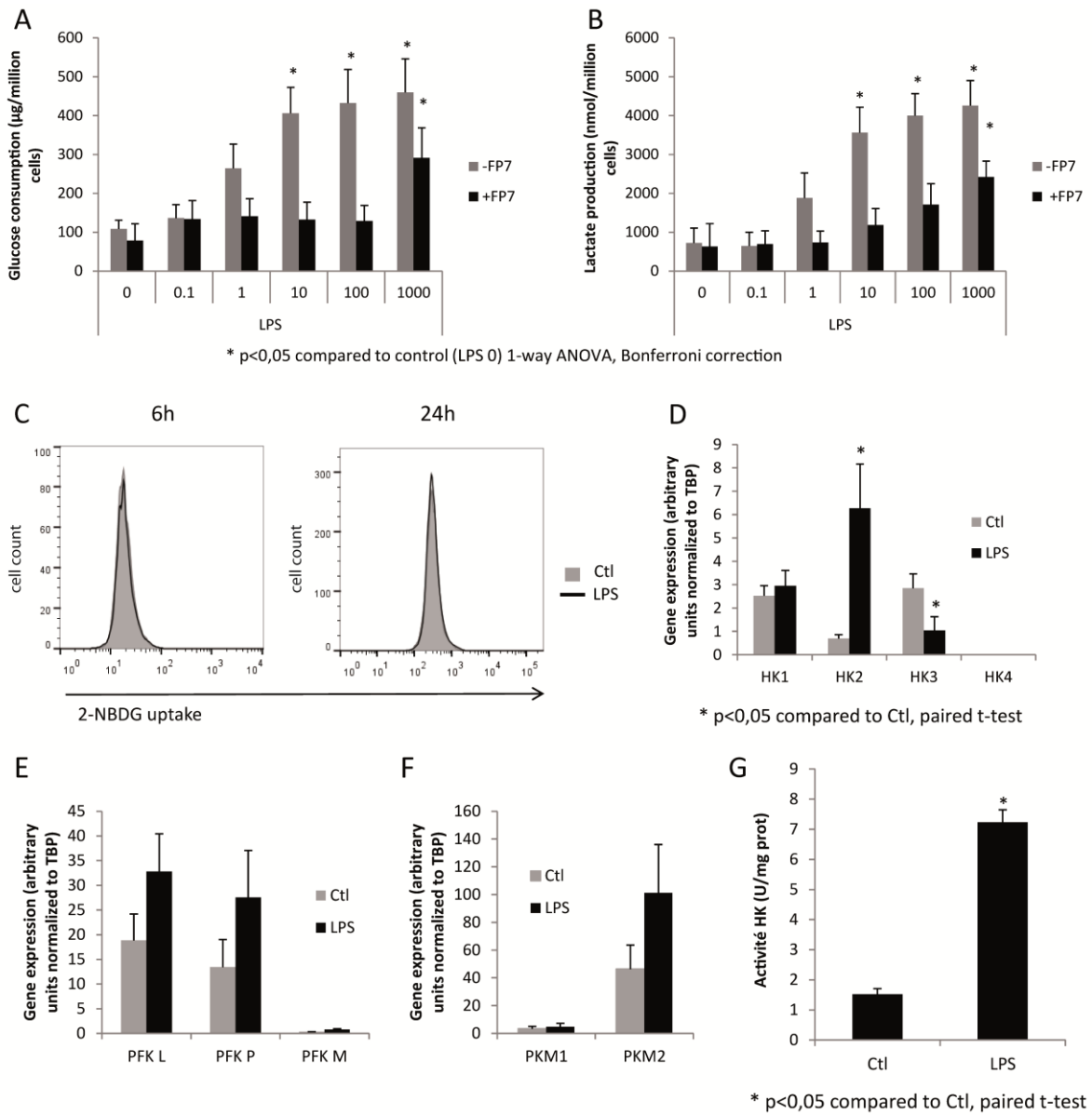
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4 **Fig. 8: TLR4 major pathway to induce a glycolytic burst in human Mo-DCs.**

5 The molecular actors involved in the glycolytic burst triggered by TLR4 in human MoDCs are  
6 indicated in grey. LPS stimulation of the TLR4 signaling pathway resulted in the  
7 phosphorylation of p38-MAPK that plays a major role in the accumulation of HIF-1 $\alpha$ . HIF-1 $\alpha$   
8 strongly enhanced the expression of HK2. Hexokinase activity and glycolytic activity were  
9 increased in LPS-stimulated MoDCs.

