ΠΑΝΕΠΙΣΤΗΜΙΟ ΚΡΗΤΗΣ
UNIVERSITY OF CRETE

Ευρωπαϊκή Ένωση
European Social Fund

Operational Programme
Human Resources Development,
Education and Lifelong Learning
Co-financed by Greece and the European Union

ΕΣΠΑ 2014-2020
ανάπτυξη - εργασία - αλληλεγγύη

ΙΔΡΥΜΑ ΚΡΑΤΙΚΩΝ ΥΠΟΤΡΟΦΙΩΝ
ΙΚΥ

Delineation of the mechanisms that promote the tolerogenic properties of Dendritic cells in autoimmune diseases

Panagiota Goutakoli

Ph.D. candidate

Laboratory of Rheumatology, Autoimmunity and Inflammation,
University of Crete Medical School, Greece

Heraklion, Greece

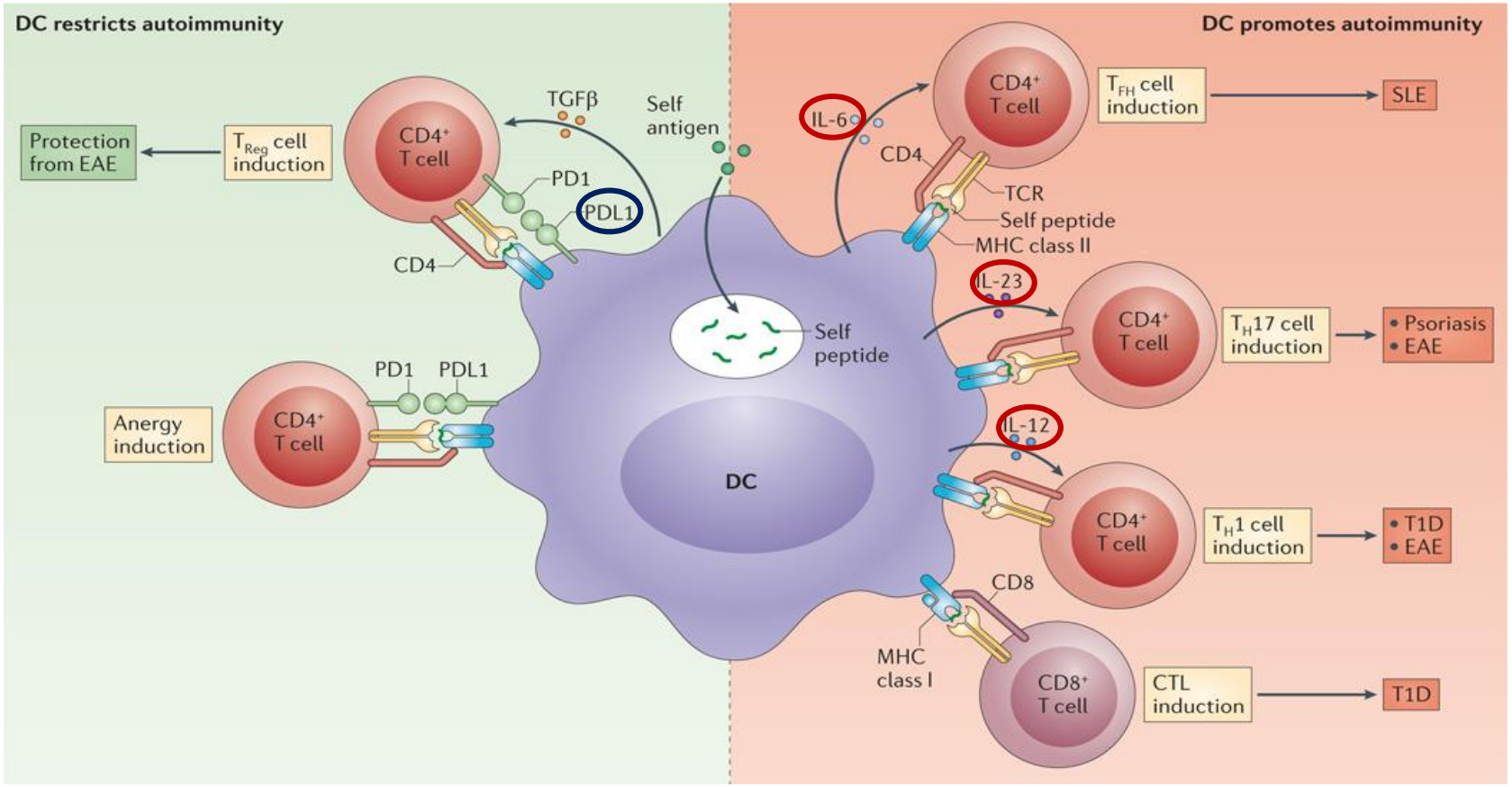
«This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research" (MIS-5000432), implemented by the State Scholarships Foundation (IKY)»

Dendritic cells (DCs) have central role in the development of autoimmune diseases

Break of self-tolerance

Anti-inflammatory signals **Pro-inflammatory signals**

Tregs
Anergy



Effector T cells

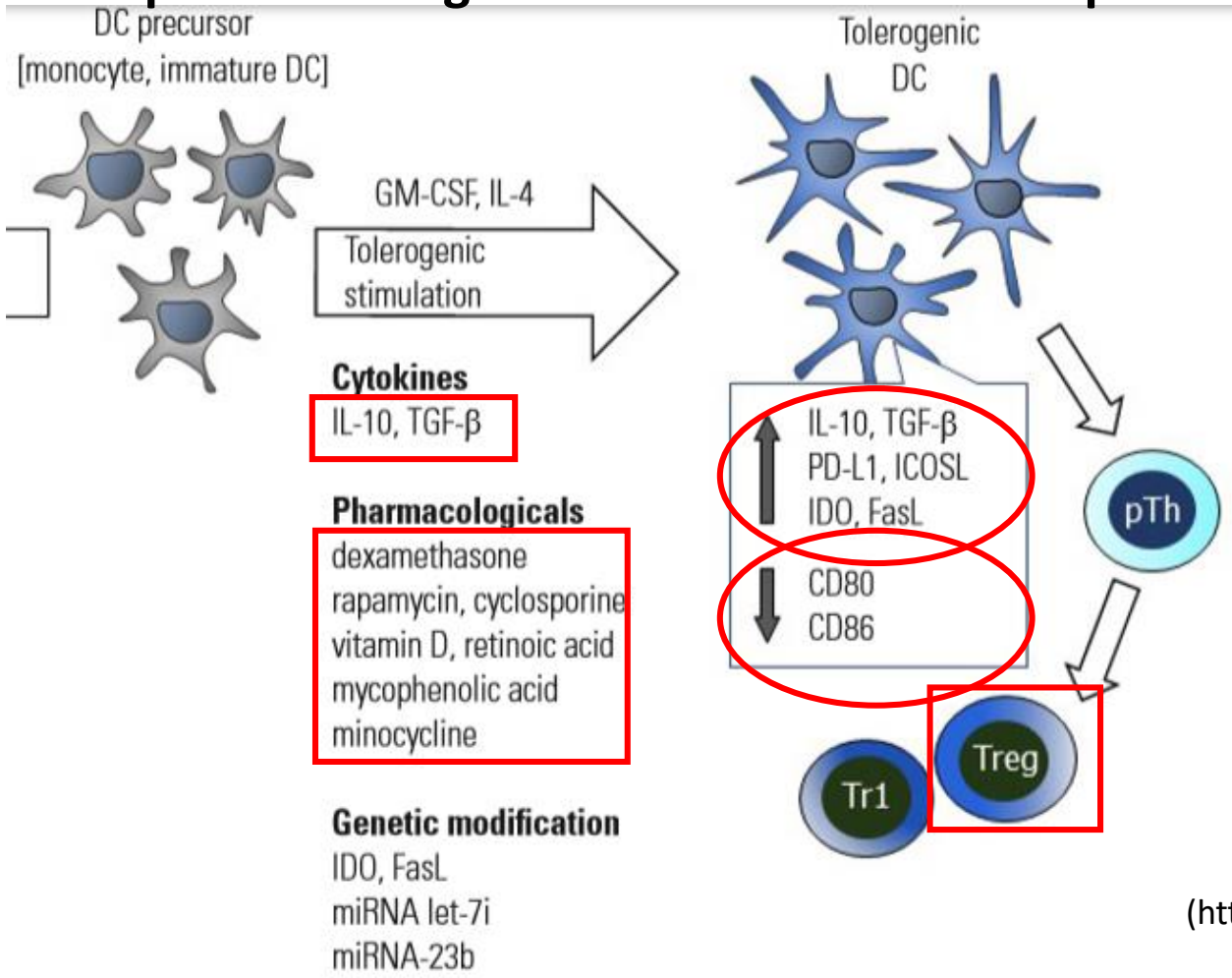
❖Autoimmune diseases develop upon aberrant activation of lymphocytes mainly due to failure to self-tolerance mechanisms.

❖DCs might promote the T and B cell-mediated autoreactive responses, because of altered cytokine secretion associated by breakdown of negative regulation against autoantigens.

❖DCs are an interesting target of study for the immunotherapy of autoimmune diseases.

(Ganguly et al. Nature Reviews Immunology. 2013)

Therapeutic strategies based on *ex-vivo* manipulated DCs



(https://doi.org/10.3349/ymj.2018.59.7.807)

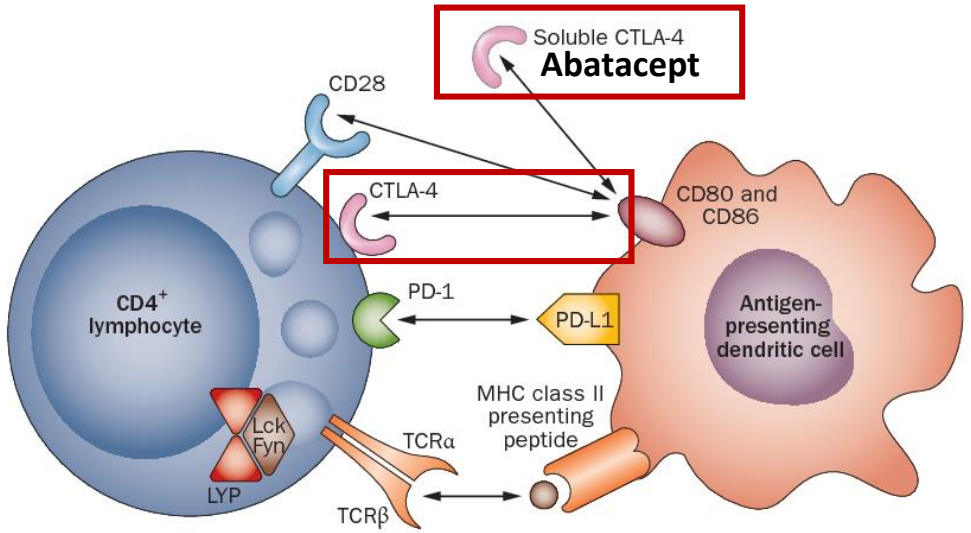
Clinical trials *ex-vivo* manipulated DCs in Rheumatoid arthritis

- **AuToDeCRA** Autologous Tolerogenic Dendritic Cells for Rheumatoid and Inflammatory Arthritis (dexamethasone and vitamin D3; synovial fluid)
- **Rheumavax** tolDCs → NFkB inhibitor; citrullinated peptide mix

(Benham H, et al. Sci Trans Med 2015;7:304;), (Bell GM, et al. Ann Rheum Dis 2017;76:227-34)

CTLA4 regulates the immune responses

- Cytotoxic T lymphocyte antigen 4 (**CTLA4**) is expressed in Tregs or induced following T cell activation via CD28 and TCR signaling.
- CTLA4 function as a negative co-stimulatory molecule and regulates the immune responses of T cells by inhibiting their activation.



