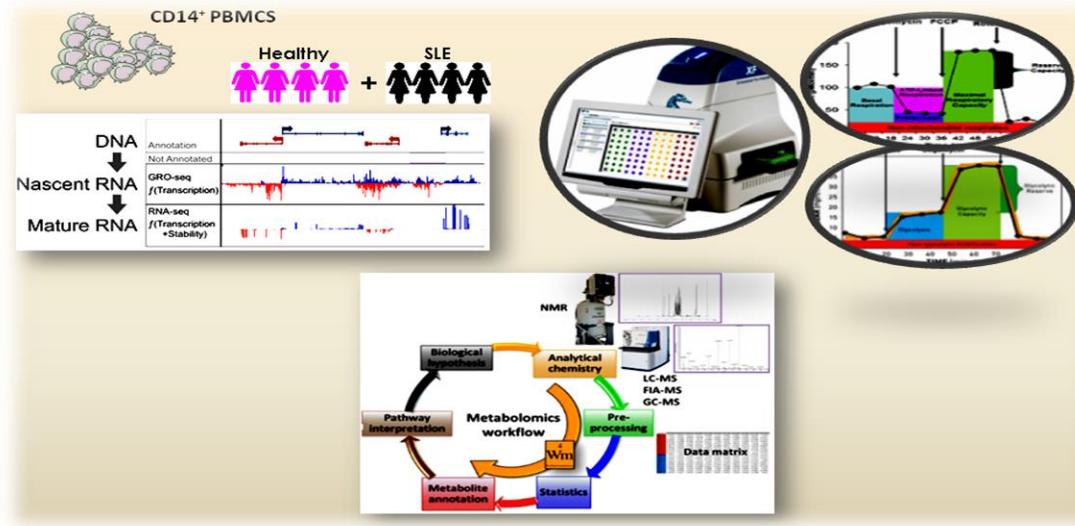


# IFN-mediated perturbations in glucose and cholesterol metabolism affect the activation and cytokine production in healthy monocytes (Mo) and SLE-Mo



Stathopoulou Chrysa

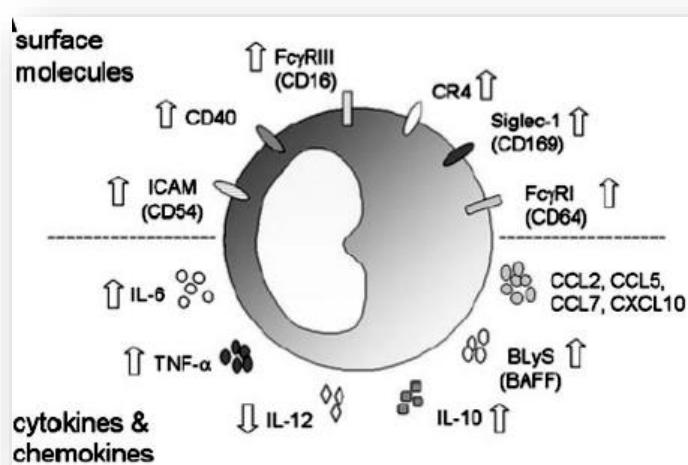
Lab. of Rheumatology, Autoimmunity & Inflammation