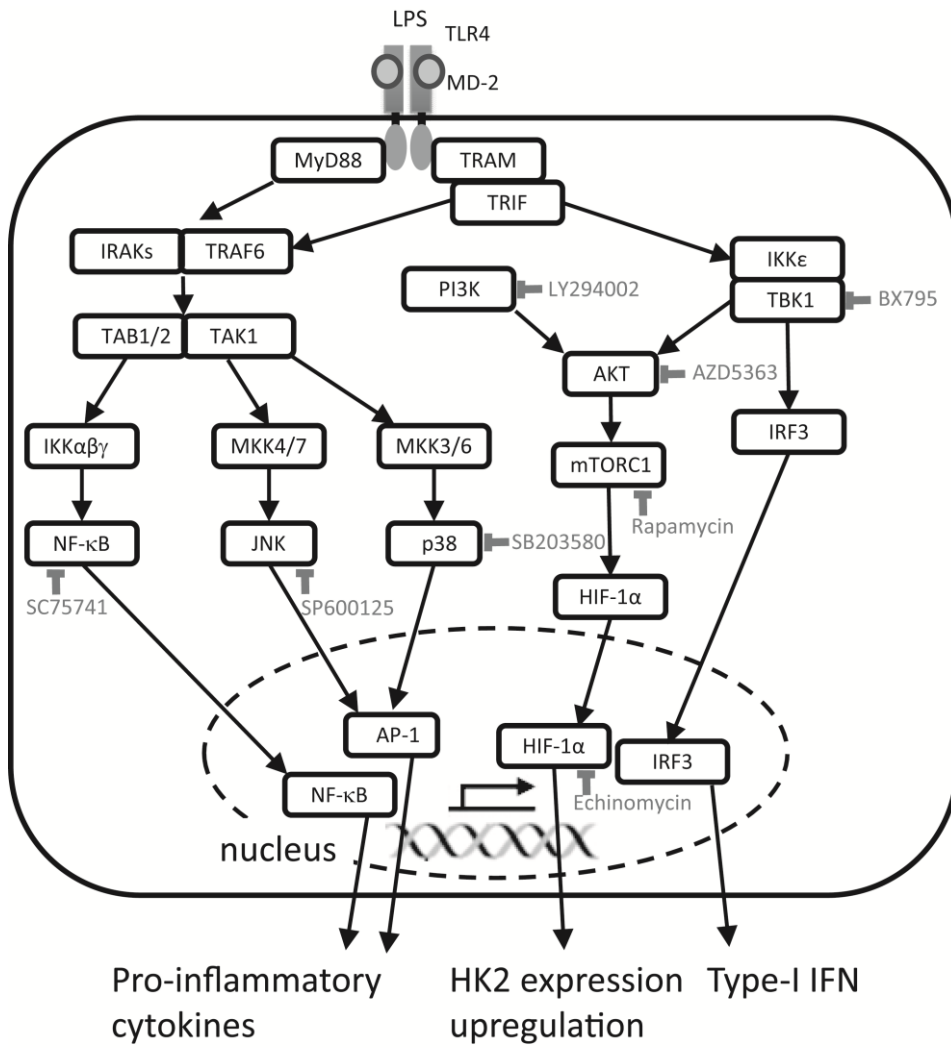
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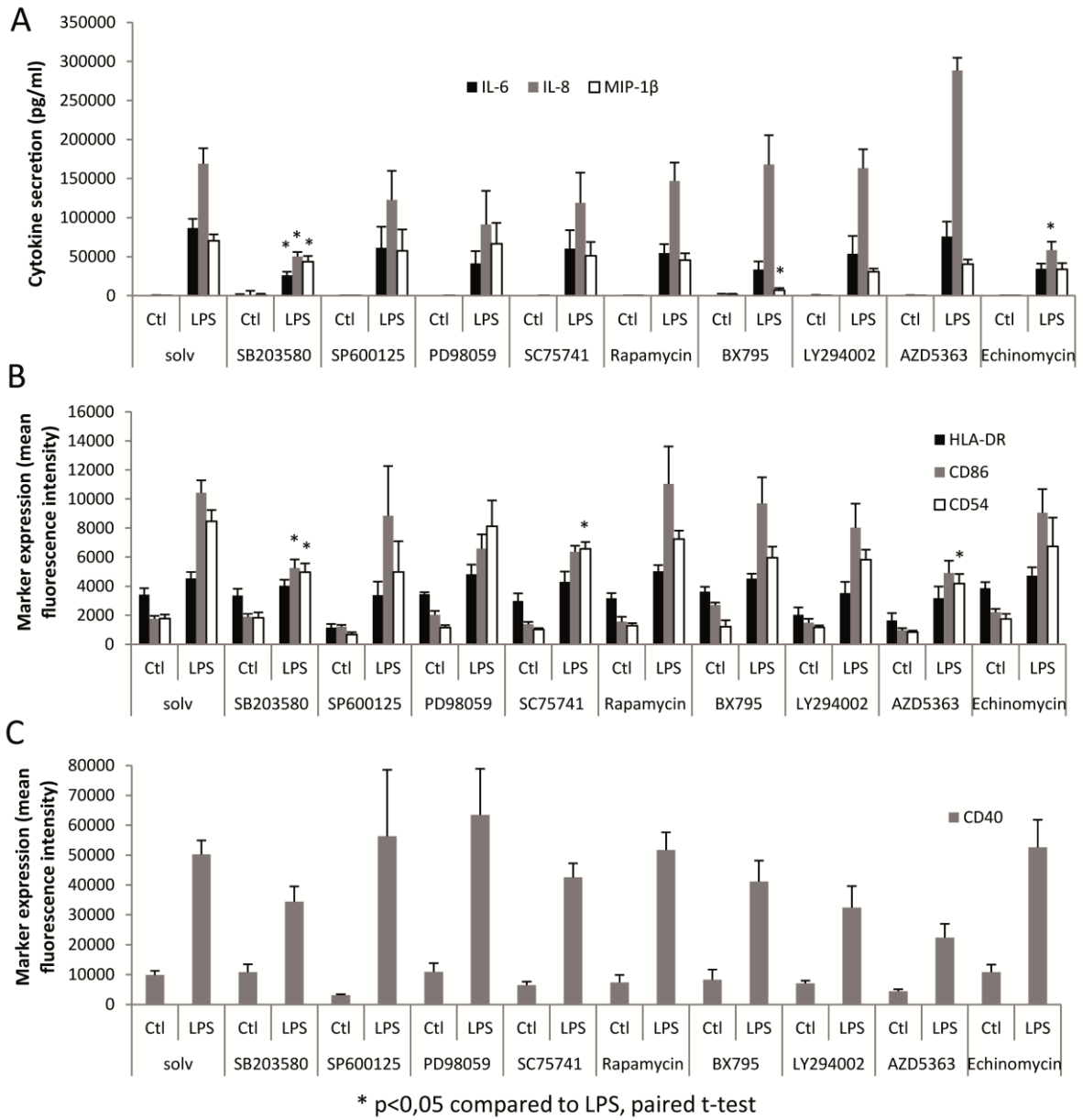
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Figure 1