➢ The significant **immunoregulatory** role of **CTLA4** molecule in the context of autoimmune diseases is confirmed through the clinical benefit upon CTLA4-Ig treatment (**abatacept**) in patients with RA

CTLA4 delivers reverse signals through CD80 and CD86 on DCs and inhibits their immunogenic potential

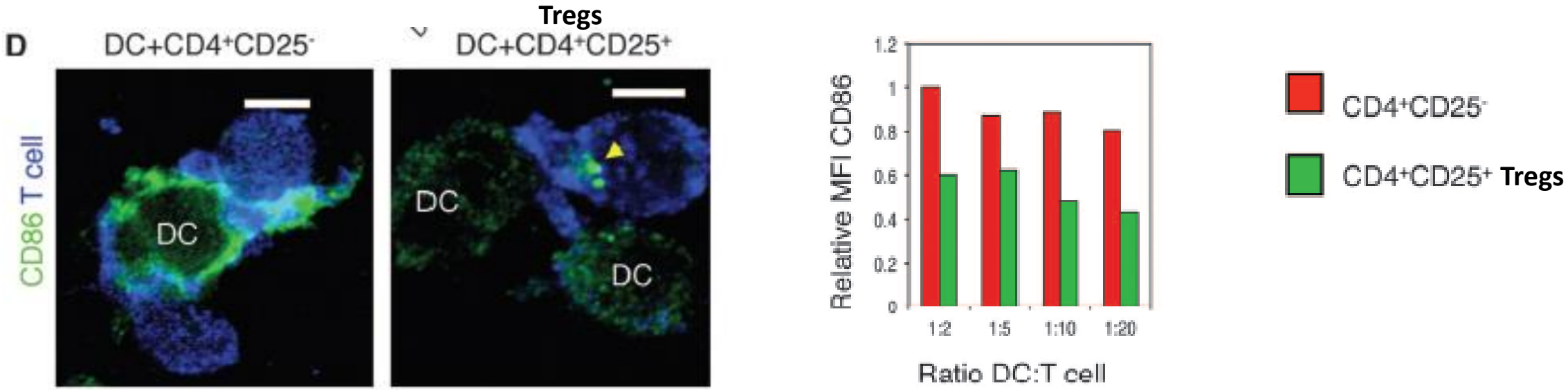
Induction of IDO through CTLA4 ligands

- Grohmann U, et al. **CTLA-4-Ig regulates tryptophan catabolism in vivo**. **Nat Immunol. 2002**.

  Engagement of CD80/CD86 by CTLA-4 induces IFN-γ production and activates STAT1- dependent transcriptional expression of the gene encoding IDO

CTLA4 captures ligands from DCs

- Qureshi OS, et al**. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4**. **Science. 2011.**

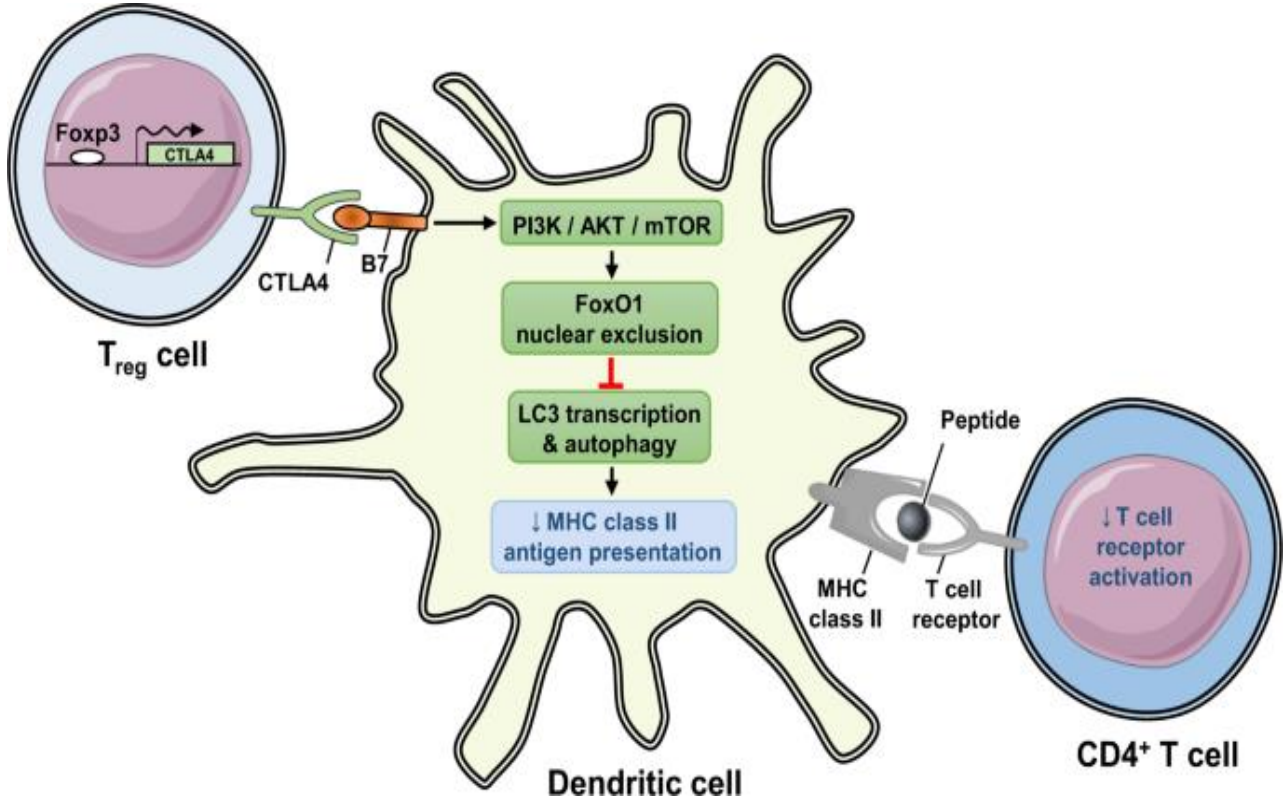


CTLA-4 acts as an effector molecule to inhibit CD28 costimulation by the cell-extrinsic depletion of ligands

CTLA4 delivers reverse signals through CD80 and CD86 on DCs and inhibits their immunogenic potential

CTLA4 impairs autophagic machinery on DCs and ameliorates immune responses

- Alissafi T, et al. **Tregs restrain dendritic cell autophagy to ameliorate autoimmunity**. **J Clin Invest. 2017**.



(Alissafi et al. JCI.2017)

Aim of the study:

Investigation of the molecular mechanisms that are involved in the induction of the tolerogenic phenotype of CTLA4-mediated effects in DCs

Objective I: Delineation of the molecular mechanisms of CTLA4-mediated tolerogenic effect on DCs

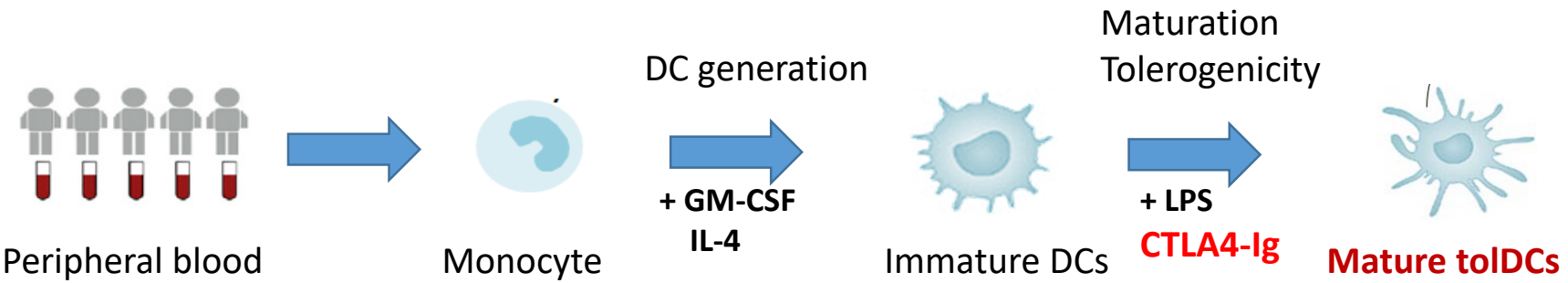
Objective II: Investigation of the role of specific molecular pathways in the antiflammatory function of DCs

- *In vitro* functional studies of targeted genes (human)
- Targeted gene modification in CIA mouse

Objective III: Characterization of mechanisms of resistance to abatacept treatment in patients with RA

Research Methodology

***In vitro* generation of tolerogenic DCs**

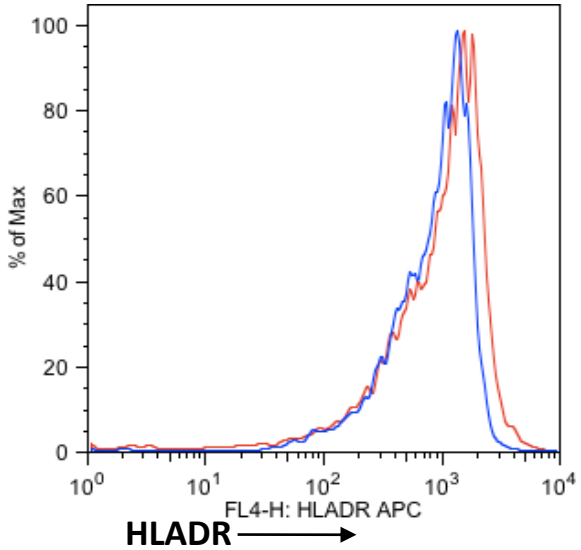


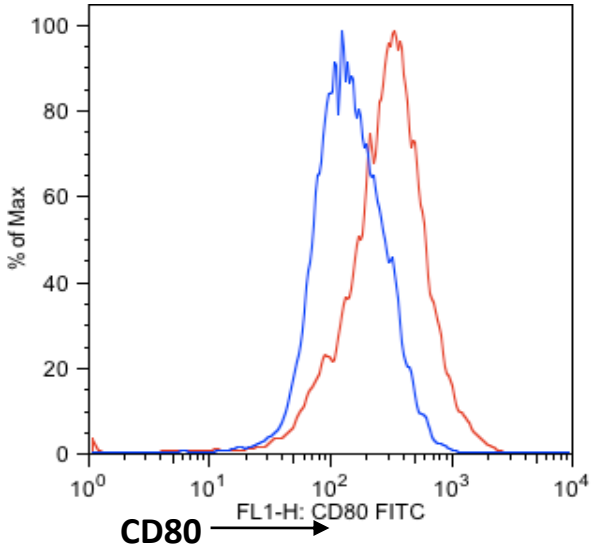
Objective I: ***Characterization of the molecular mechanisms of CTLA4-mediated tolerogenic effect on DCs***