Heraklion 10/10/2021

# Introduction

## Lupus-Mo are altered compared to Healthy-Mo

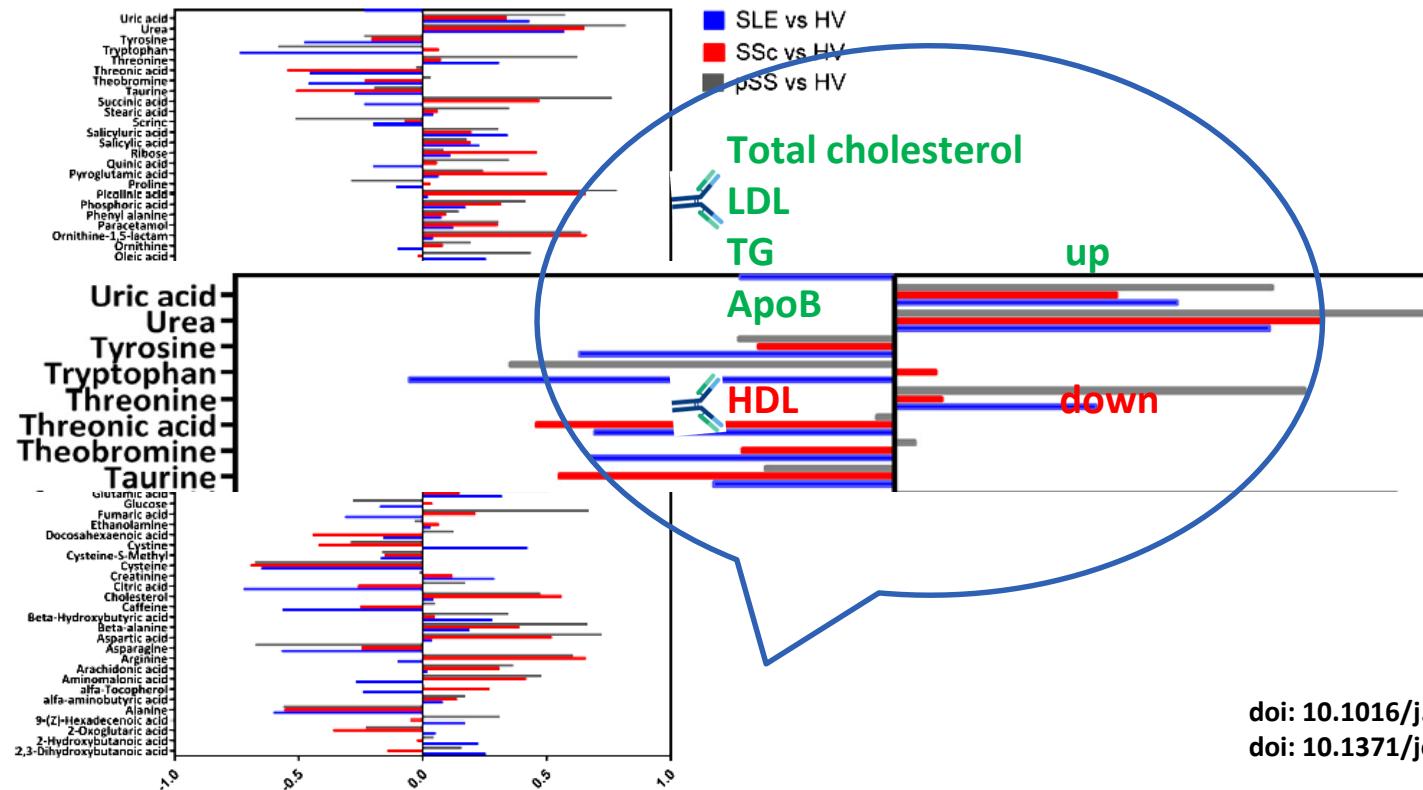
- Increased number of intermediate Mo (CD14<sup>+</sup>CD16<sup>+</sup>)
- Defective Phagocytosis:
  - defective engulfment : ↓C1q, C3, C4
  - impaired Fcγ-R-mediated ICs phagocytosis
  - Impaired autophagic flux
- Increased production of inflammatory cytokines and IFNs
- ISG expression during inactive disease
- Increased NO secretion
- Increased ROS production