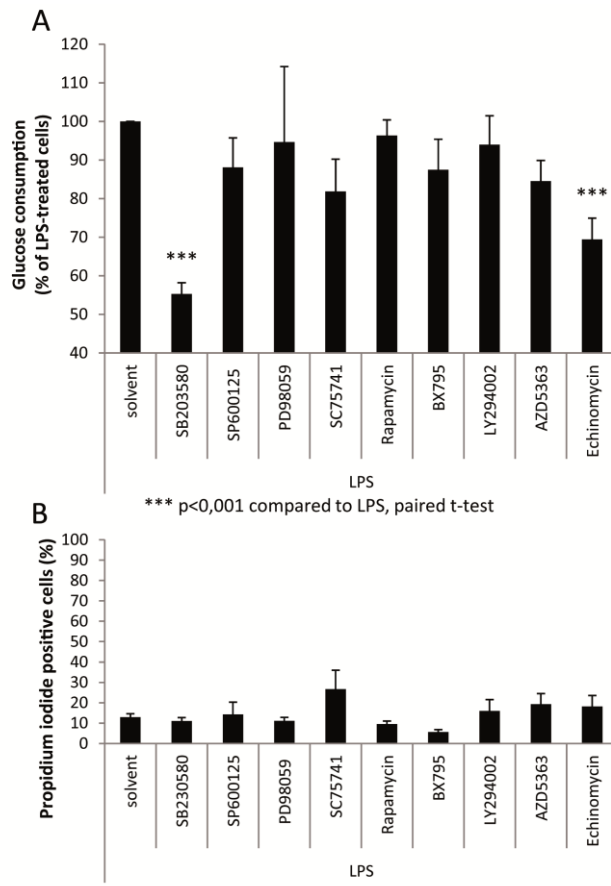
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Figure 2



