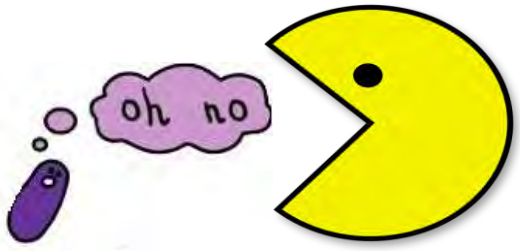
Mitochondrial dysfunction prevents repolarization of inflammatory macrophages



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Experimental Vascular Biology group

funded by the dutch heart foundation
Hartstichting
Postdoc Grant 2013T003

NWO

Universiteit van Amsterdam

amc

Macrophage functions

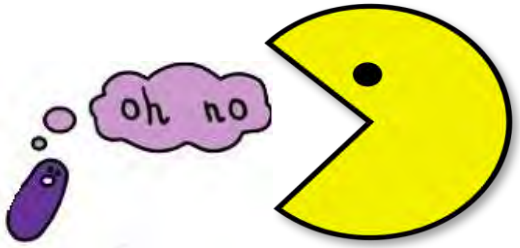
Anti-inflammatory

Promote inflammation

Mediate Type 2 inflammation in allergy

Bacterial killing

Tissue repair



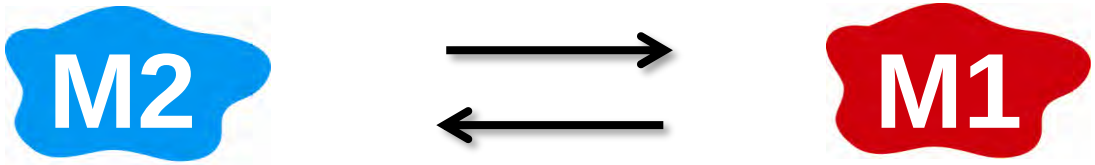
Wound healing

Kill tumor cells

Promote tumor growth

Host defense

Macrophage plasticity and repolarization are key features of macrophages



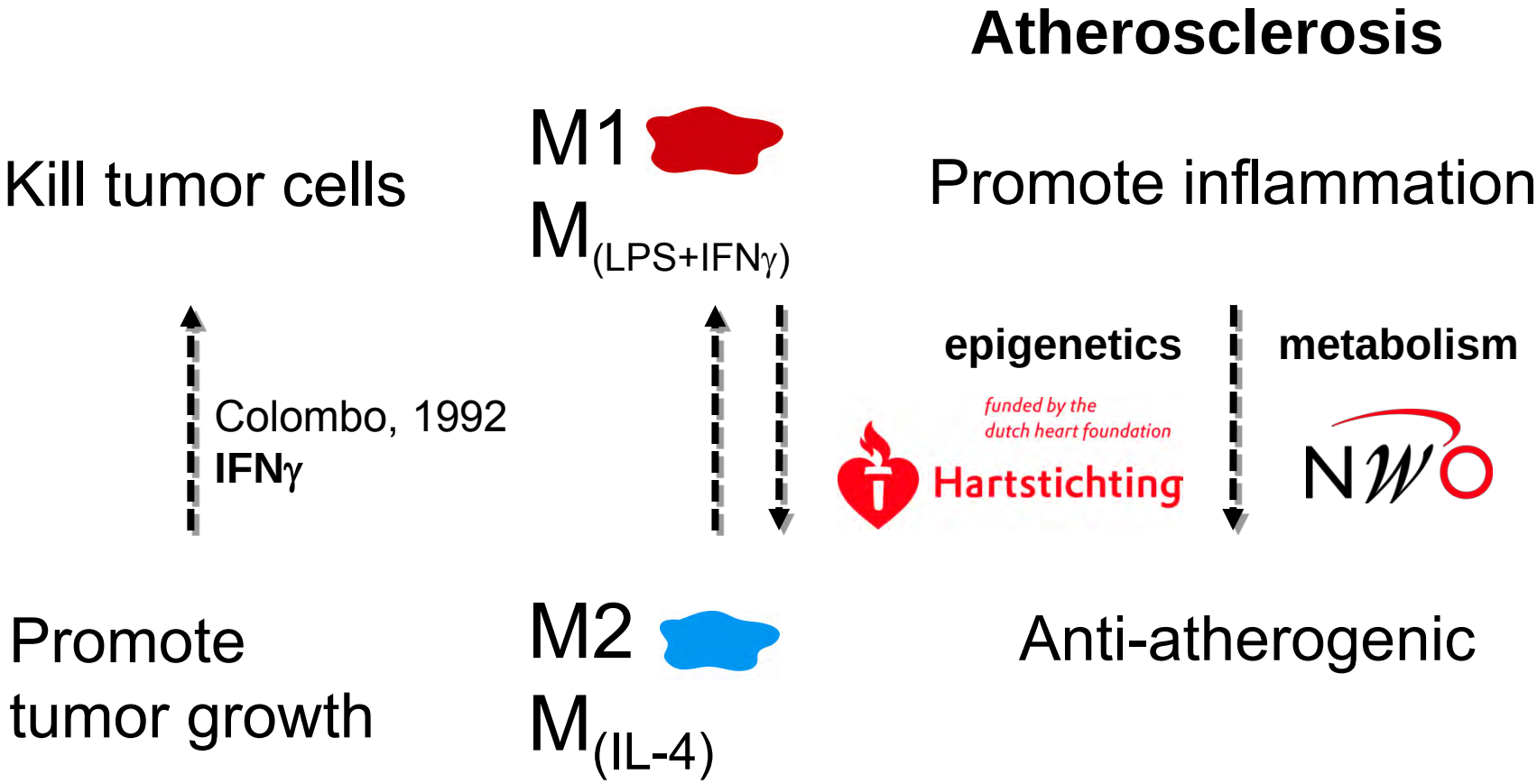
Reorienting and reshaping deranged macrophage polarization is the holy grail of macrophage therapeutic targeting (129). Polarized phenotypes are reversible in vitro and in vivo (29, 130–132).