doi: 10.1016/j.semarthrit.2008.11.002  
doi:10.1007/s00005-010-0093-y

# Introduction

SLE sera reveal an altered metabolic phenotype compared to HC

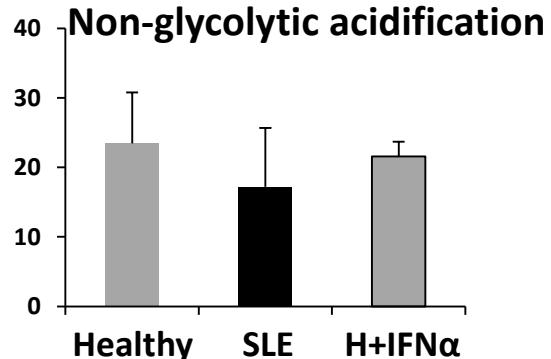
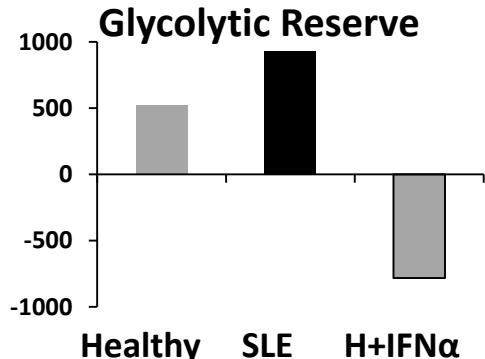
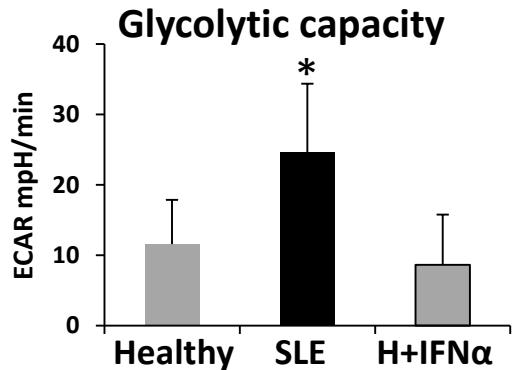
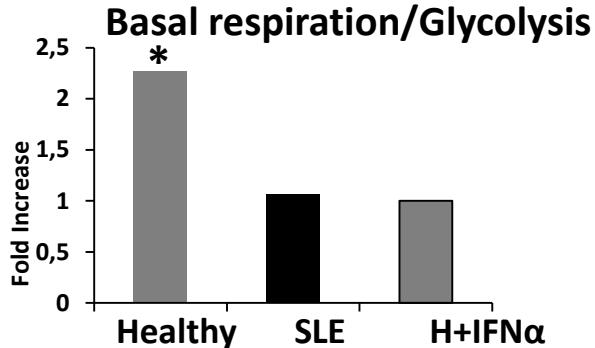
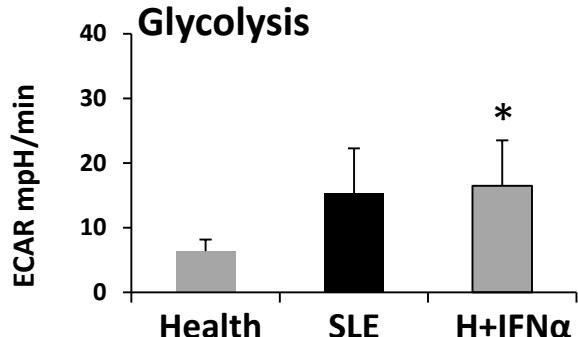
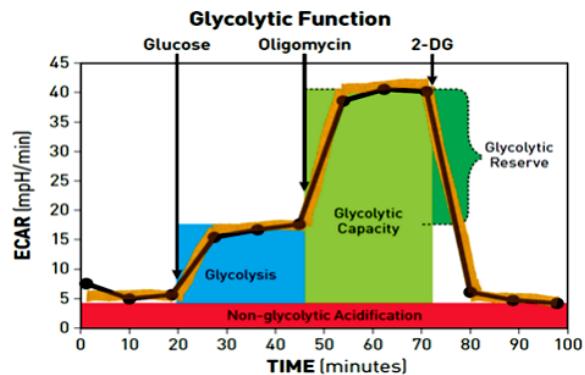


## Working Hypothesis

- IFN signature is predominant in SLE monocytes irrespectively of DA
- IFNs interfere with cellular autophagy to control immune responses
- Monocytes can serve as energy biomarkers of the systemic exposure to metabolic stressors or inflammatory cytokines

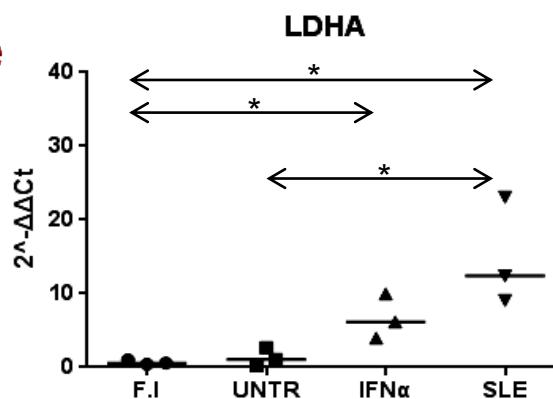
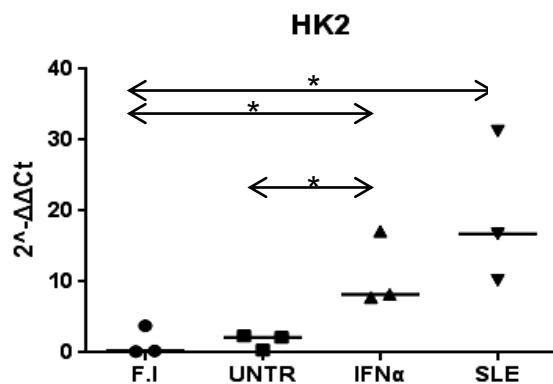
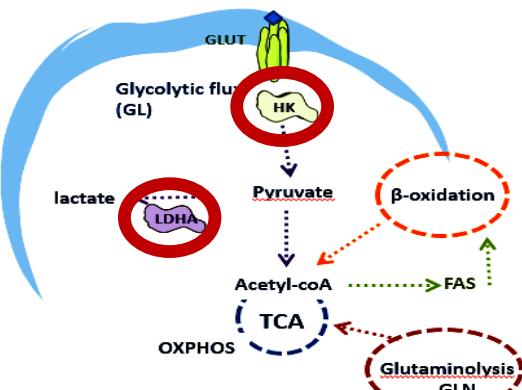
**Studying the metabolism of Monocytes in response to IFN could reveal implicated metabolic pathways serving as candidates for therapeutic targeting in interferonopathies such as SLE**