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Figure 3

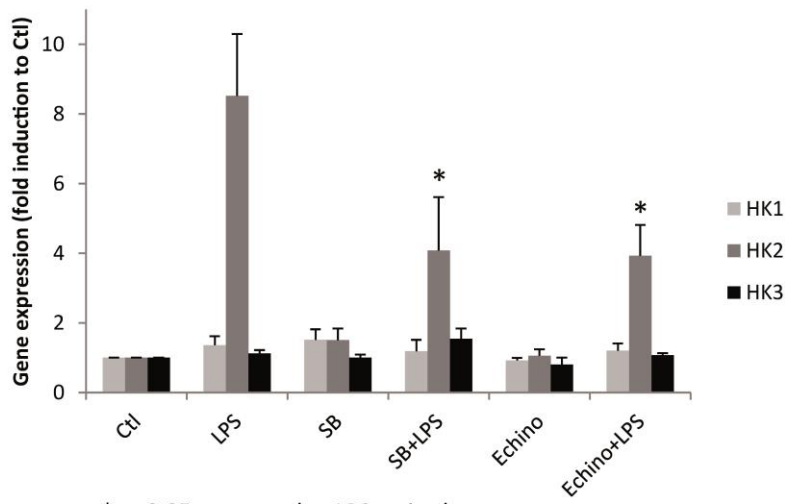


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Figure 4

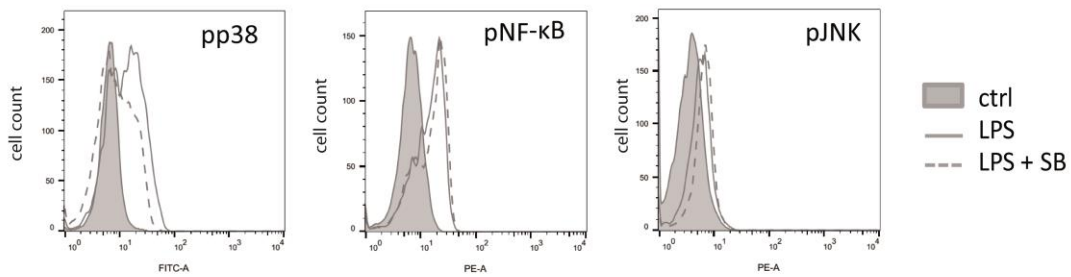
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A