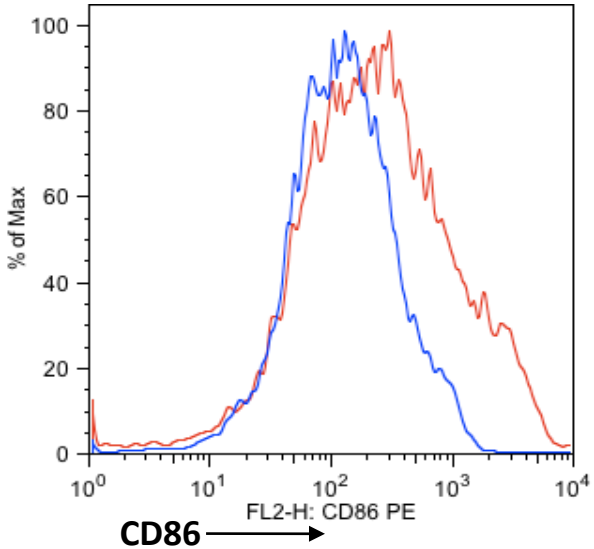
- Confirm the tolerogenic effect of CTLA4-Ig on DCs
- Characterize the metabolic profile of DCs-treated with CTLA4-Ig
- Investigate the role of AKT/mTOR pathway on the function of CTLA4-treated DCs

Objective I: ***Characterization of the molecular mechanisms of CTLA4-mediated tolerogenic effect on DCs***

Investigation of the effect of CTLA4-Ig on major features of antigen presenting DCs

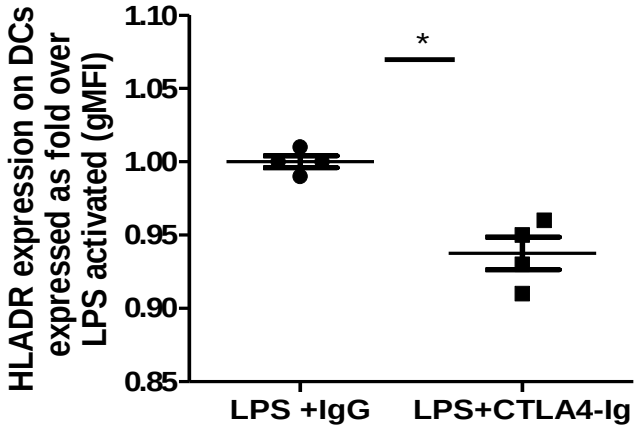


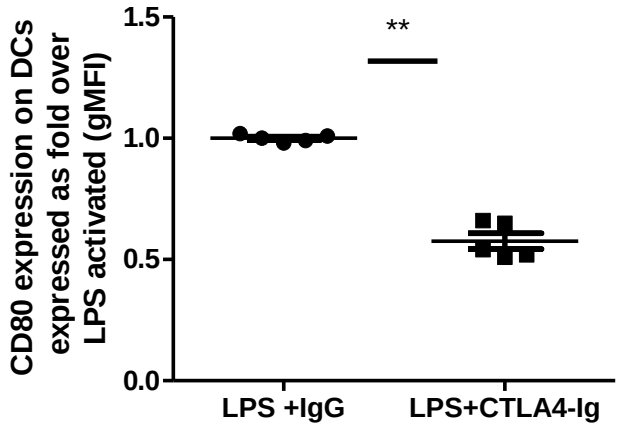


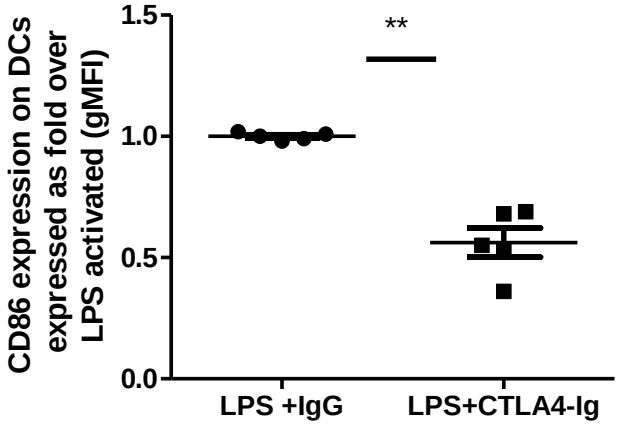


DCs LPS+IgG

DCs LPS+CTLA4-Ig







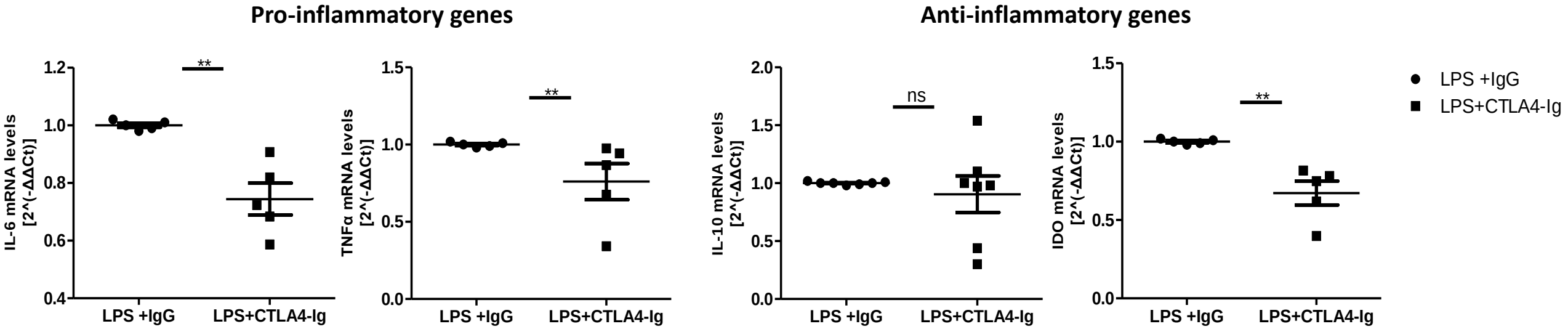
LPS +IgG

LPS+CTLA4-Ig

❖ **Regulatory DCs downregulated the expression of antigen presenting and co-stimulatory molecules**

Objective I: ***Characterization of the molecular mechanisms of CTLA4-mediated tolerogenic effect on DCs***

Investigation of the effect of CTLA4-Ig on the expression of pro- and anti-inflammatory genes



❖**CTLA4-Ig reduced the mRNA levels of pro-inflammatory genes *IL6* and *TNFα*, whereas the expression of anti-inflammatory genes varied upon CTLA4 treatment on DCs.**

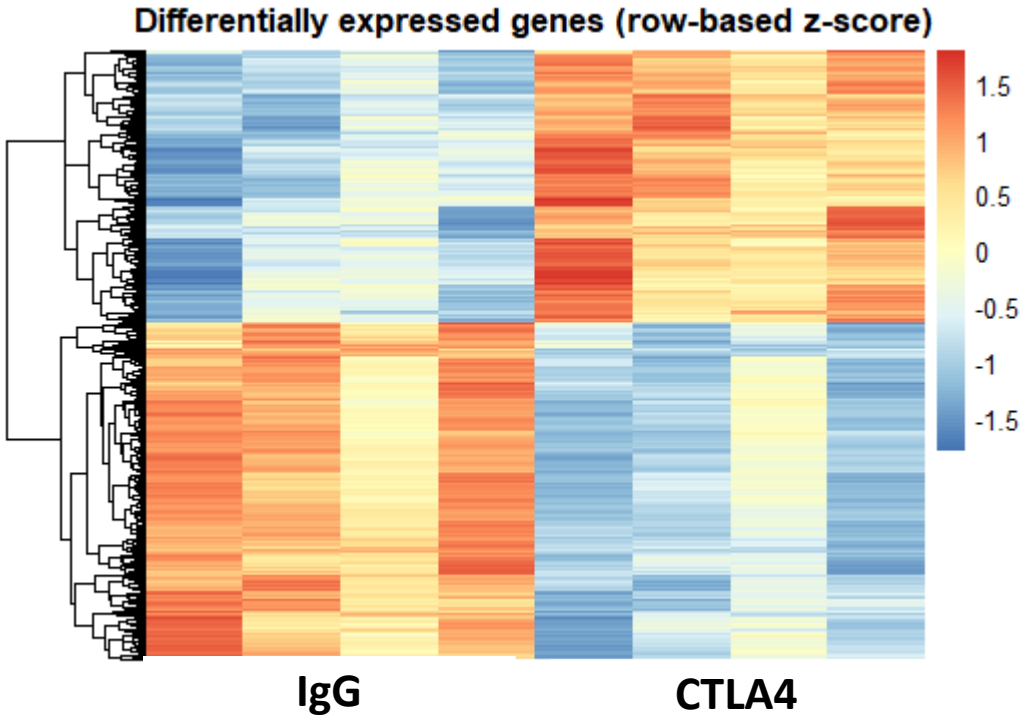
Objective I: ***Characterization of the molecular mechanisms of CTLA4-mediated tolerogenic effect on DCs***