Sica and Mantovani, JCI, 2012

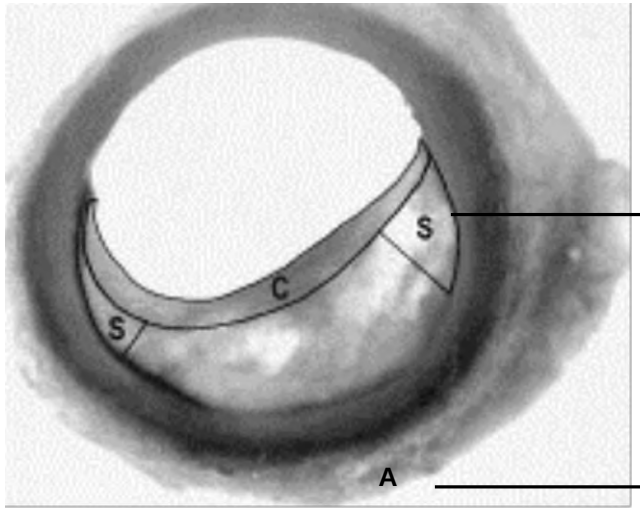
Macrophages are plastic

1/ Respond to distinct environmental stimuli

2/ Reverse their phenotype = repolarization



Reshaping macrophage (re)polarization for atherosclerosis therapy



Stöger et al., 2012

M1

iNOS+

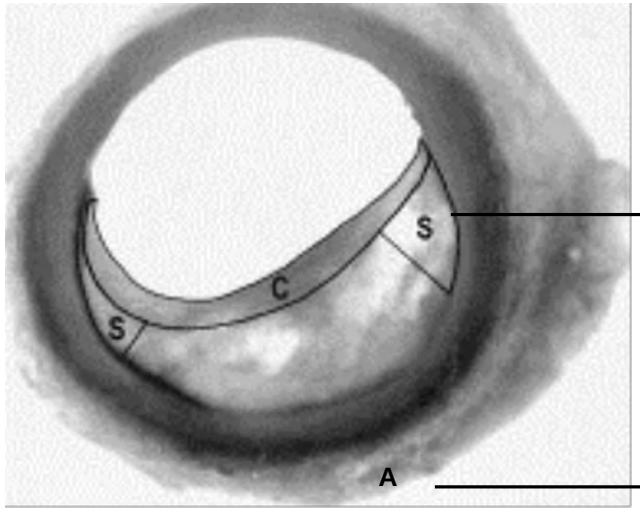
$M_{(LPS+IFN\gamma)}$

M2

CD206+

$M_{(IL\text{-}4)}$

Reshaping macrophage (re)polarization for atherosclerosis therapy



Stöger et al., 2012

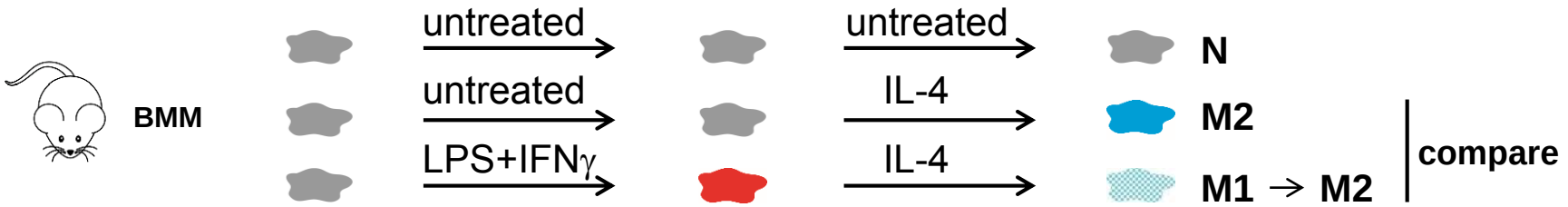
iNOS+

$M_{(LPS+IFN\gamma)}$

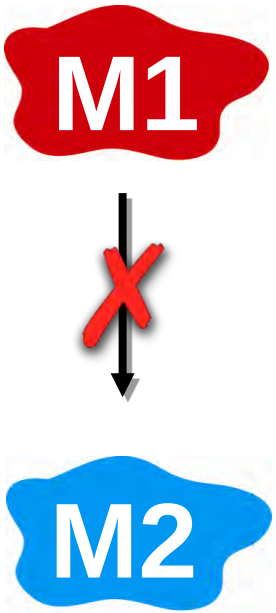
CD206+

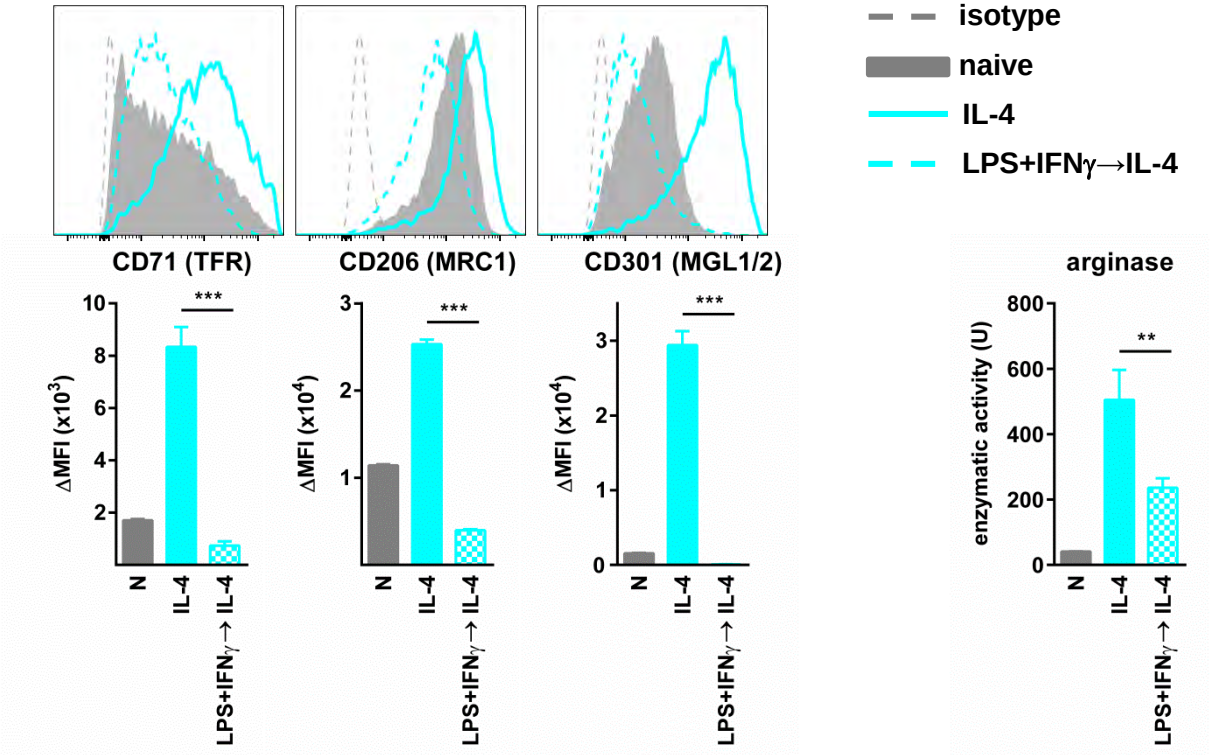
$M_{(IL\text{-}4)}$

M1s fail to repolarize to M2s



M2 surface markers and function





M1s fail to repolarize to M2s *in vivo*

CD45.1

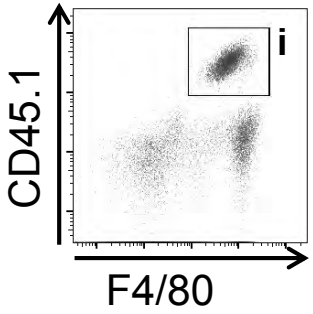
BMM

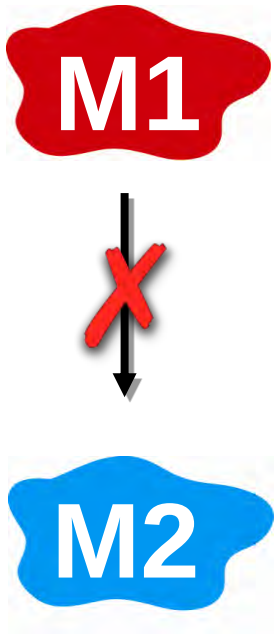
Ex vivo stimulation
- Naive (N)
- LPS+IFNγ