## Results: Seahorse ECAR. IFN increases glucose uptake in healthy Monocytes



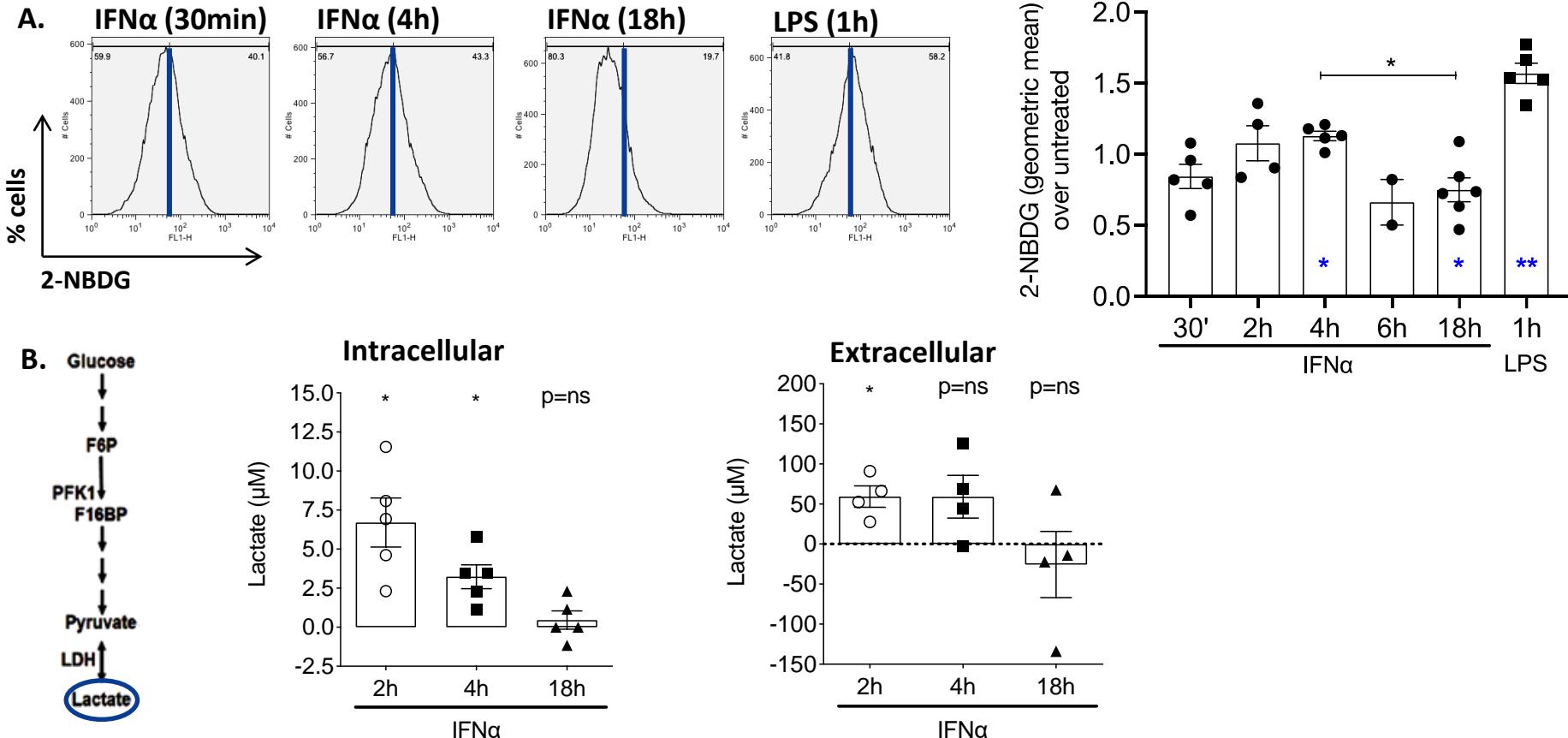
# Results: IFN upregulates the mRNA expression of genes involved in aerobic glycolysis