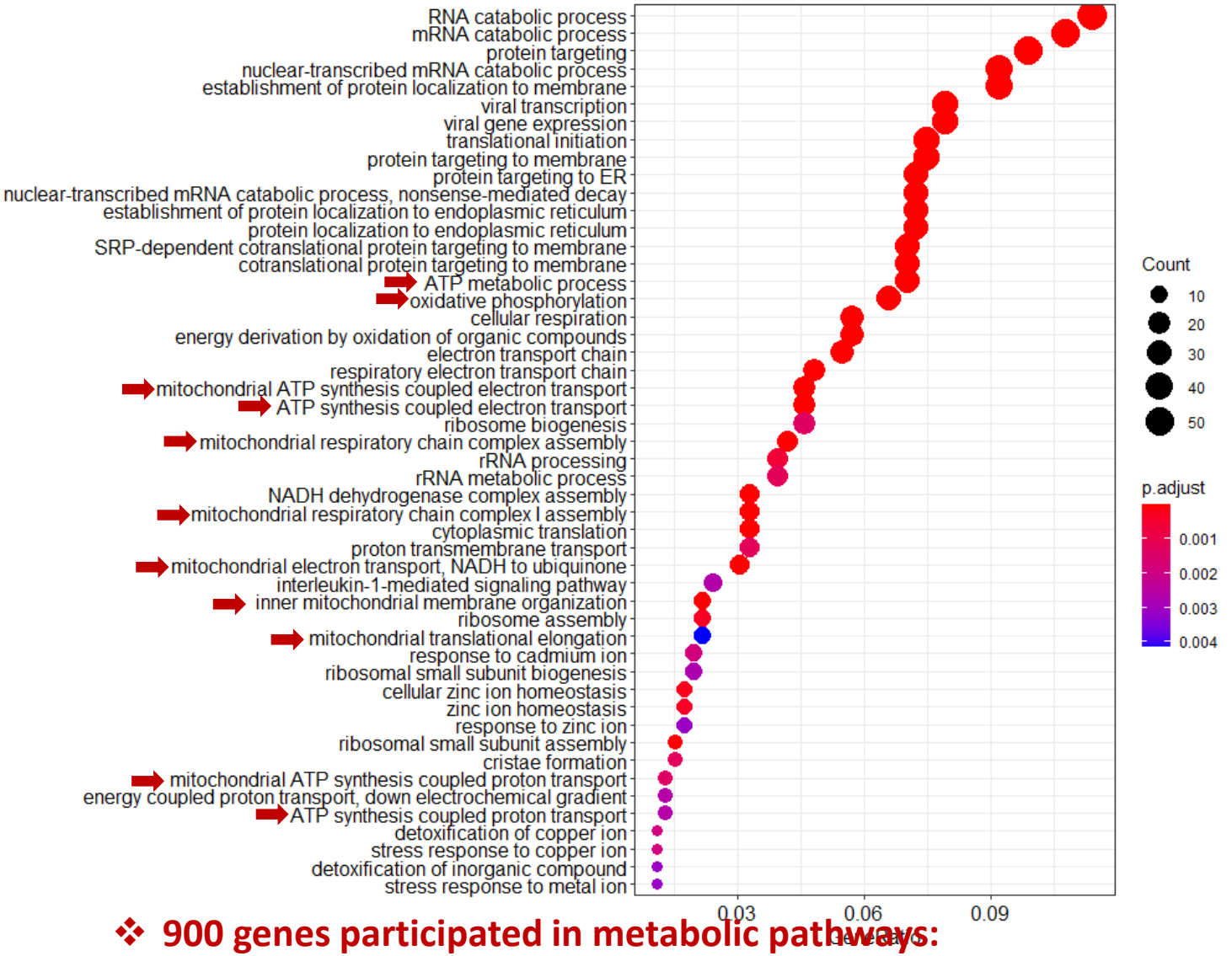
RNAseq

1270 genes

Biological-process enrichment among deregulated genes p-value <0.05 counts>10

900 genes participate in metabolic pathways



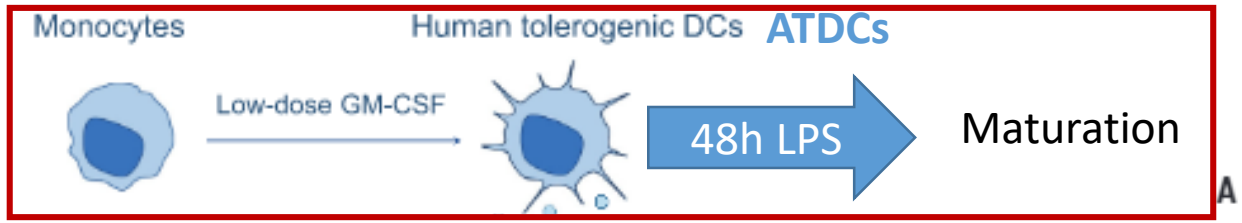


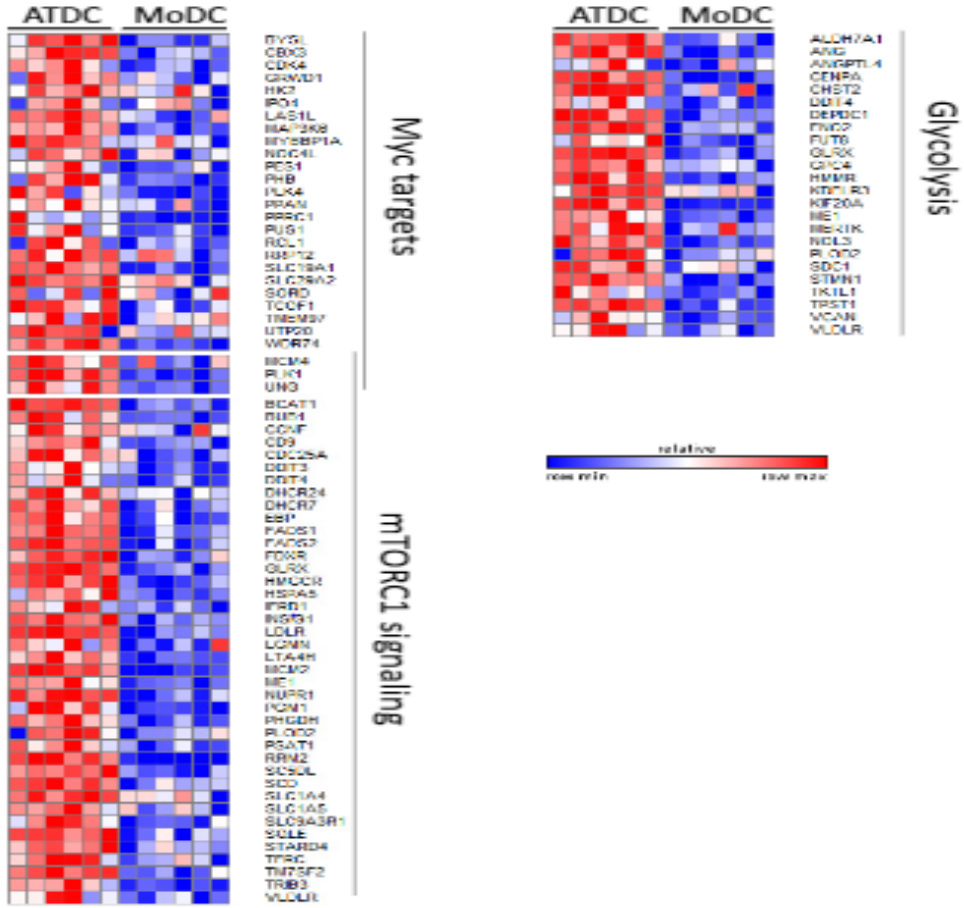
❖ **The anti-inflammatory function of DCs controlled statistically significant the expression of 1270 genes**

❖ **900 genes participated in metabolic pathways: OxPHOS, mitochondrial function**

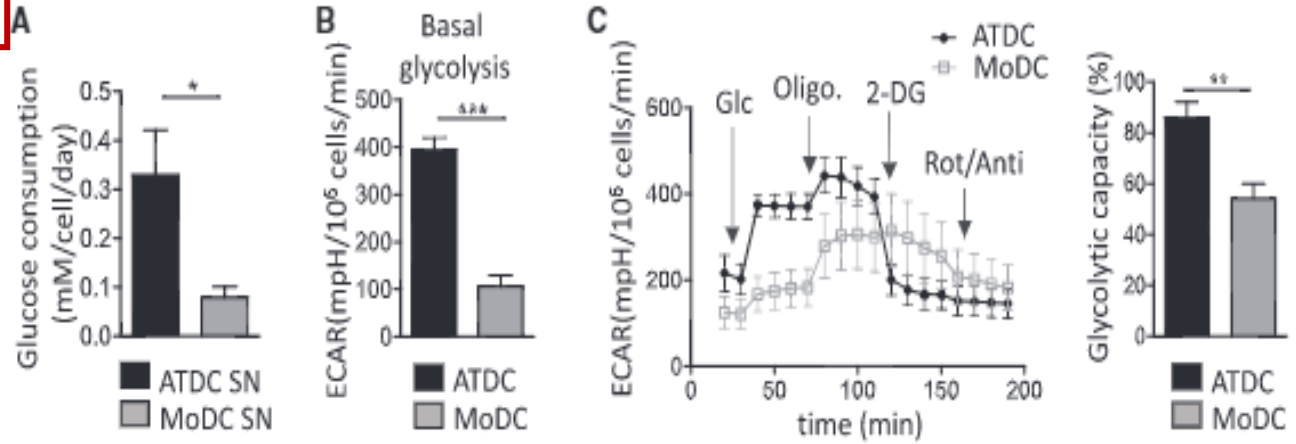
Cell Metabolism

Human Tolerogenic Dendritic Cells Regulate Immune Responses through Lactate Synthesis

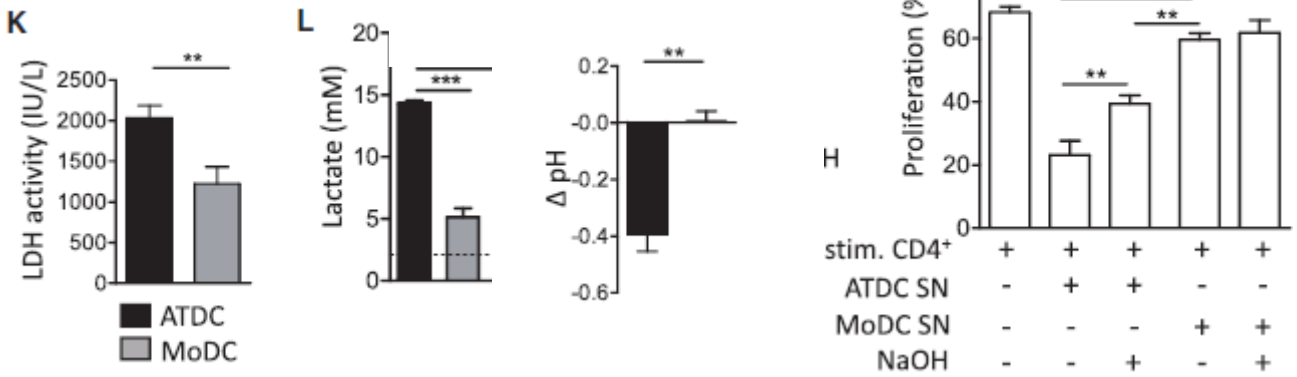




Human tolerogenic DCs (ATDCs) are highly glycolytic cells



Lactate secreted by ATDCs is involved in their suppressive activity



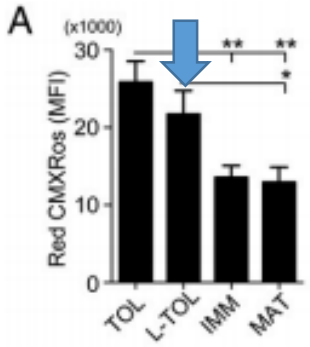
The Journal of Immunology

High Mitochondrial Respiration and Glycolytic Capacity Represent a Metabolic Phenotype of Human Tolerogenic Dendritic Cells

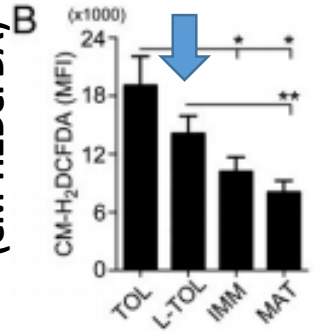
CD14+ cells → (5 days; GM-CSF, IL4) → imDCs → (24h; Dex, Vit D3) → Tol DCs → (24h; LPS) → L-tol DCs

tol moDCs have increased mitochondrial content and OXPHOS activity

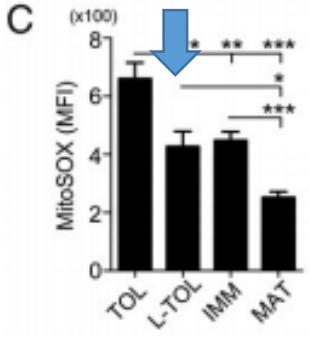
Mitotracker

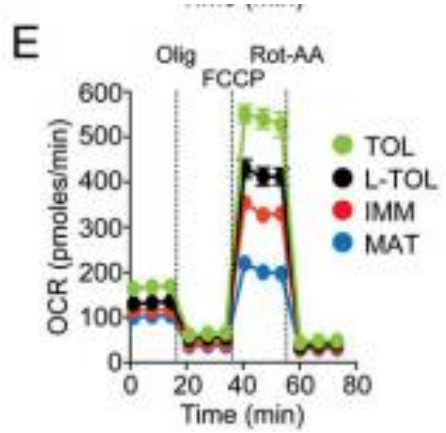


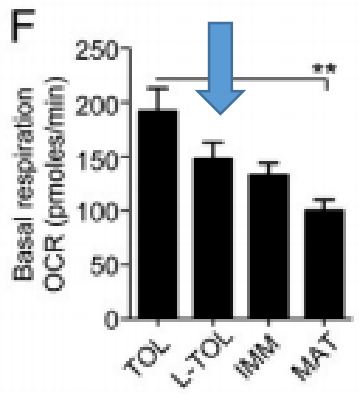
ROS (CM-H2DCFDA)