transfer

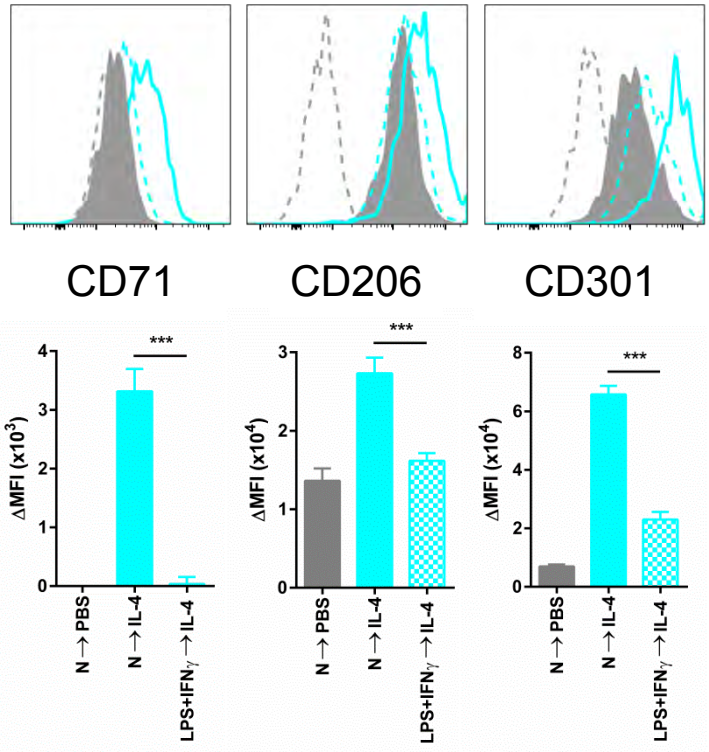
CD45.2

IL-4c/PBS

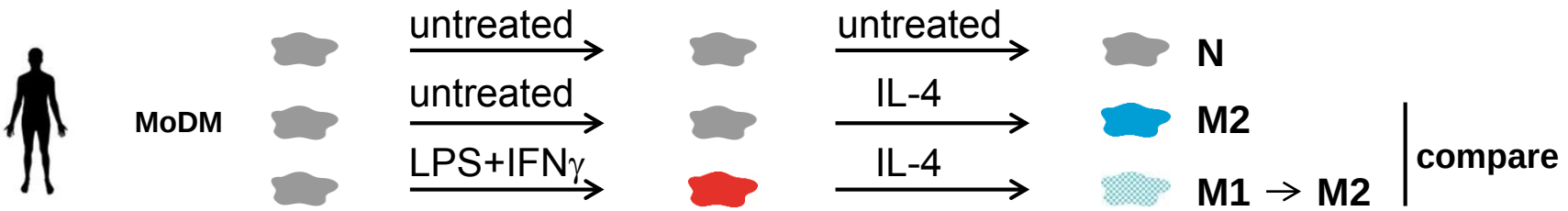


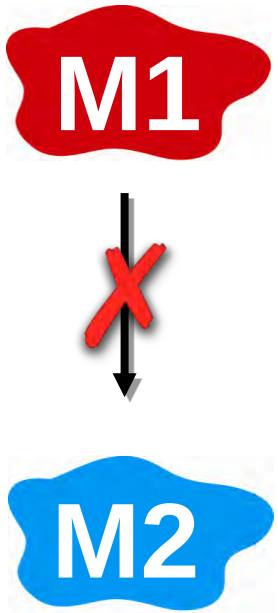


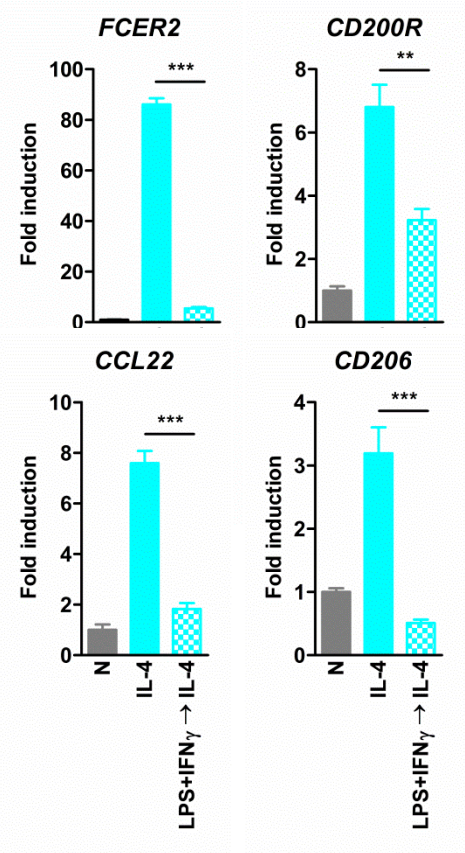
i: CD45.1⁺ F4/80⁺ macrophages

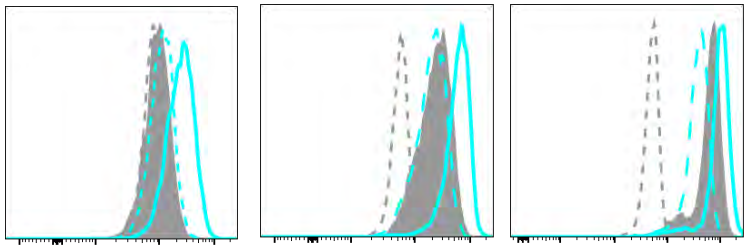


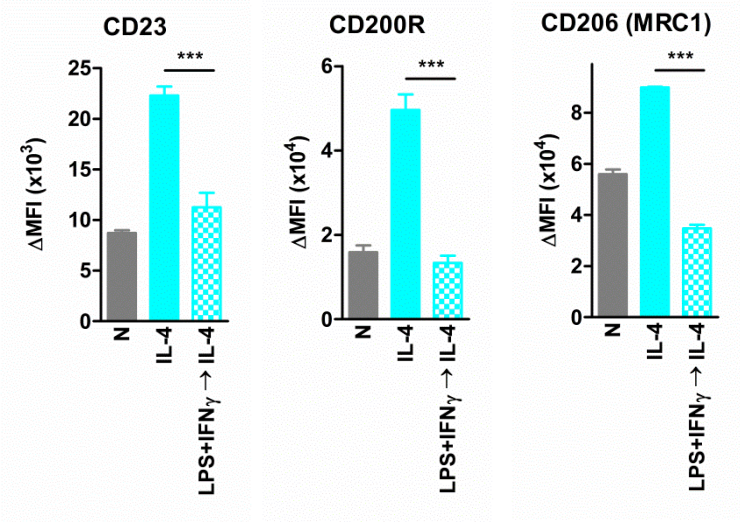
Human M1s fail to repolarize to M2s





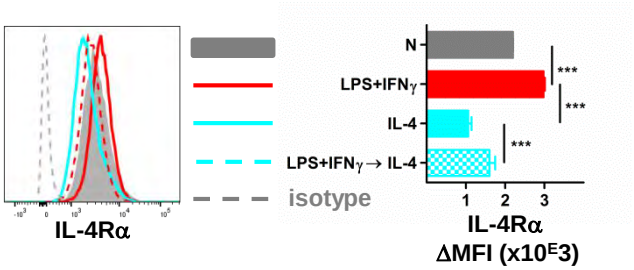


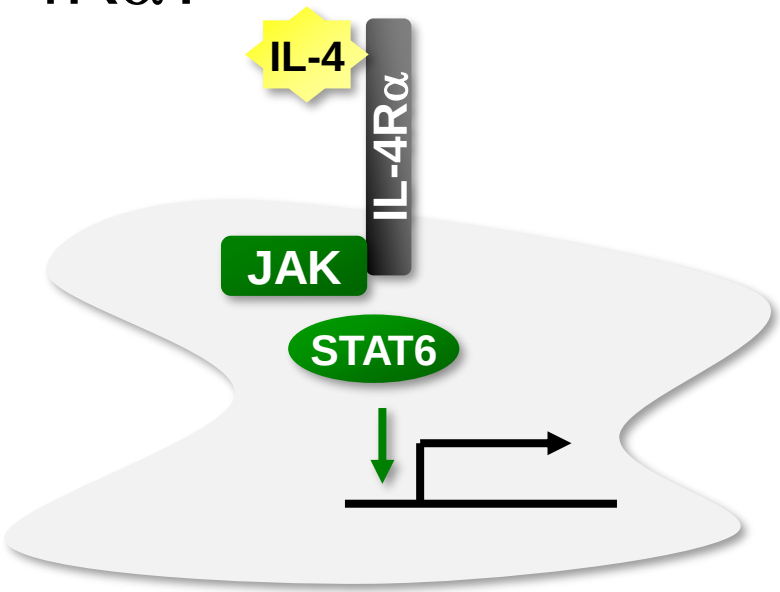




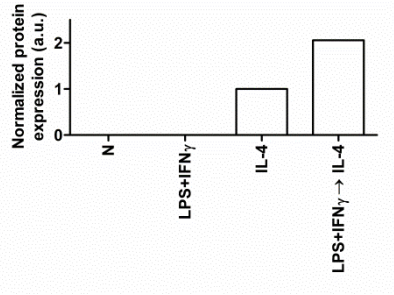
Why are M1 macrophages not plastic?

Because they don't have a IL-4Rα?



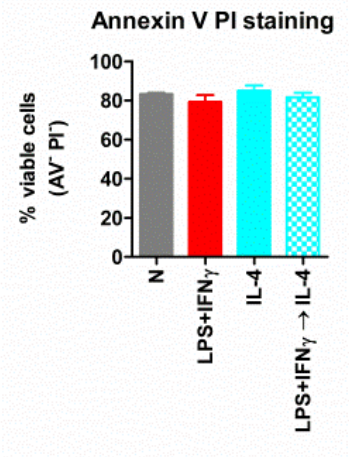


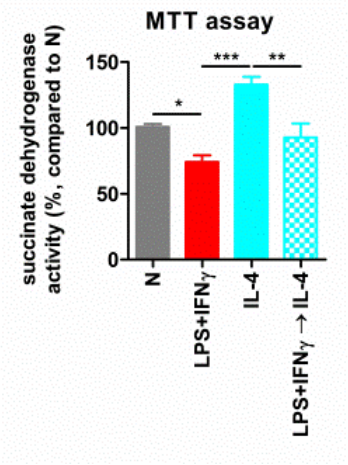
Because they have dysfunctional STAT6 activation?

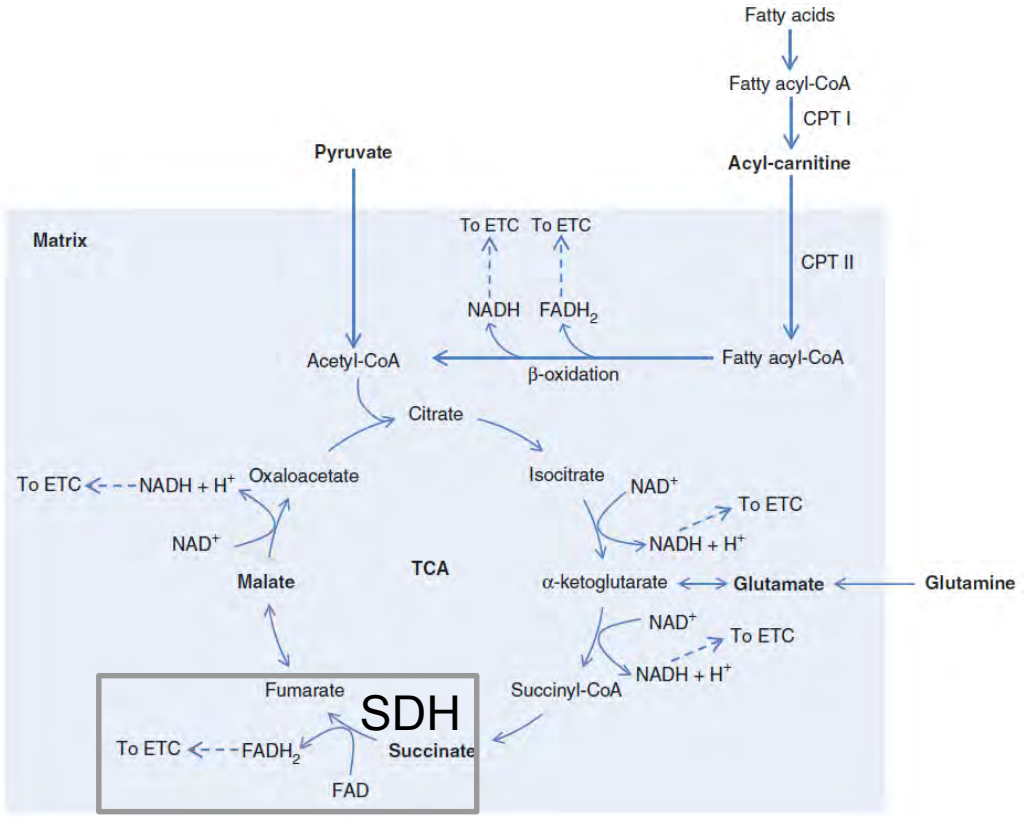


Why are M1 macrophages not plastic?

Because they are dead?



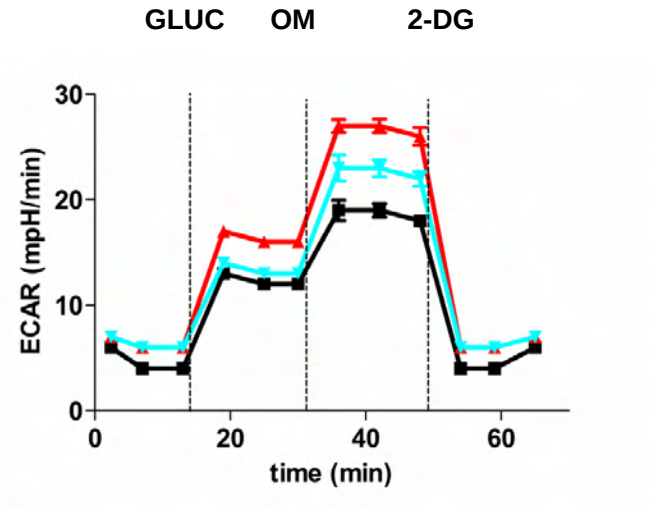




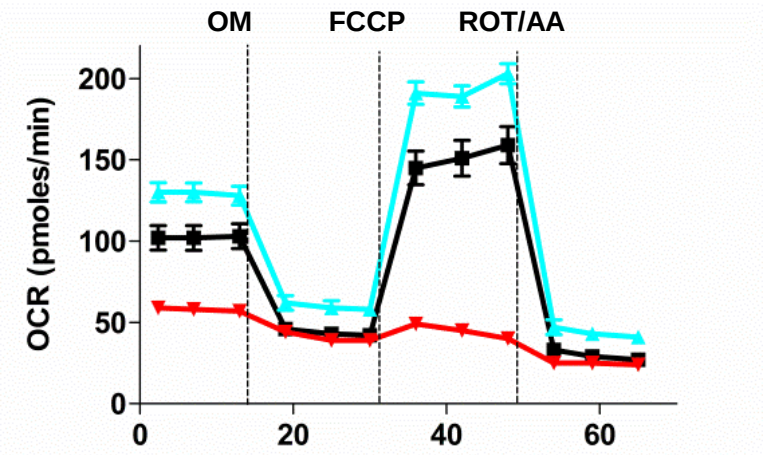
Why are M1 macrophages not plastic?