## Glycolytic transcripts are also increased in SLE-Mo

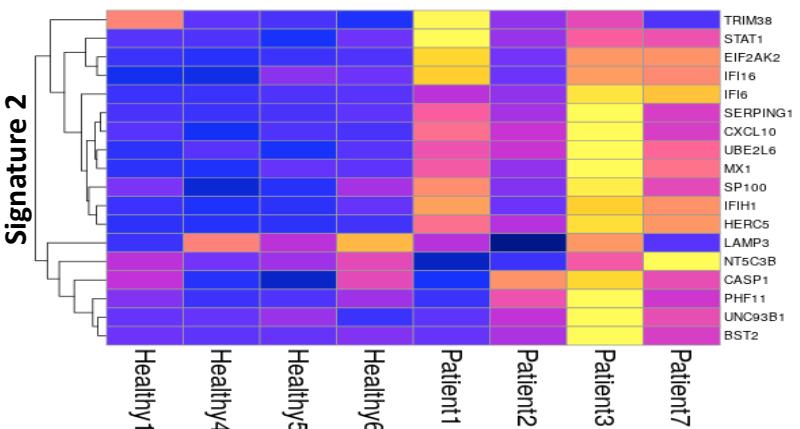
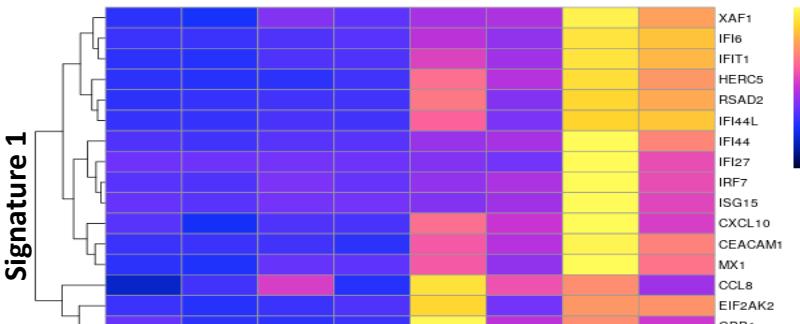


Donor selection  
on CXCL10

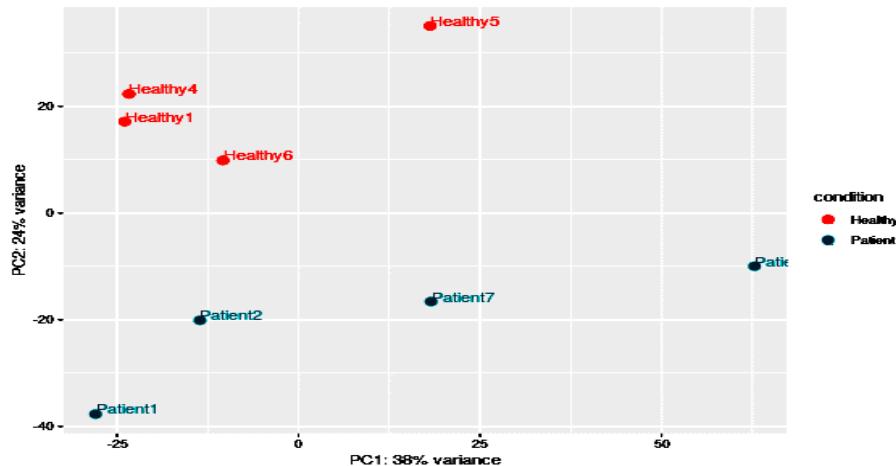
# Results: IFN increases glucose influx and lactate production in Healthy Monocytes



# Methods: Selection of Patients used for RNA-seq analysis based on increased IFN signature

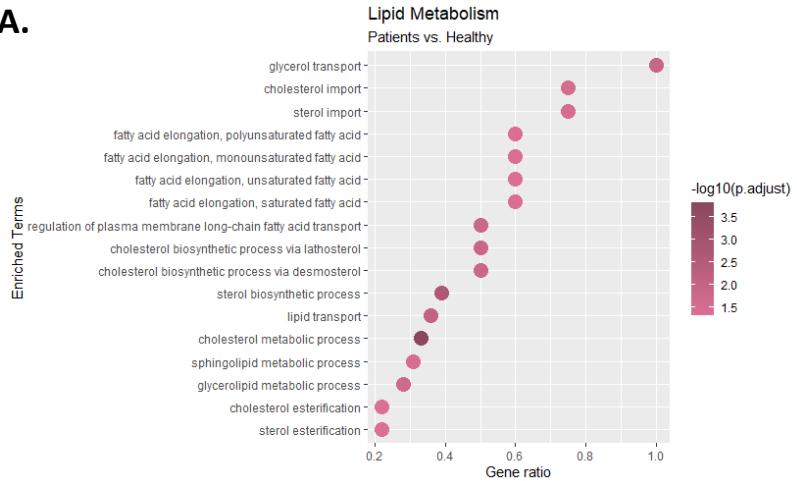


Patient ID	Sledai-2k	Symptoms	Anti-ds DNA
P1	6	Malar rash, Arthritis	-
P2	6	Arthritis	+
P3	5	skin rash, fatigue, lymphocytopenia	+
P7	9	Arthritis, fever, mouth ulcers	+