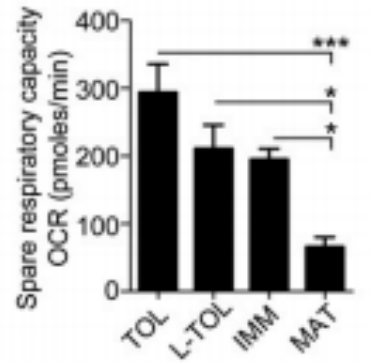


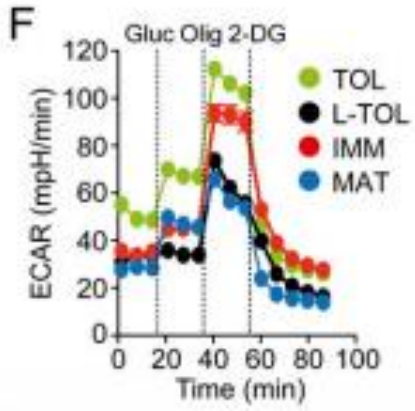
MitoSOX

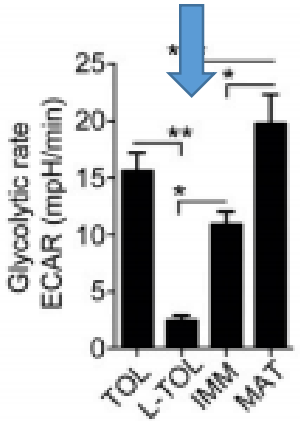


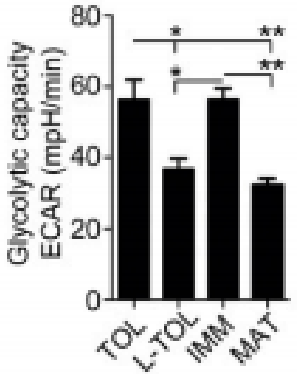


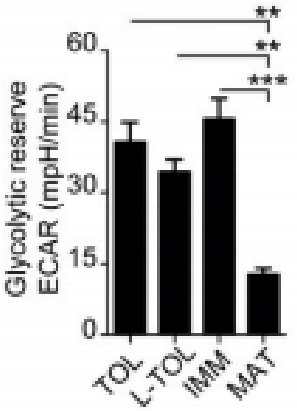






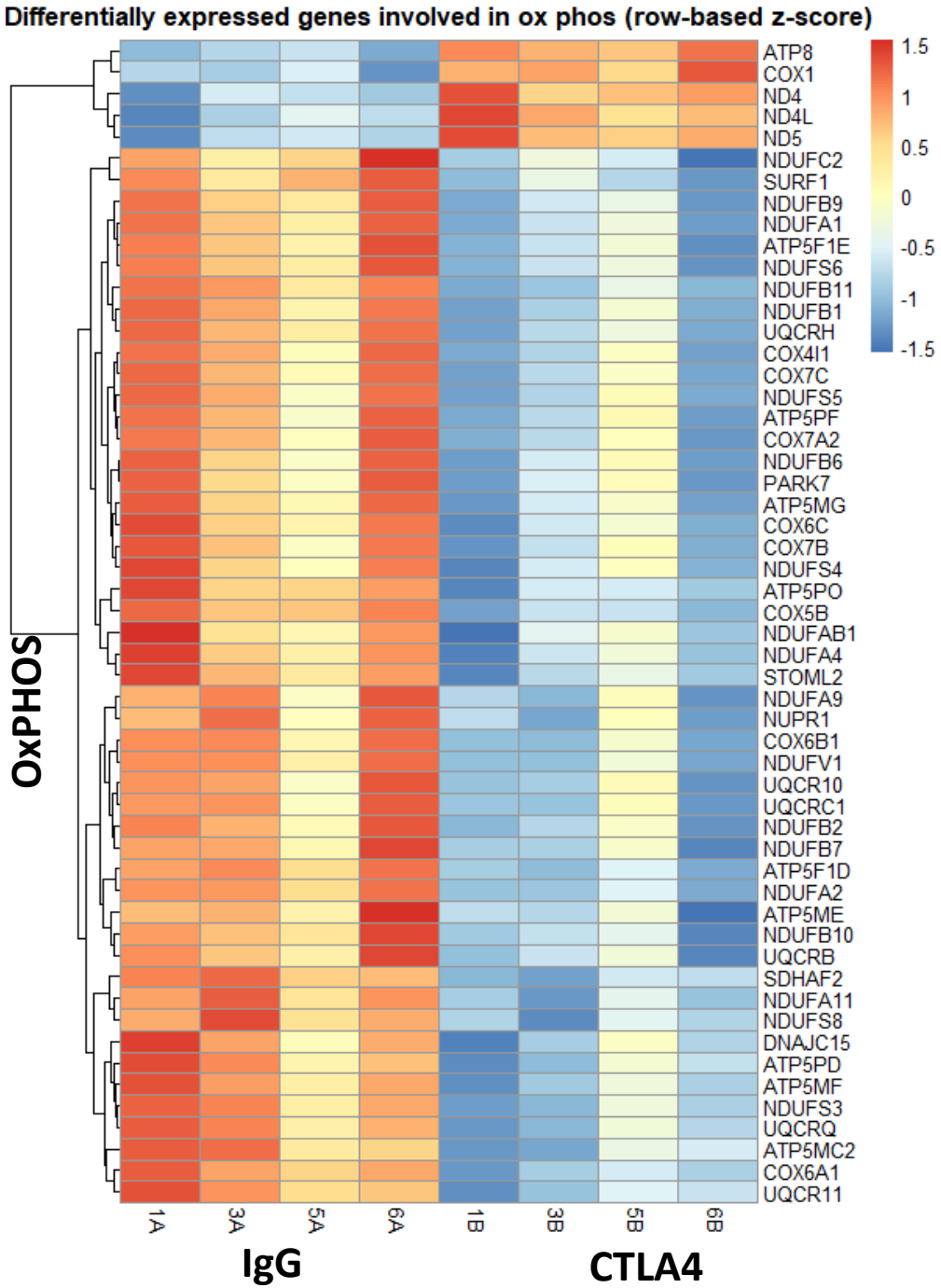


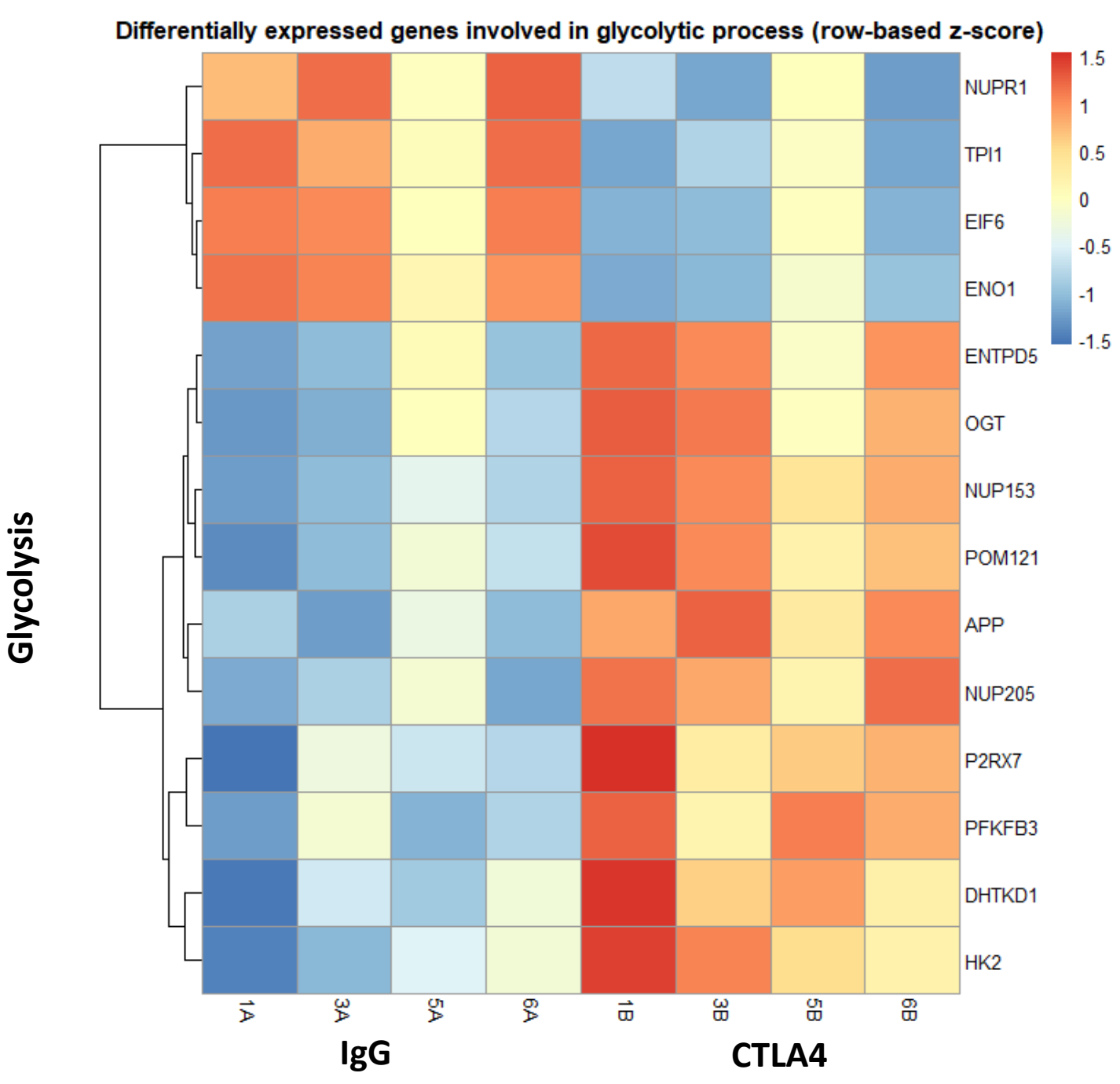




tol- moDCs have higher mitochondrial activity, produce higher levels of ROS and MtROS