Because their metabolism doesn't allow it?

Glycolysis stress test



Mito stress test

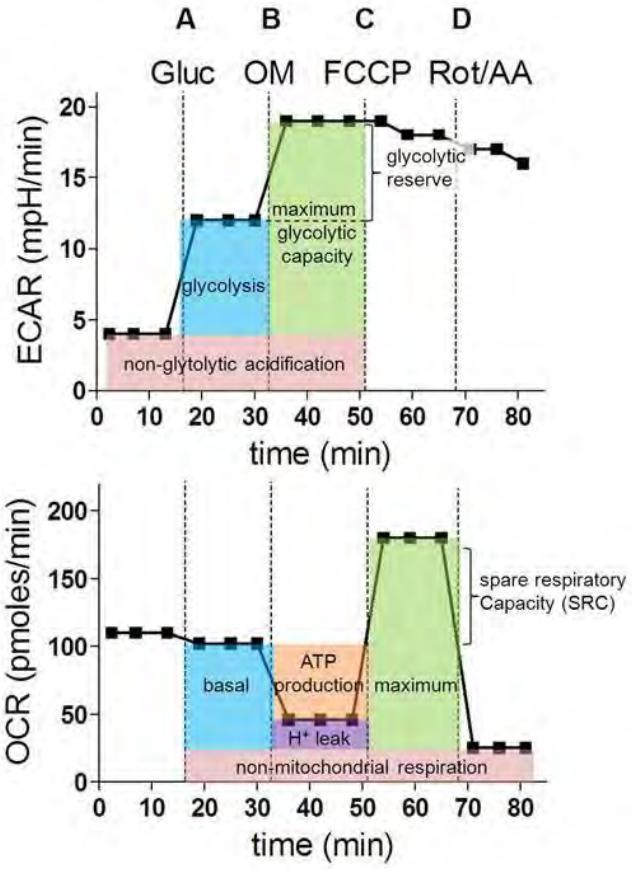


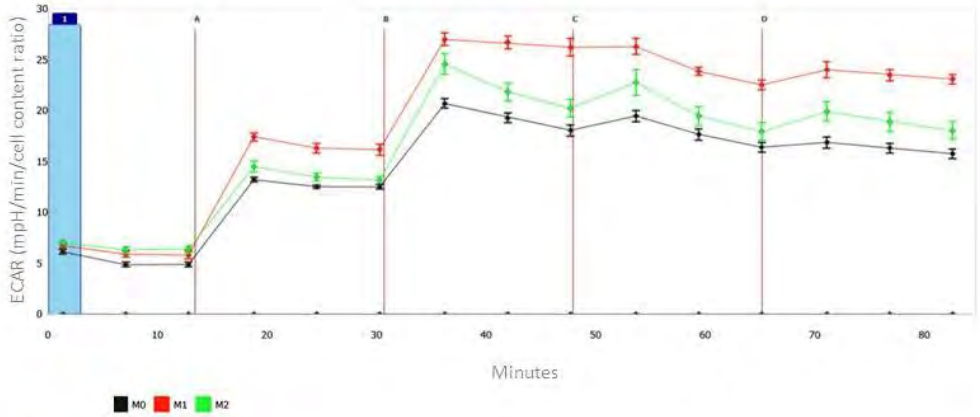
M1 = high glycolysis, low OXPHOS
M2 = high OXPHOS

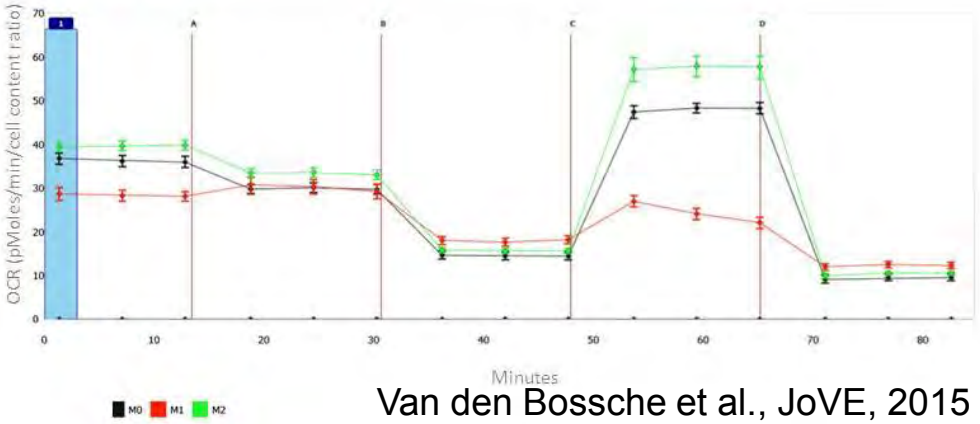
Seahorse intermezzo

Because their metabolism doesn't allow it?

Glycolysis & mito stress test combined





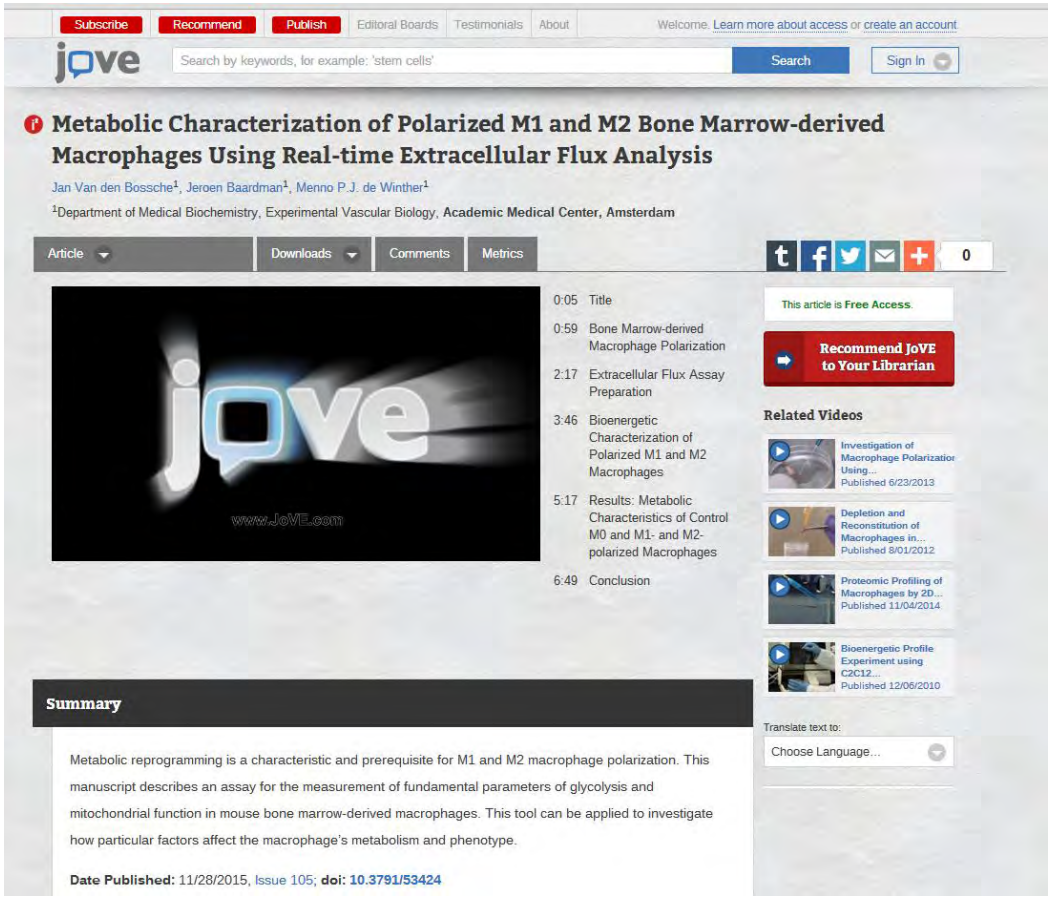


Van den Bossche et al., JoVE, 2015

Seahorse intermezzo

Because their metabolism doesn't allow it?

Glycolysis & mito stress test combined :

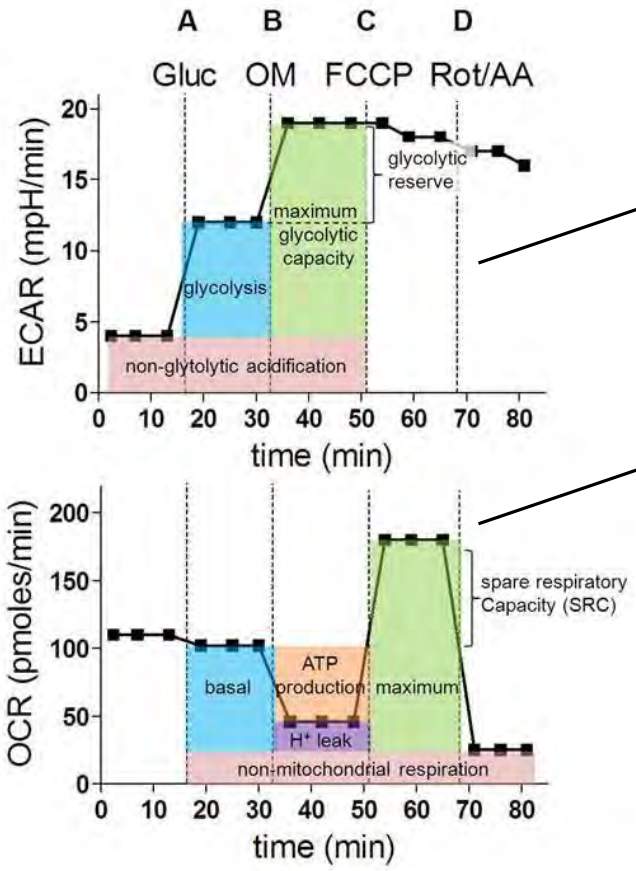


Van den Bossche et al., JoVE, 2015

Seahorse intermezzo

Because their metabolism doesn't allow it?