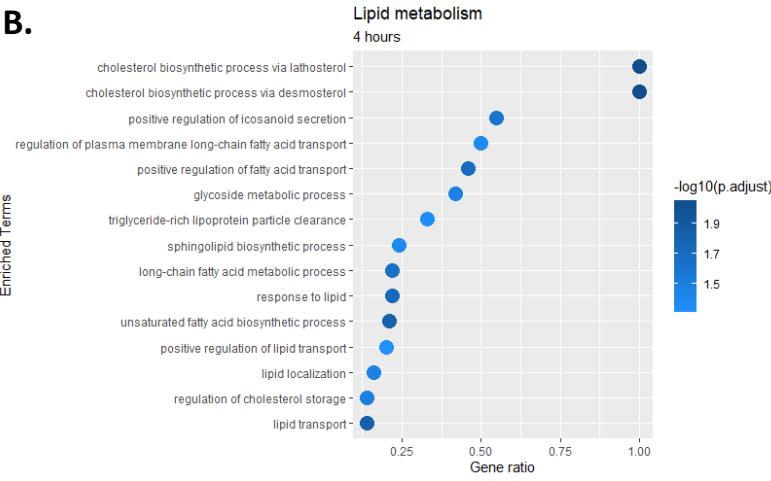


# Results: Interferon activation alters lipid metabolism in Mo

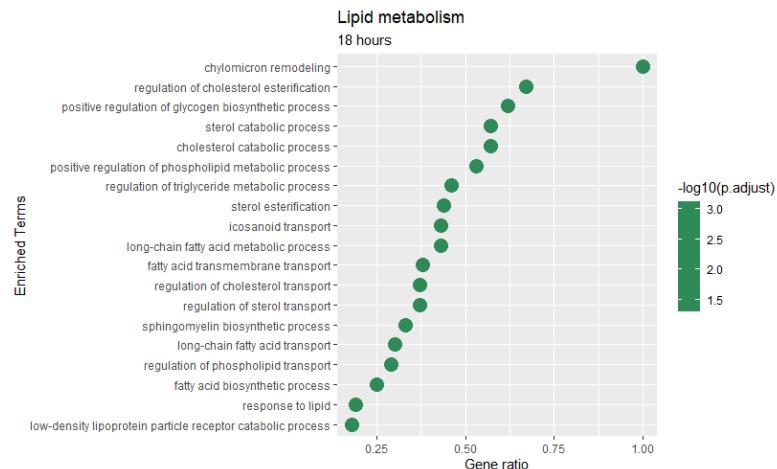
**A.**



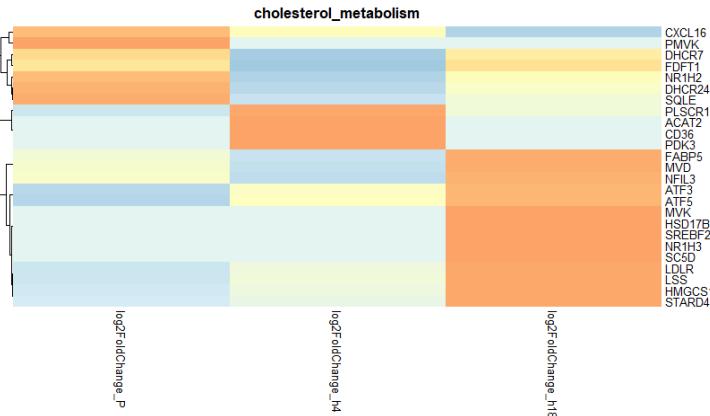
**B.**



**C.**



**D.**

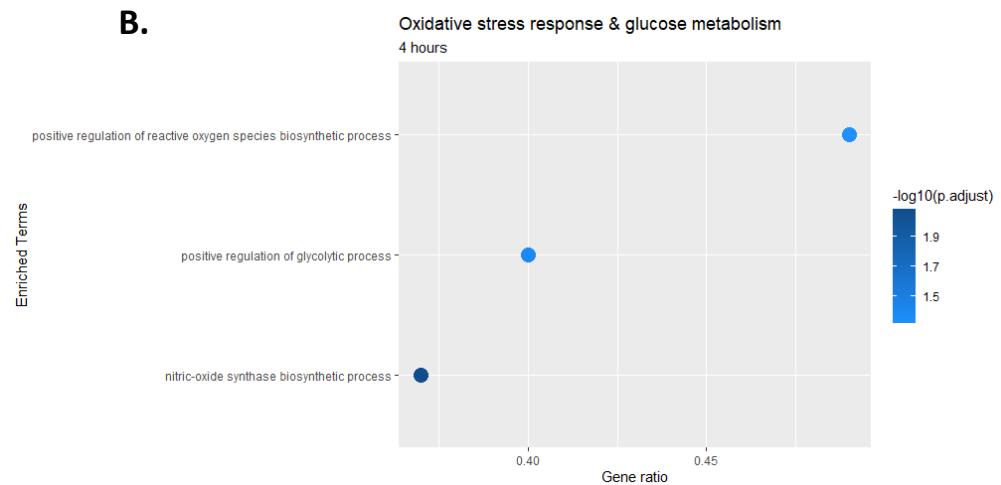