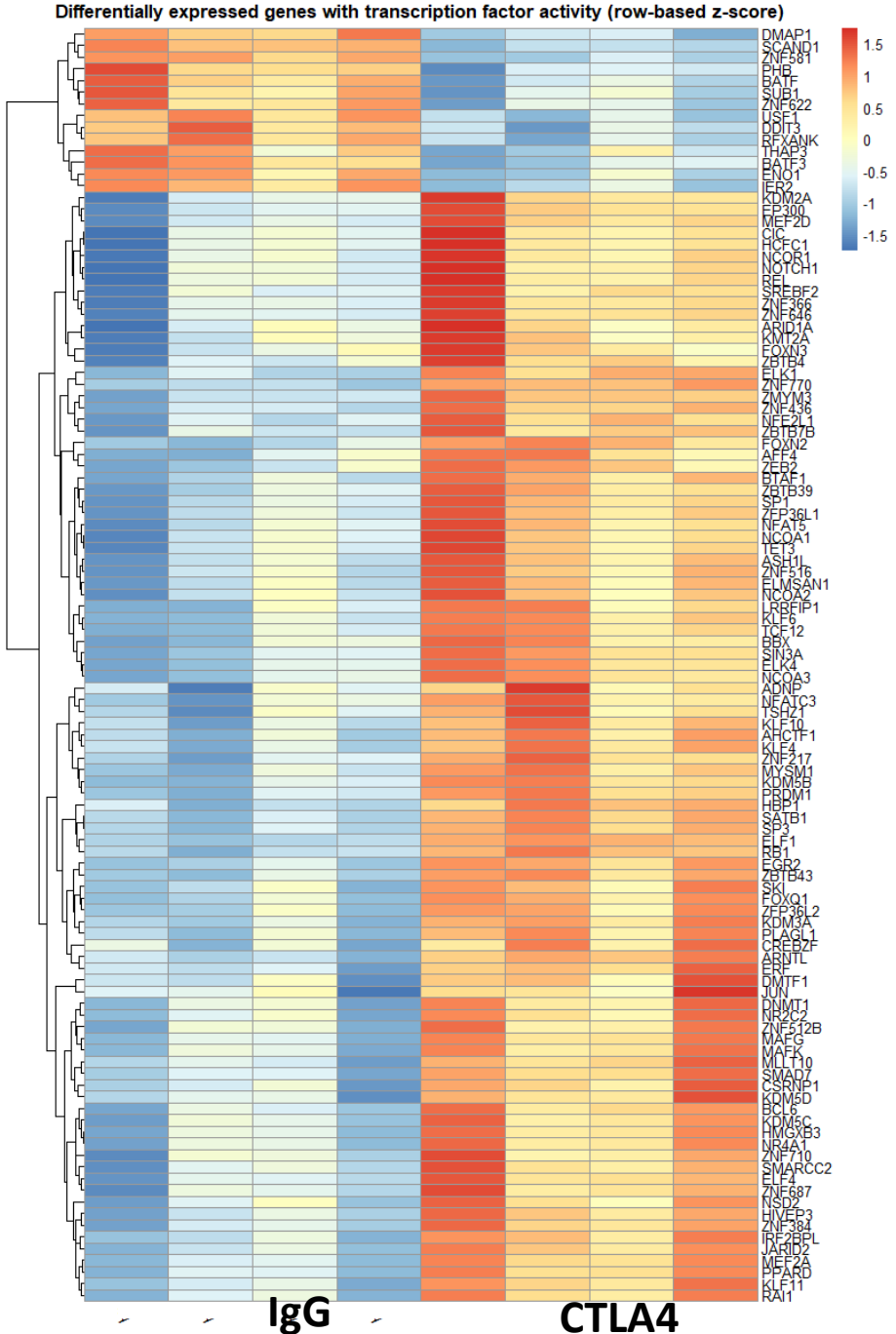
Objective I: ***Characterization of the molecular mechanisms of CTLA4-mediated tolerogenic effect on DCs***





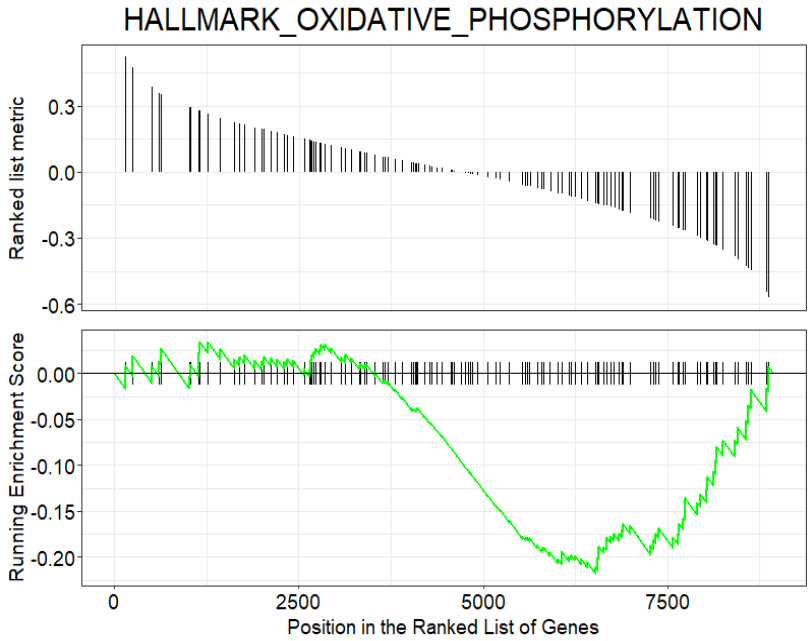
Objective I: *Characterization of the molecular mechanisms of CTLA4-mediated tolerogenic effect on DCs*

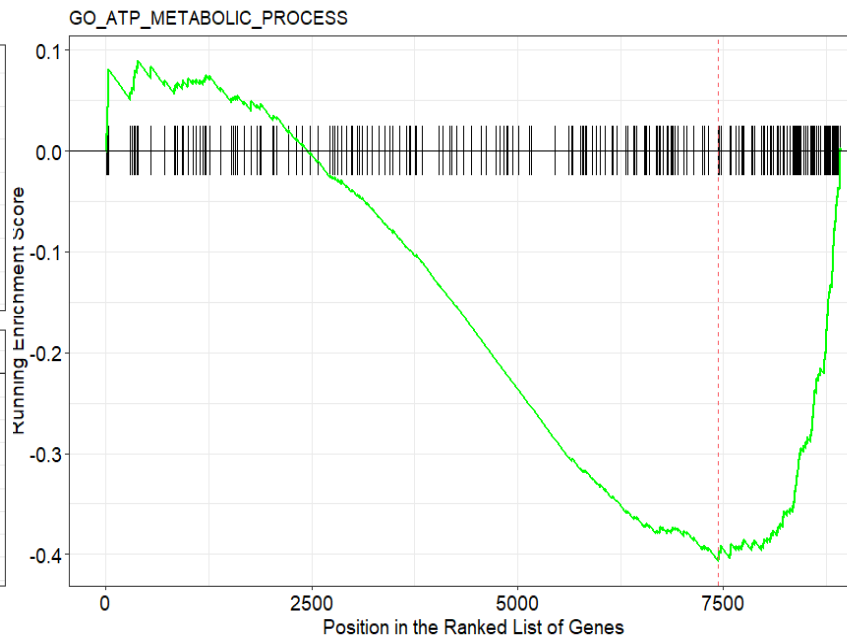
Transcription factors

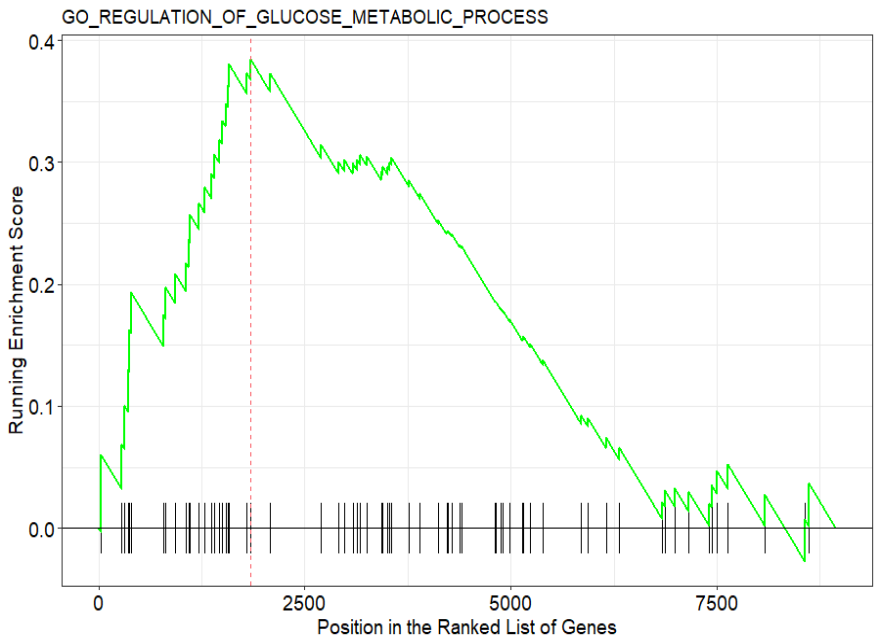


Objective I: *Characterization of the molecular mechanisms of CTLA4-mediated tolerogenic effect on DCs*

Gene Set Enrichment Analysis







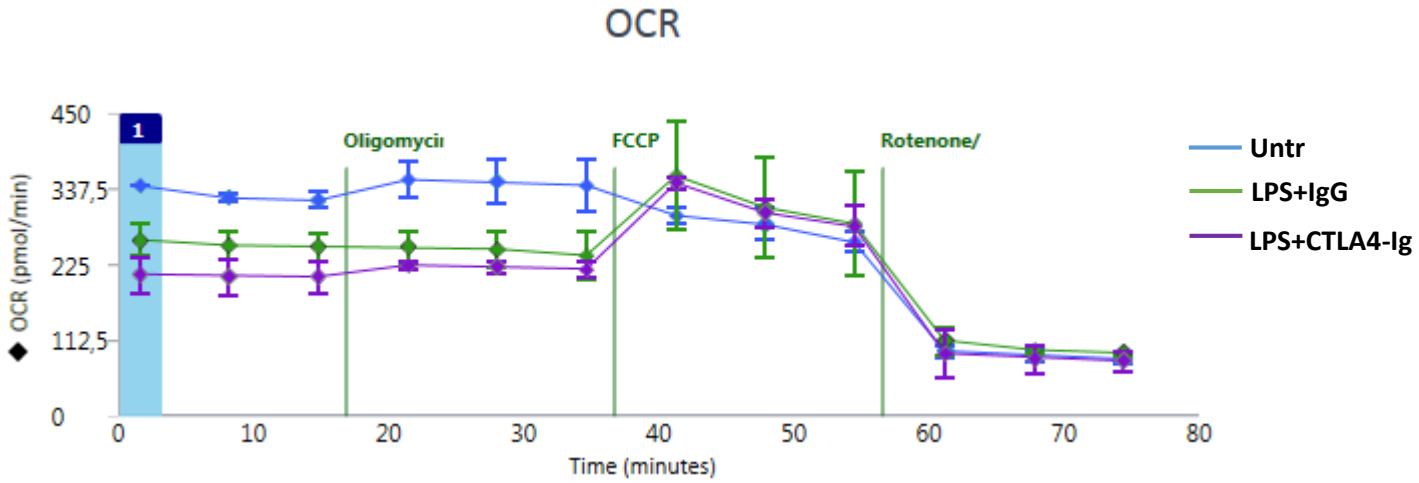
Tolerogenic DCs characterized by:

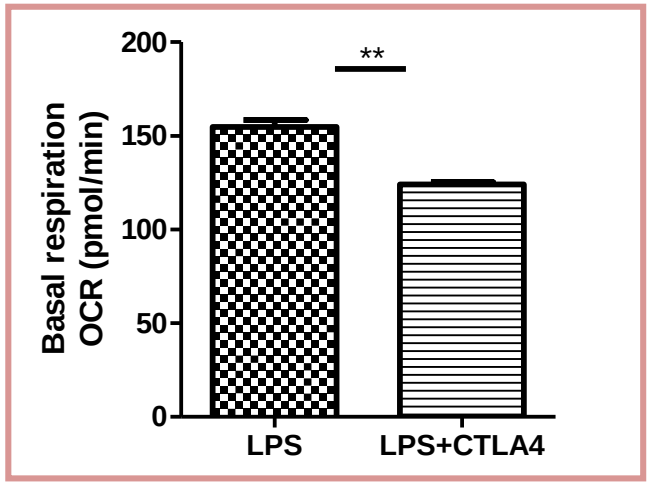
- **Enrichment in downregulated genes in OXPHOS**
- **Enrichment in downregulated genes in ATP metabolic process**
- **Enrichment in glucose metabolic process**

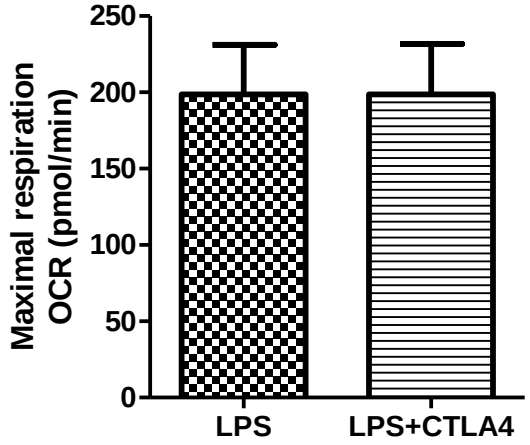
Objective I: ***Characterization of the molecular mechanisms of CTLA4-mediated tolerogenic effect on DCs***

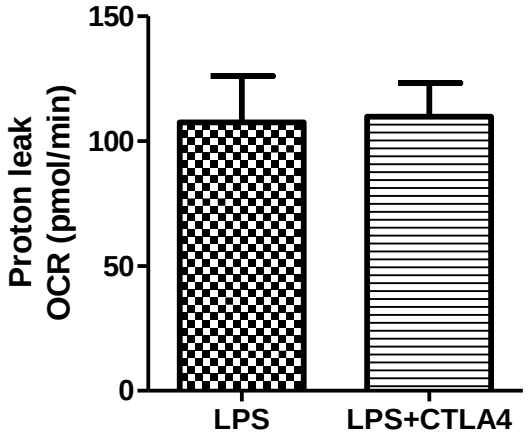
Investigation of the metabolic profile of DCs-treated with CTLA4-Ig

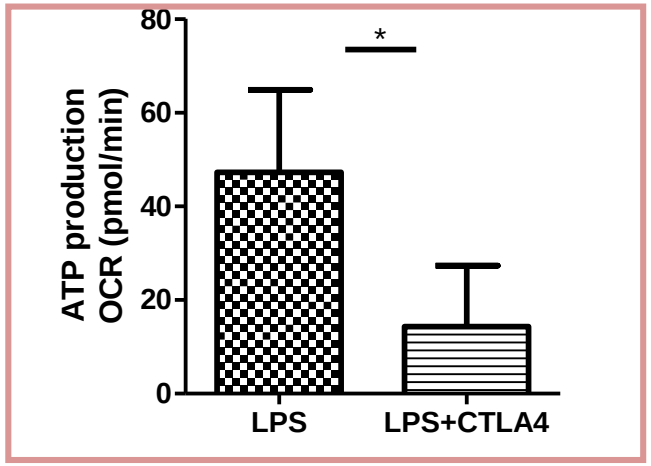
Seahorse XF Cell Mito Stress Test









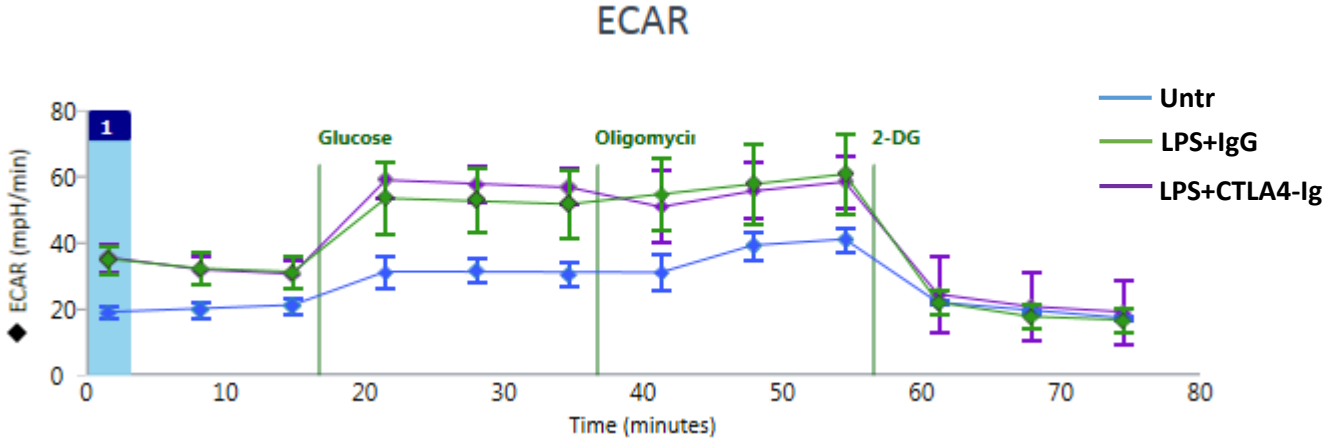


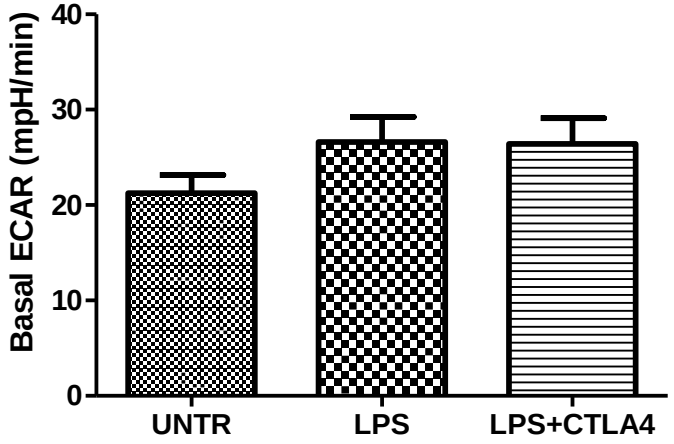
❖ **CTLA4-treated DCs showed metabolic signatures of decreased oxidative phosphorylation programing and lower ATP production.**

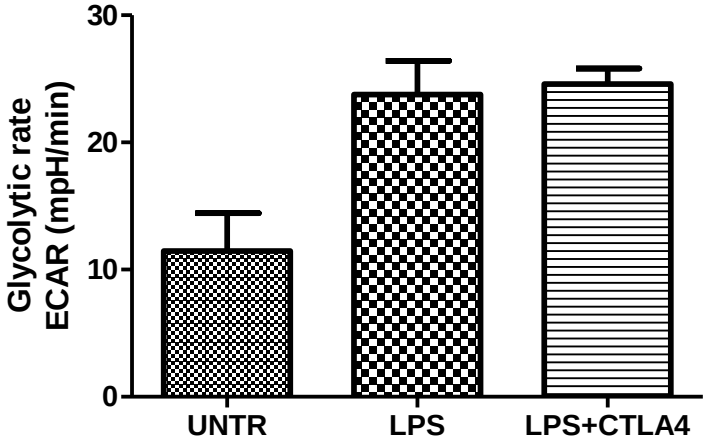
Objective I: ***Characterization of the molecular mechanisms of CTLA4-mediated tolerogenic effect on DCs***

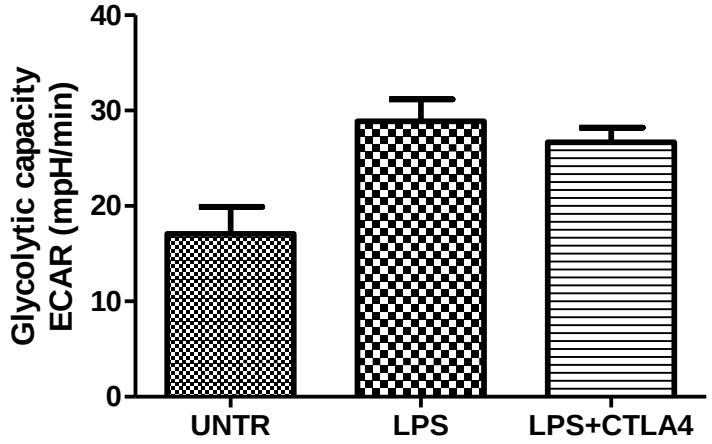
Investigation of the metabolic profile of DCs-treated with CTLA4-Ig

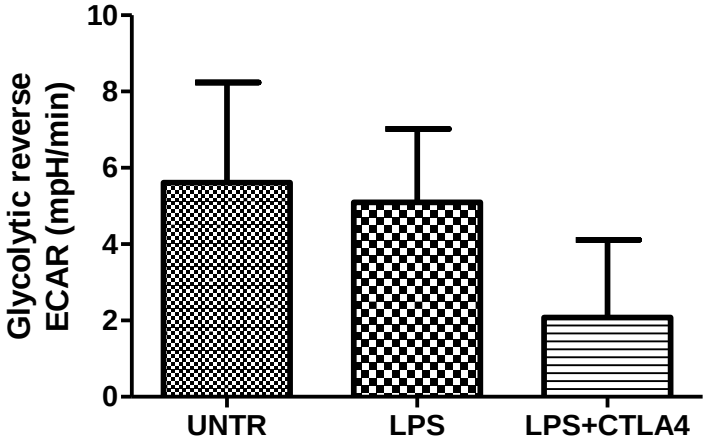
Seahorse XF Glycolysis Stress Test











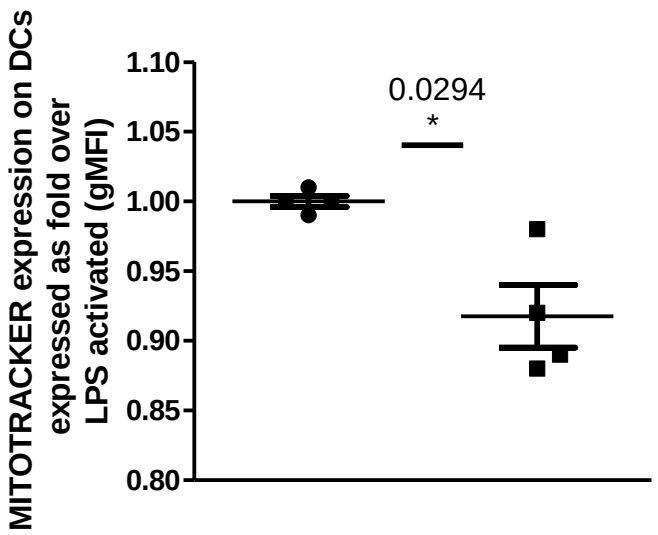
❖ **Glucose metabolic pathway was not altered on activated regulatory DCs.**