Glycolysis & mito stress test combined



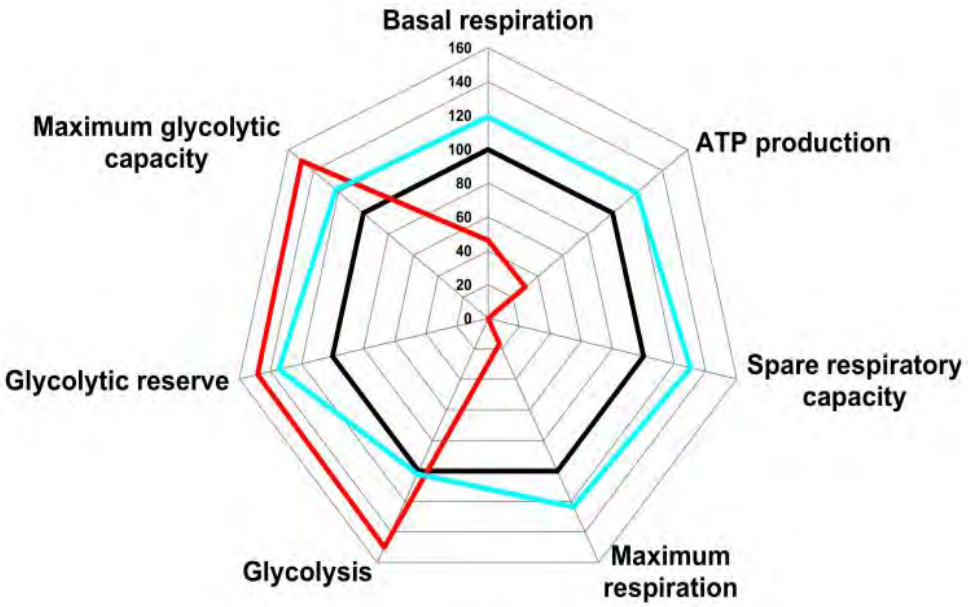
Use 2-DG to verify that ECAR is indeed 'true' glycolysis

Add pyruvate together with FCCP to allow max respiration

Van den Bossche et al., JoVE, 2015

Why are M1 macrophages not plastic?

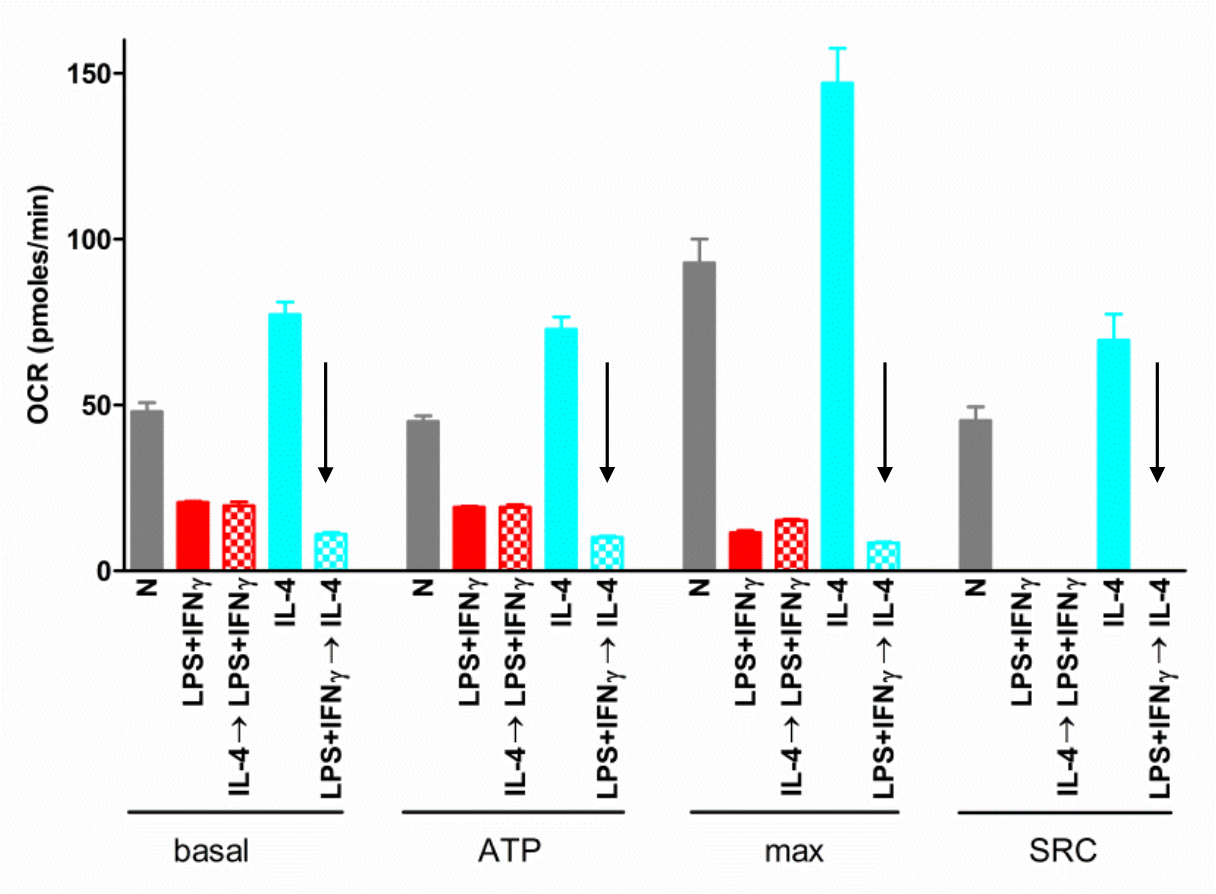
Because their metabolism doesn't allow it?



M1 = high glycolysis, low OXPHOS
M2 = high OPXHOS

Why are M1 macrophages not plastic?

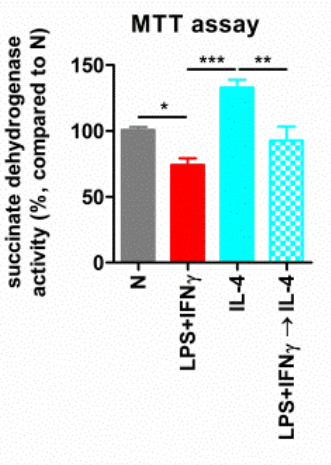
IL-4 does not induce OXPHOS upon M1→M2 **repolarization**



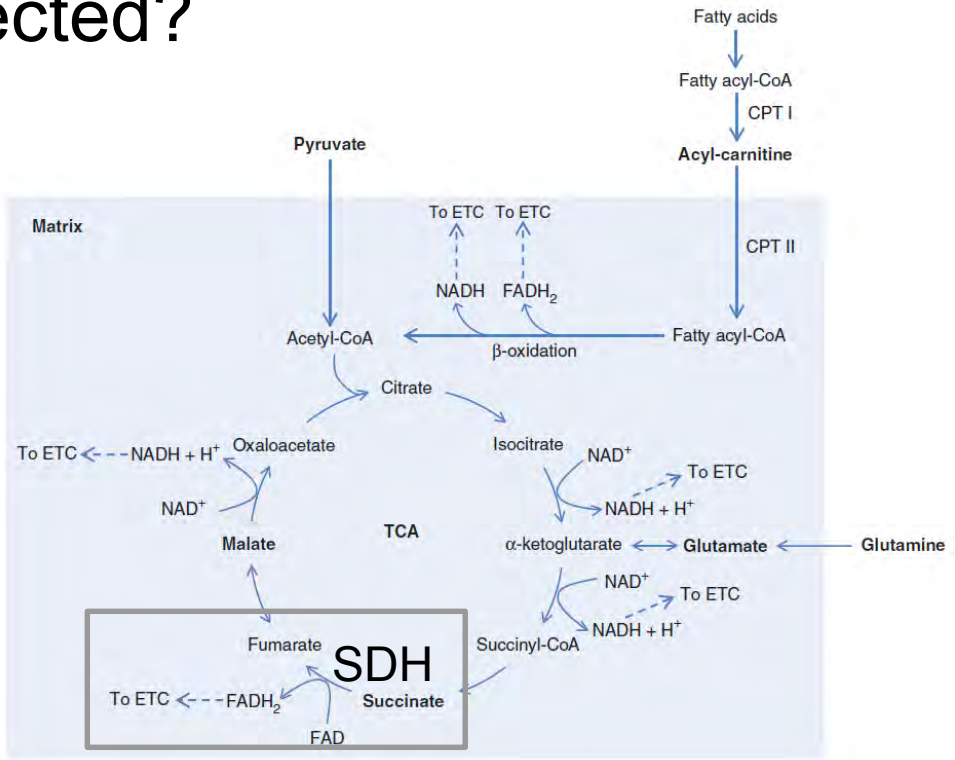
Why are M1 macrophages not plastic?