# Results: Interferon activation alters glucose metabolism in Mo

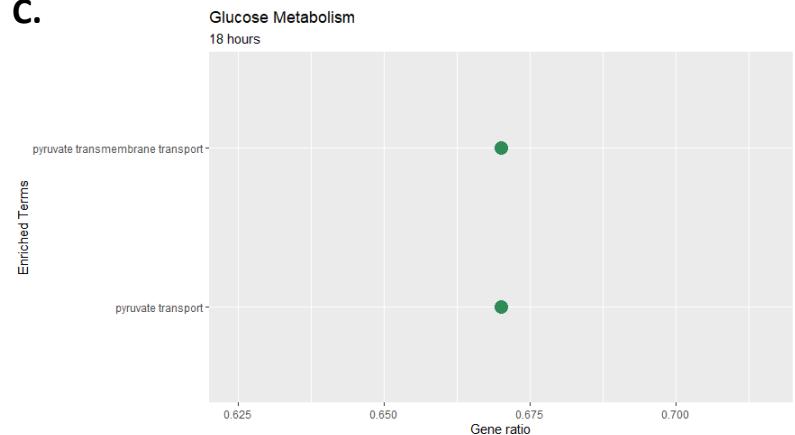
A.



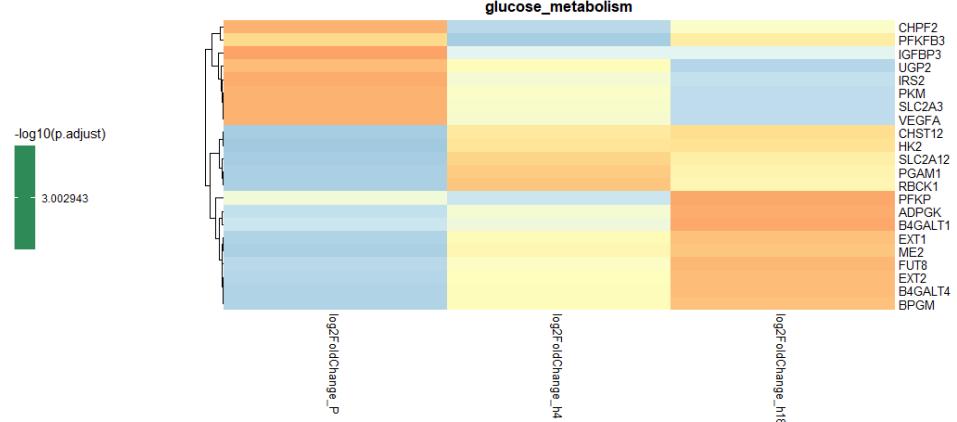
B.



C.

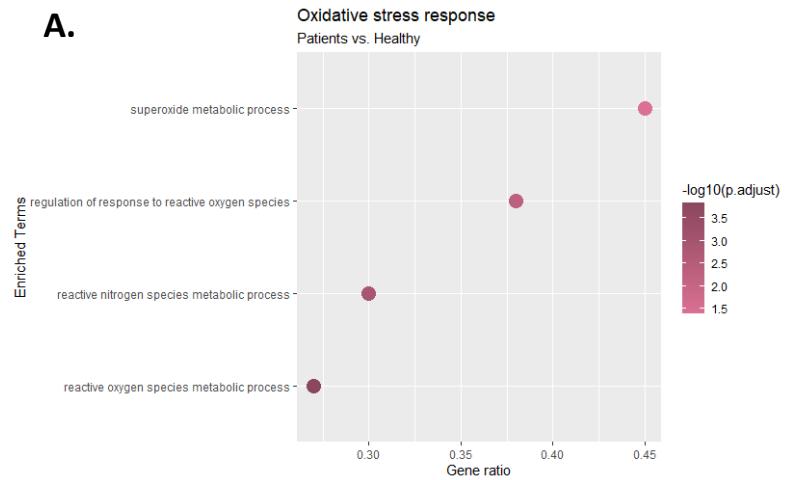


D.