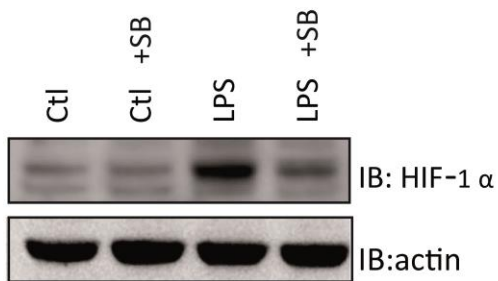


\* p<0,05 compared to LPS, paired t-test

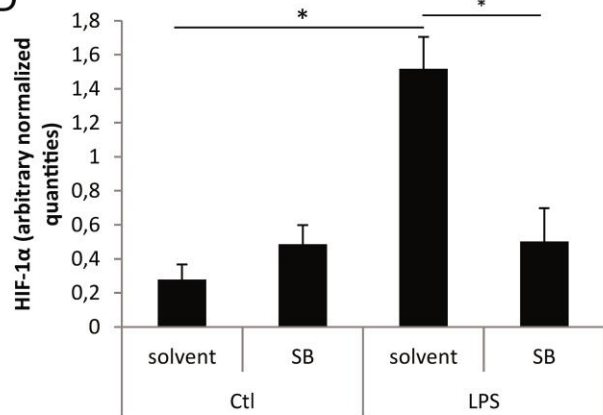
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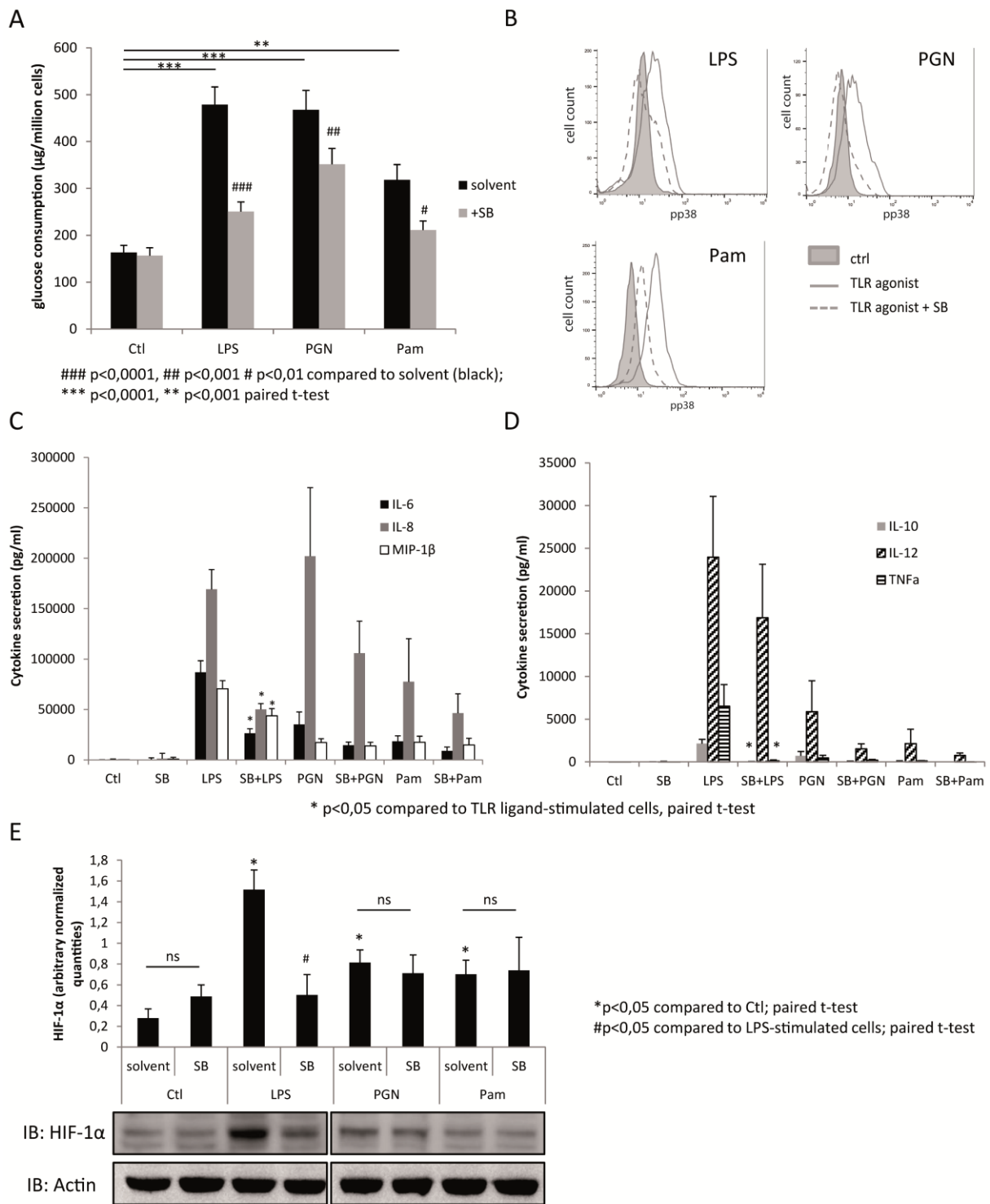
C