IFNγ+LPS induces mitochondrial dysfunction

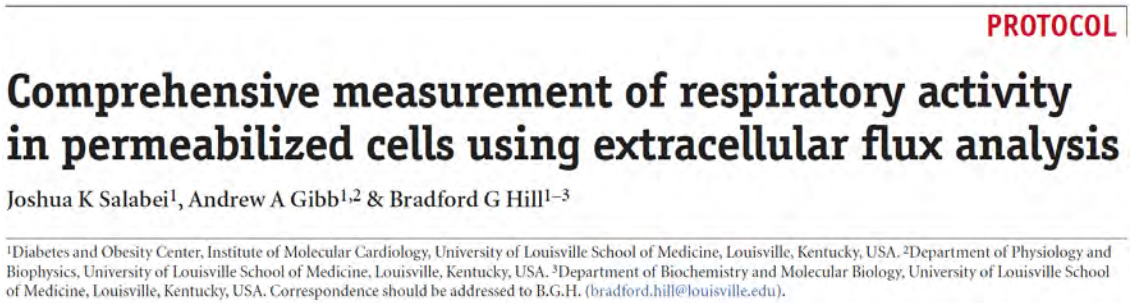
Which complexes are affected?



= complex II assay



Measure complex I-IV activity of the ETC with the Seahorse



PROTOCOL

Comprehensive measurement of respiratory activity in permeabilized cells using extracellular flux analysis

Joshua K Salabei[1], Andrew A Gibb[1,2] & Bradford G Hill[1–3]

[1]Diabetes and Obesity Center, Institute of Molecular Cardiology, University of Louisville School of Medicine, Louisville, Kentucky, USA. [2]Department of Physiology and Biophysics, University of Louisville School of Medicine, Louisville, Kentucky, USA. [3]Department of Biochemistry and Molecular Biology, University of Louisville School of Medicine, Louisville, Kentucky, USA. Correspondence should be addressed to B.G.H. (bradford.hill@louisville.edu).

Nature protocols, 2014

Technical Brief

Using XF Plasma Membrane Permeabilizer (PMP) to Measure Mitochondrial Respiratory Complexes in Limited Biomaterial Without Isolating Mitochondria

Measure complex I-IV activity of the ETC with the Seahorse

PROTOCOL

Comprehensive measurement of respiratory activity in permeabilized cells using extracellular flux analysis

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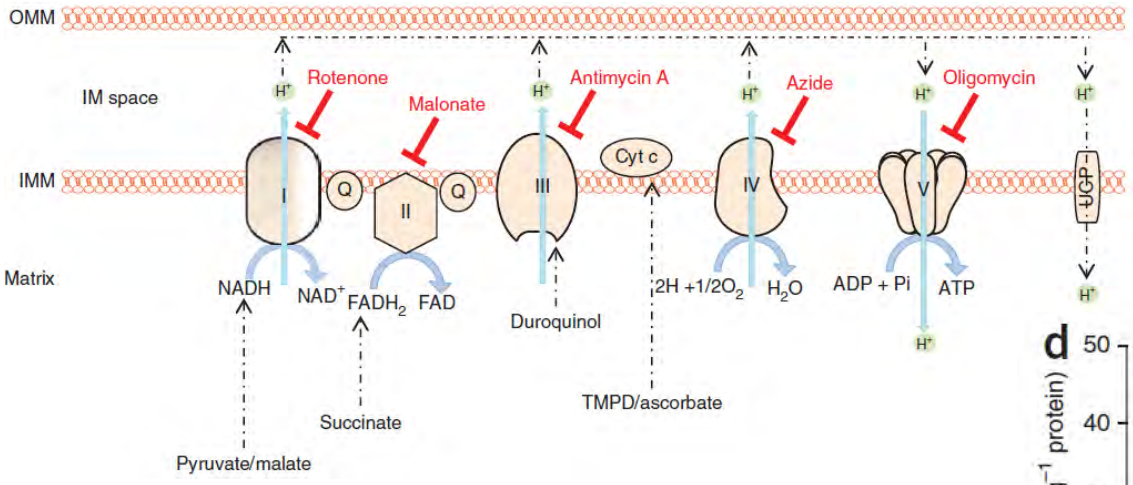
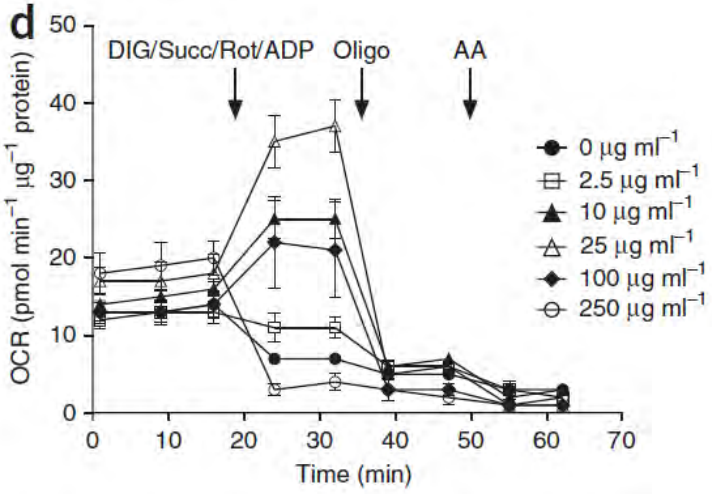
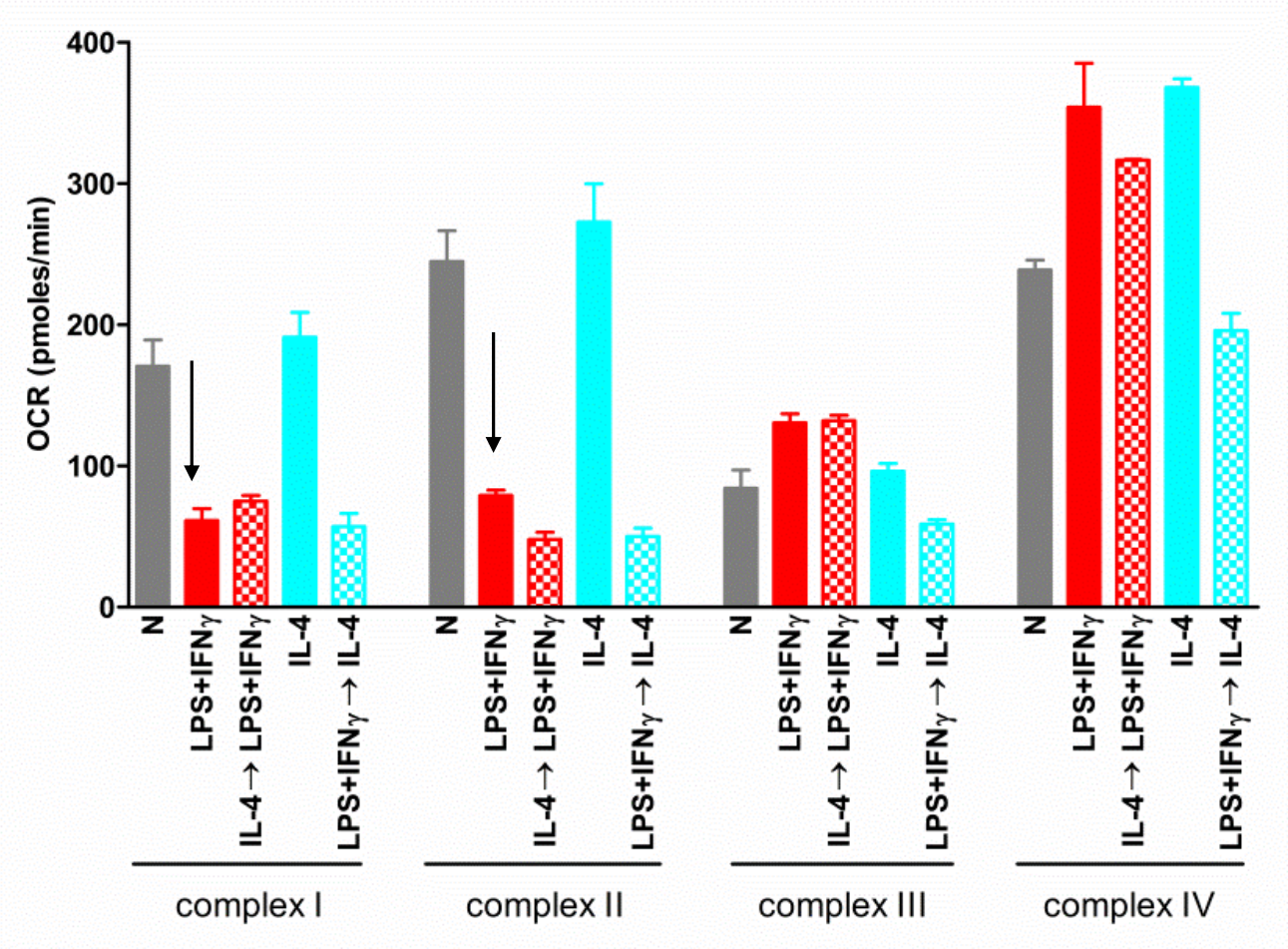
Nature protocols, 2014