Objective I: ***Characterization of the molecular mechanisms of CTLA4-mediated tolerogenic effect on DCs***

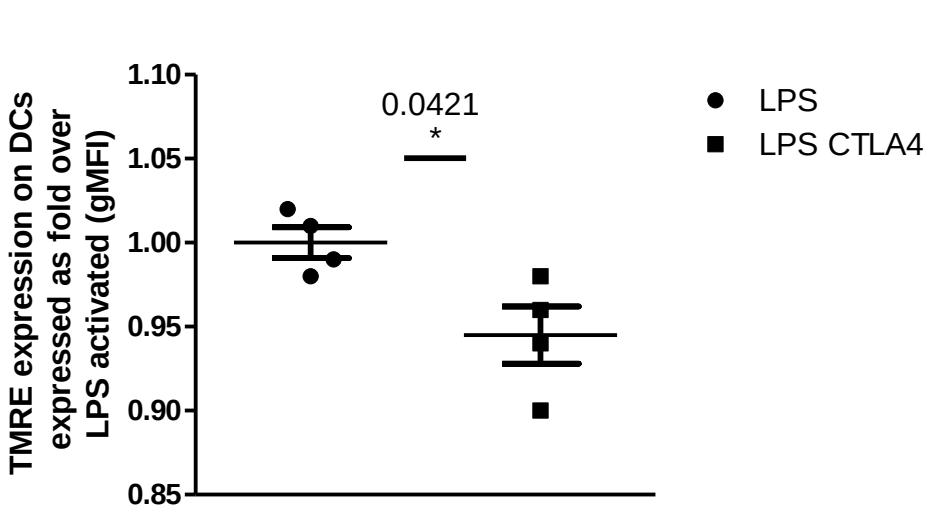
Investigation of mitochondrial changes in regulatory DCs upon CTLA4 signaling

4 h Flow Cytometry

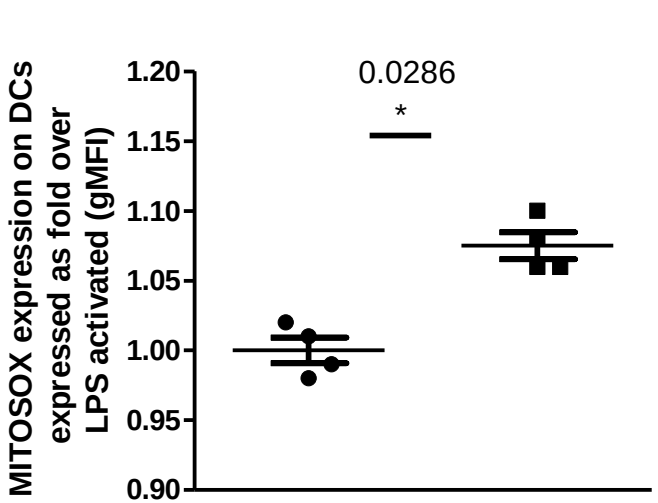
Mitochondria Mass



Mitochondrial membrane potential



Mitochondrial ROS



❖ **Regulatory DCs had reduced mitochondrial mass accompanied with low membrane potential. However regulatory DCs produced high mtROS.**

mTOR acts as a negative regulator of proinflammatory responses in DCs

- Weichhart T, et al. **The TSC-mTOR signaling pathway regulates the innate inflammatory response**. **Immunity. 2008**.

mTOR inhibition pathway rapamycin treatment, led to an **increase in IL-12p40 production and decrease in IL-10 production** in activated myeloid DCs.

- Fukao T, et al. **PI3K-mediated negative feedback regulation of IL-12 production in DCs**. **Nat Immunol. 2002**.

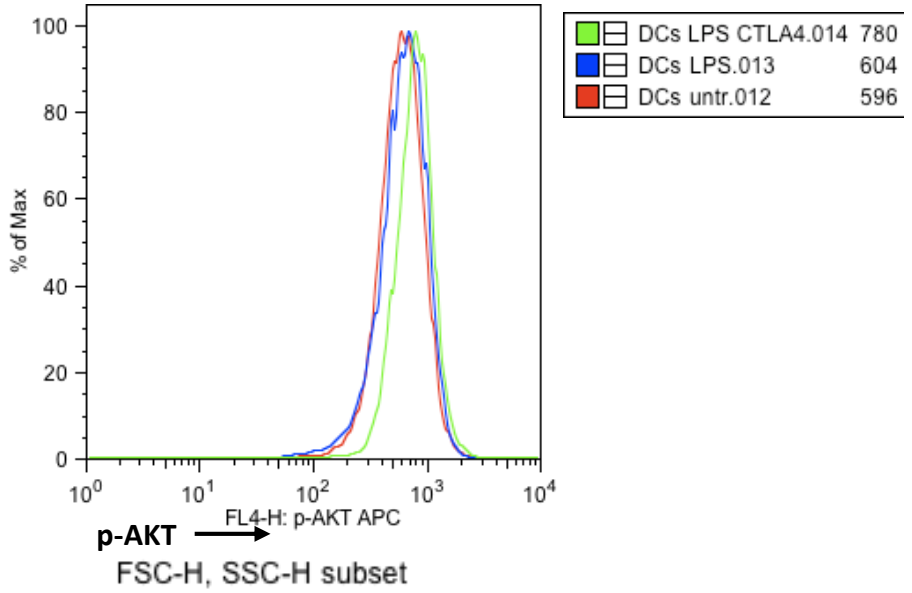
PI3K negatively regulated IL-12 synthesis by DCs and both PI3K -/- and PI3 inhibitor-treated DCs show increased IL-12 production and enhanced Th1 response

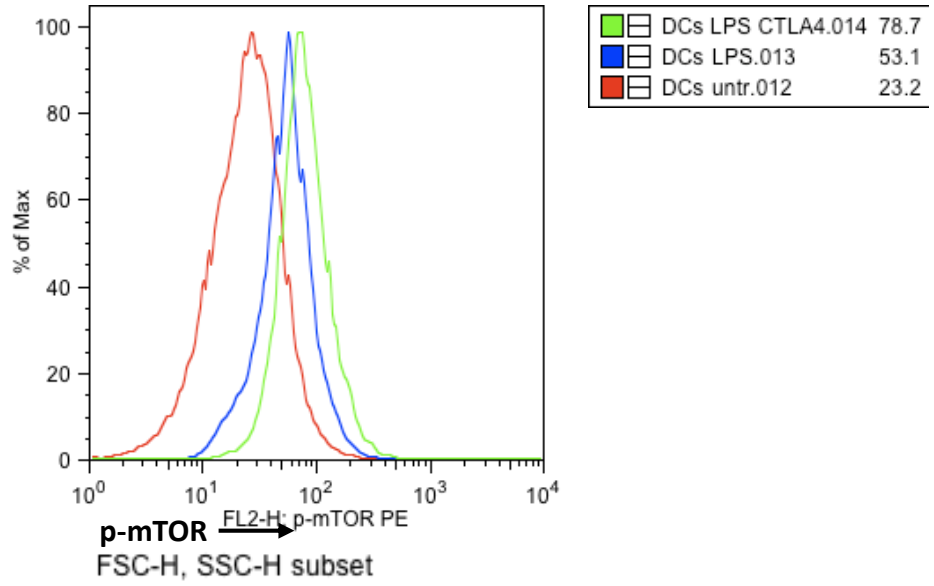
- Alissafi T, et al. **Tregs restrain dendritic cell autophagy to ameliorate autoimmunity**. **J Clin Invest. 2017**.

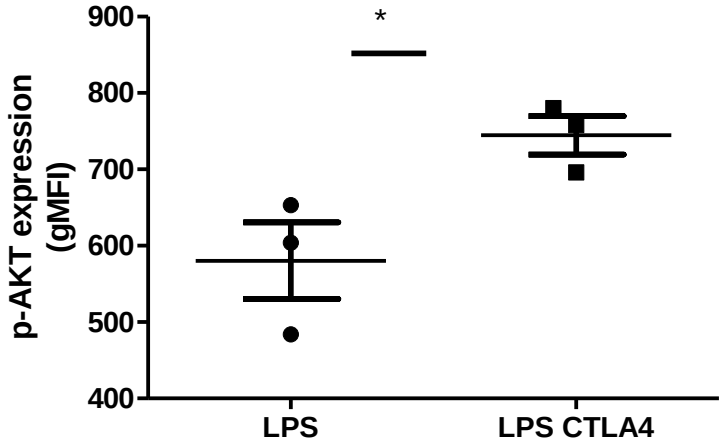
CTLA4 upregulated AKT/**mTOR signaling on DCs**, thereby impairing the autophagosome formation and **ameliorated autoimmune responses**.

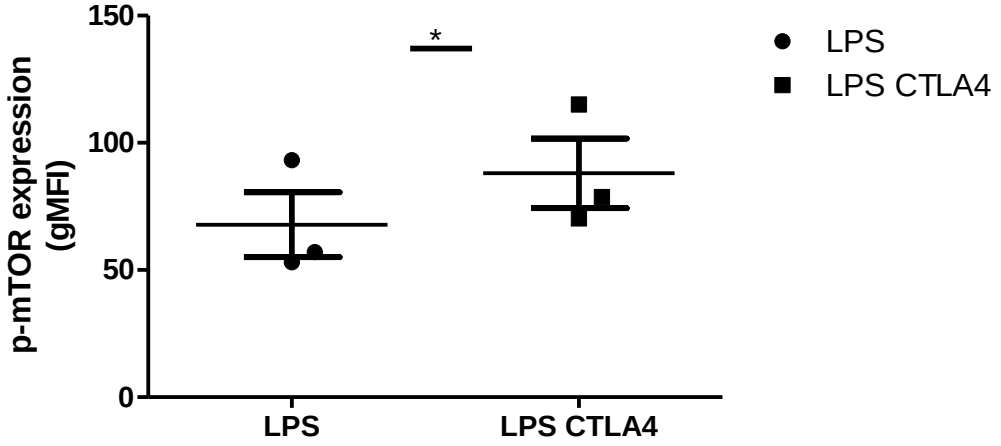
Objective I: ***Characterization of the molecular mechanisms of CTLA4-mediated tolerogenic effect on DCs***

Investigation of the metabolic profile of DCs-treated with CTLA4-Ig









❖ **CTLA4-treated DCs showed increased phospho -AKT and -mTOR expression.**

Hypothesis

The Akt/mTOR signaling pathway deteriorates the engagement in OXPHOS and controls mitochondrial fitness, contributing to the regulatory function of DCs.

Ongoing experiments

- Investigation the role of reduced OXPHOS pathway in the CTLA4-mediated regulatory features of DCs
- Investigation of the role of mTOR in the regulatory features of CTLA4-mediated DCs
  Inhibition of mTOR pathway→ proinflammatory cytokines, metabolic alterations
- Study upstream pathways that lead to increased mTOR signaling in regulatory DCs

THANK YOU