D



\* p<0,05 paired t-test





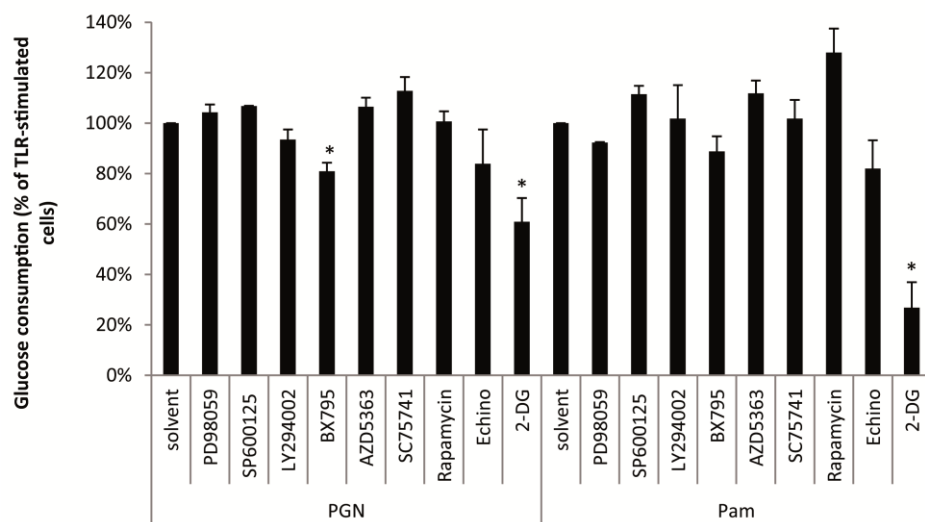
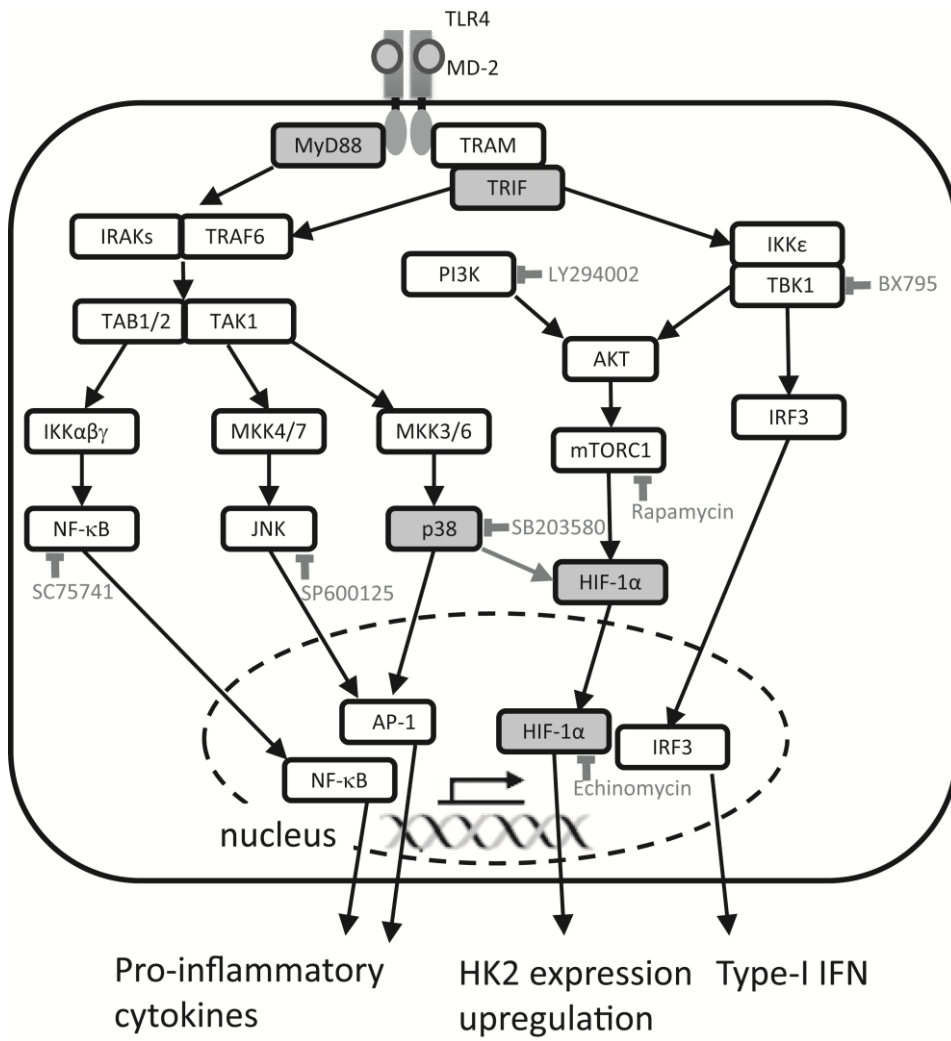


Figure 7

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2

1



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Figure 8