complex II activity

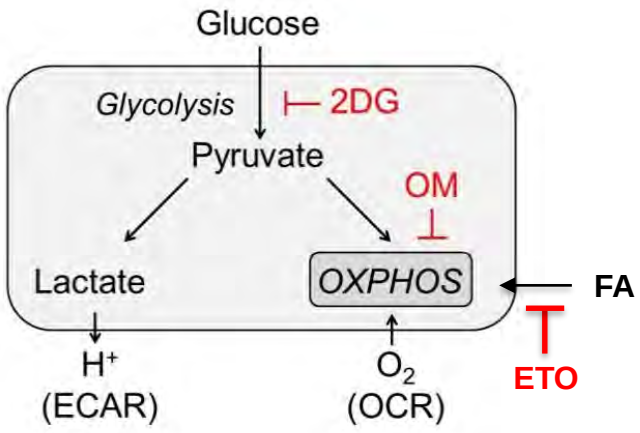


Measure complex I-IV activity of the ETC with the Seahorse



IFNγ+LPS mainly hit complex I and II activity

M2 polarization needs mitochondrial function

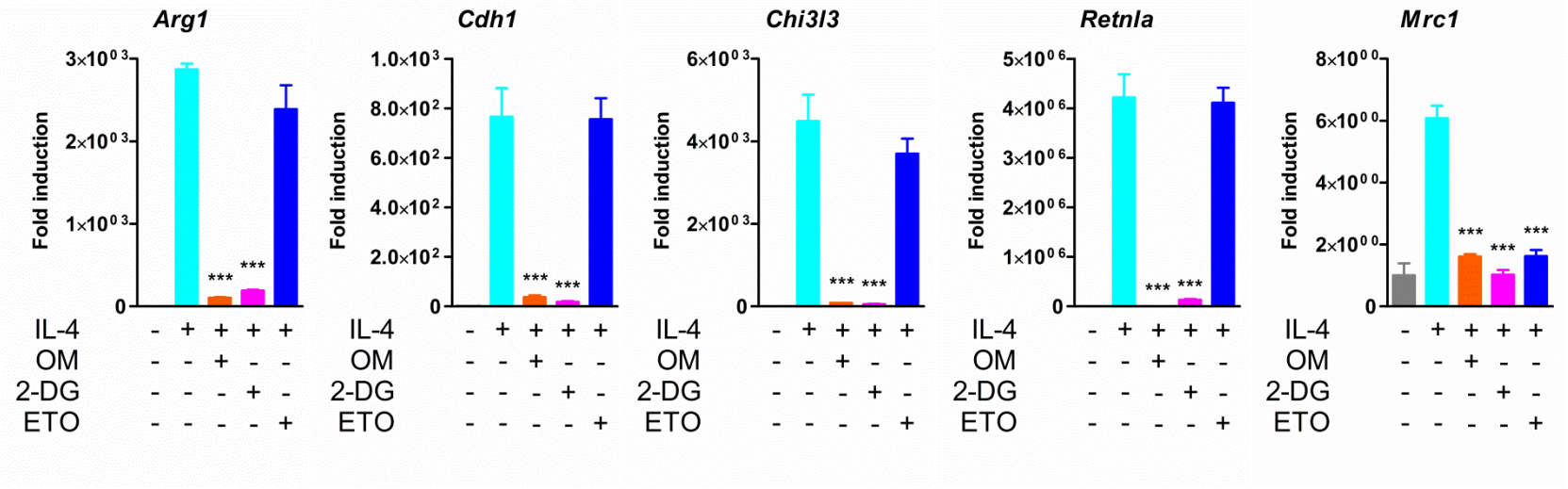


Oxidative metabolism and PGC-1β attenuate macrophage-mediated inflammation

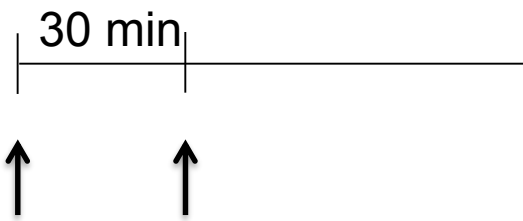
Divya Vats,[1,4] Lata Mukundan,[1,4] Justin I. Odegaard,[1,4] Lina Zhang,[1] Kristi L. Smith,[1] Christine R. Morel,[1] David R. Greaves,[2] Peter J. Murray,[3] and Ajay Chawla[1,*]

Pyruvate Dehydrogenase Kinase 1 Participates in Macrophage Polarization via Regulating Glucose Metabolism

Zheng Tan,*† Na Xie,* Huachun Cui,* Douglas R. Moellering,‡ Edward Abraham,§ Victor J. Thannickal,* and Gang Liu*



iNOS inhibition prevents the drop in OXPHOS in M1

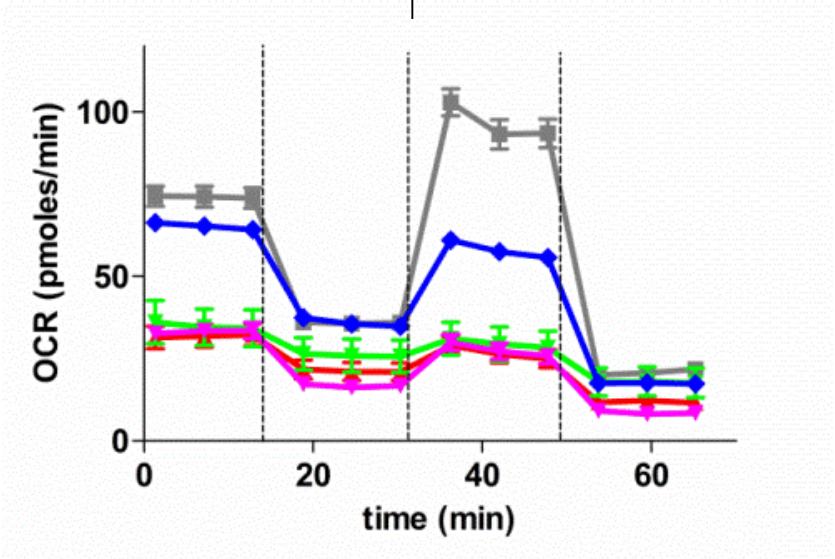


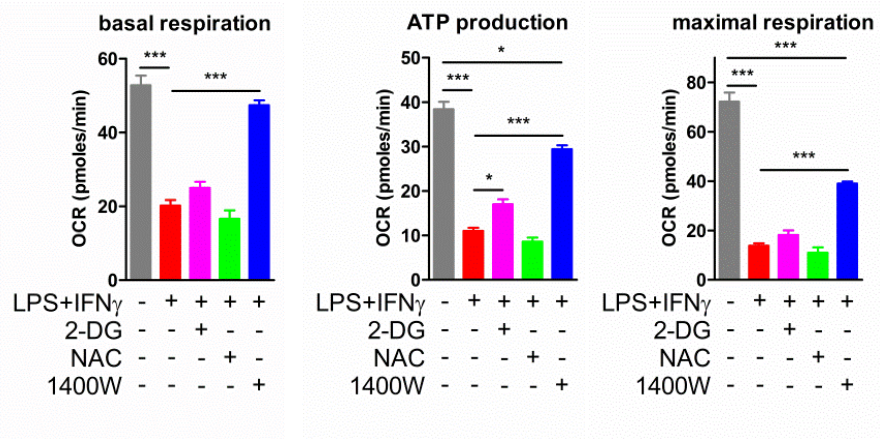
N M1 (or naive)

2DG

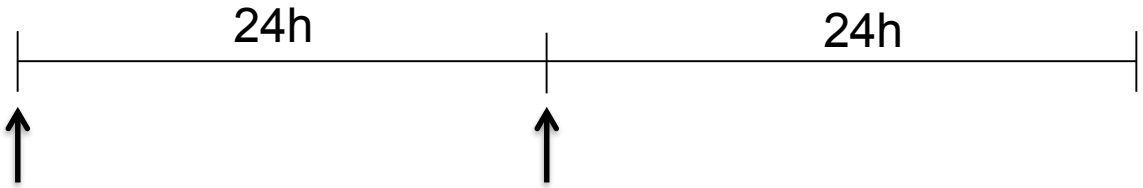
NAC (ROS scavenger)

1400W (iNOS inhibitor)



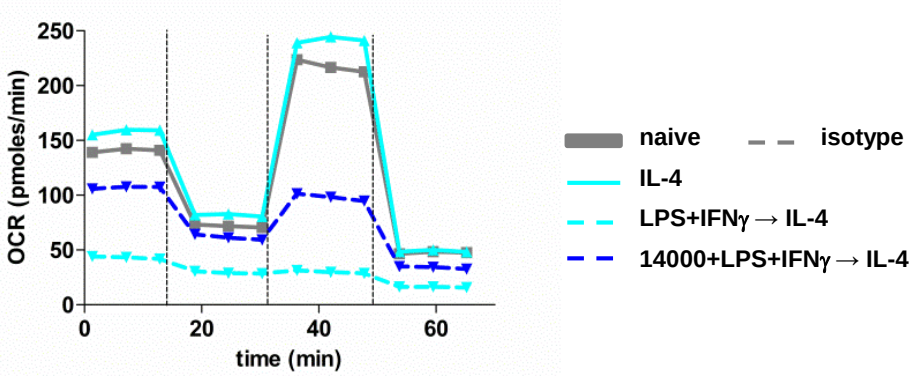


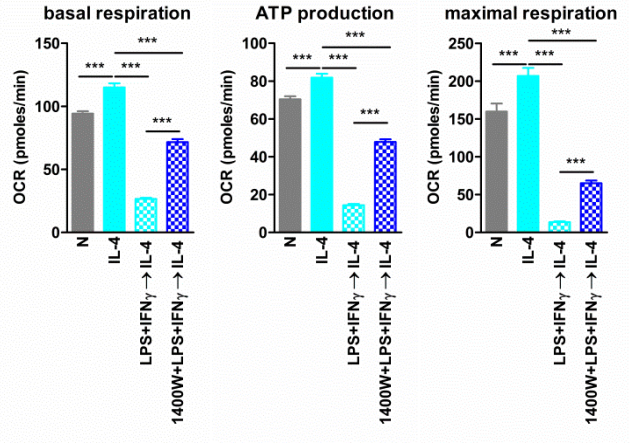
iNOS inhibition improves metabolic reprogramming by IL-4



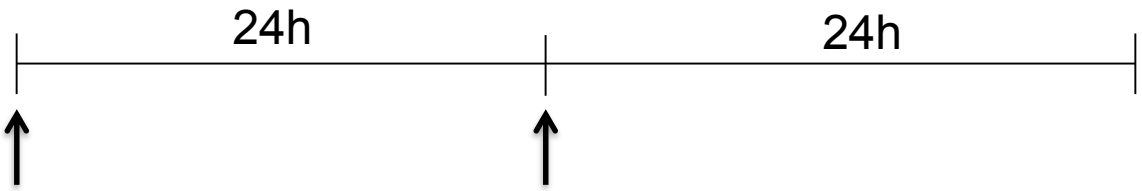
Stimulation 1
M1
1400W + M1

Stimulation 2 (after wash)
M2



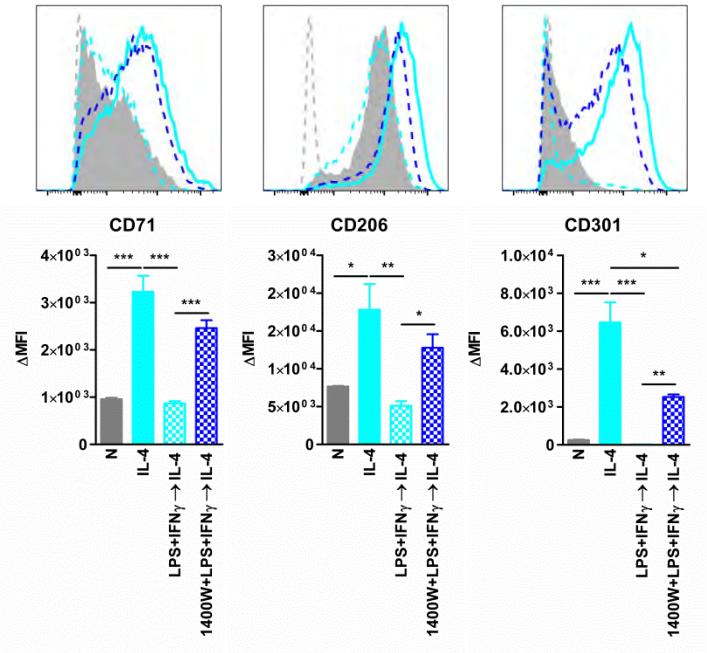


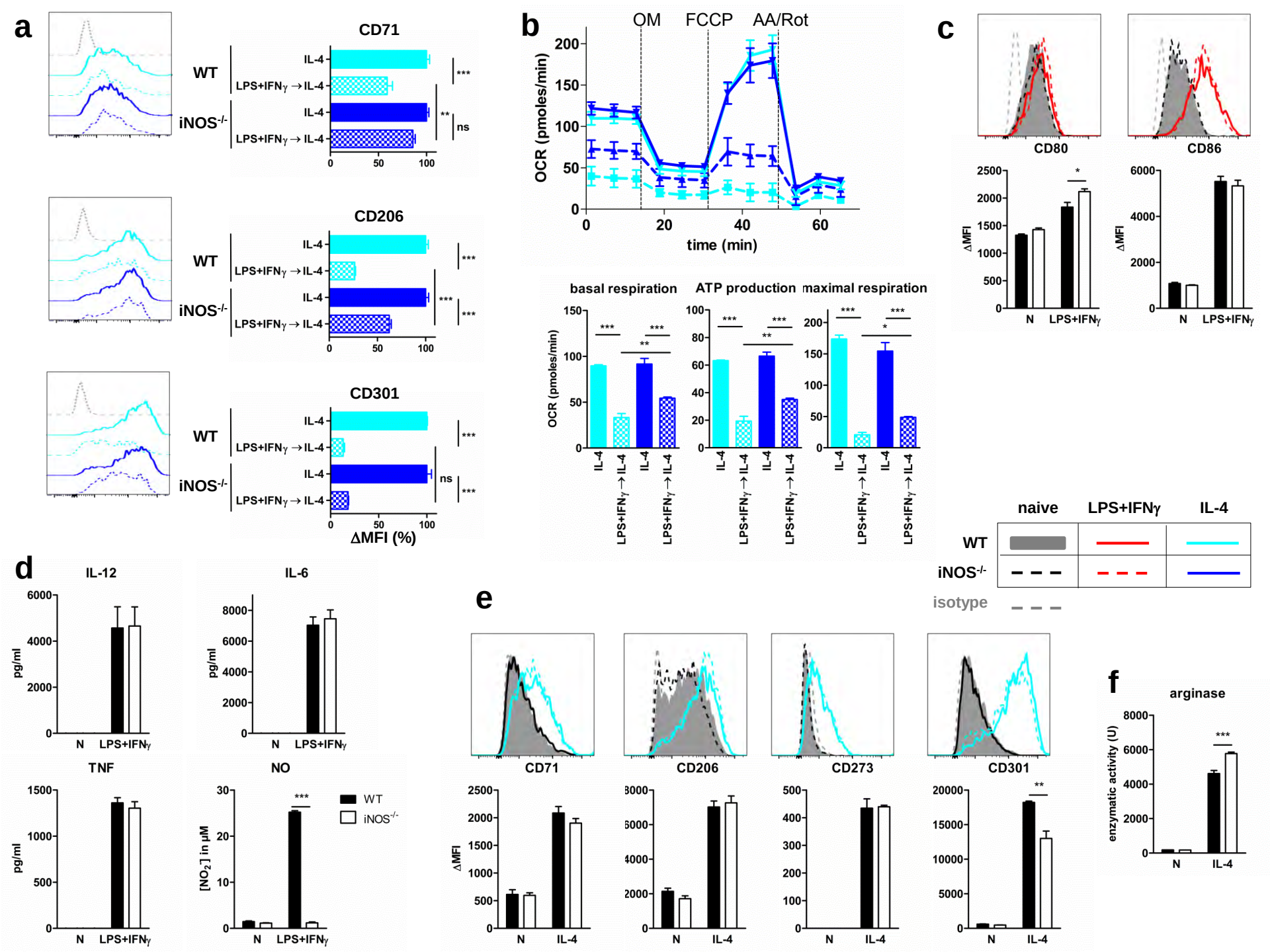
iNOS inhibition improves M1 to M2 repolarization



Stimulation 1
M1
1400W + M1

Stimulation 2 (after wash)
M2




a
CD71
WT
iNOS-/-
IL-4
LPS+IFNγ → IL-4
***
**
ns
CD206
CD301
ΔMFI (%)
b
OM
FCCP
AA/Rot
OCR (pmoles/min)
time (min)
basal respiration
ATP production
maximal respiration
c
CD80
CD86
ΔMFI
N
LPS+IFNγ
naive
IL-4
isotype
d
IL-12
IL-6
TNF
NO
pg/ml
[NO2] in µM
e
CD273
f
arginase
enzymatic activity (U)

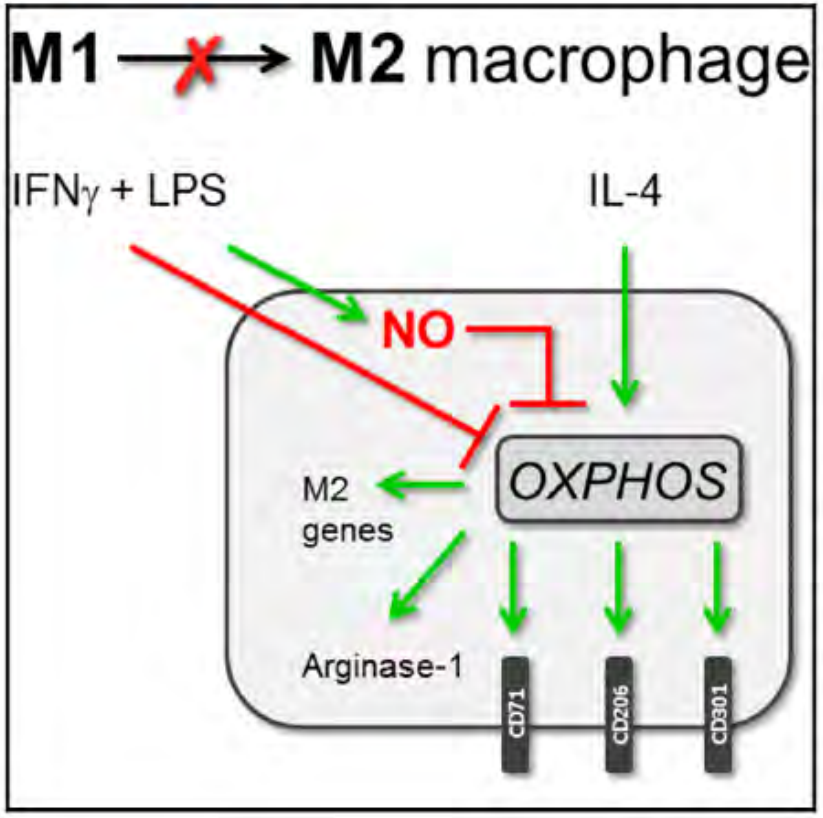
Macrophage Immunometabolism : Where Are We?

Article

Cell Reports

Mitochondrial Dysfunction Prevents Repolarization of Inflammatory Macrophages

Graphical Abstract



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In Brief

Editing macrophage polarization is an emerging concept for the treatment of inflammatory diseases. Van den Bossche et al. show that inflammatory M1 macrophage activation dampens mitochondrial function, thereby preventing the repolarization to an anti-inflammatory M2 phenotype. Inhibiting nitric oxide production improves mitochondrial function and reprogramming to M2 macrophages.

Van den Bossche et al., Cell Reports, 2016

Trends in Immunology

CellPress

ReviewSeries: Immunometabolism

Macrophage Immunometabolism: Where Are We (Going)?

Jan Van den Bossche,[1,*] Luke A. O'Neill,[2] and Deepthi Menon[2]

Outstanding Questions

To what extent can immunometabolism in mouse macrophages be translated to humans?

What are the metabolic characteristics of macrophages *in vivo* and how do they relate to LPS(+IFNγ)- and IL-4-induced macrophages *in vitro*?

What is the molecular mechanism that links increased FAO to IL-4-induced gene expression?

How important is epigenetic modification as a target for altered metabolism in macrophages?

Could targeting of macrophage metabolism be used for therapy?

Van den Bossche et al., Trends in Immunology, 2017

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Medical Biochemistry

Experimental Vascular Biology group

Jeroen Baardman

Menno de Winther & Esther Lutgens

Genetic Metabolic diseases

Michel van Weeghel
Riekelt Houtkooper

Vincent de Boer