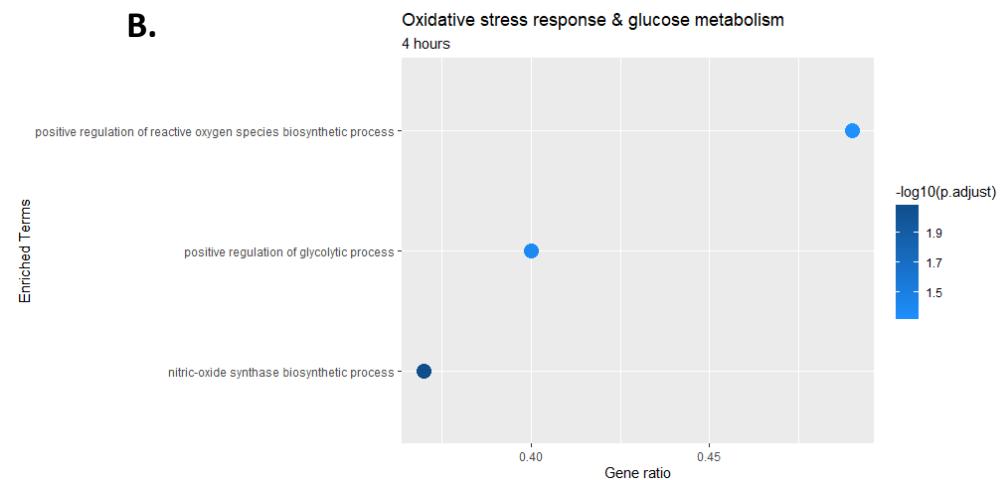


# Results: Altered ROS metabolism in SLE-Mo compared to healthy counterparts

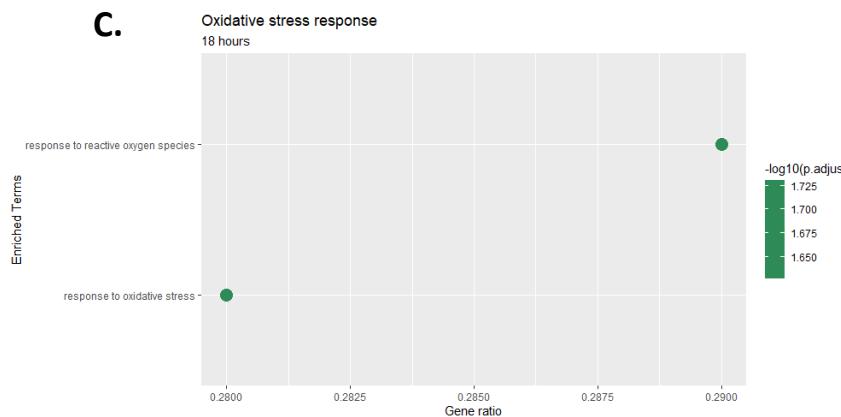
A.



B.

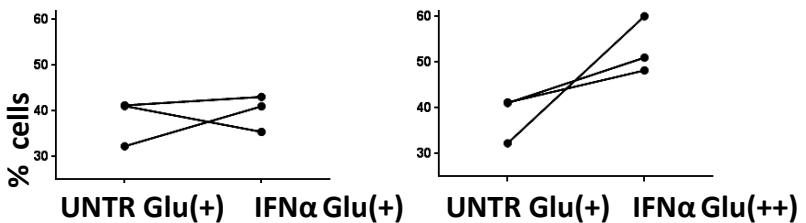


C.

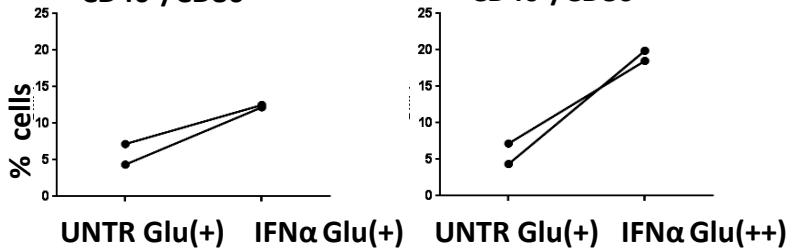


## Results: Glucose supplementation increases IFN-mediated inflammation in Healthy Monocytes

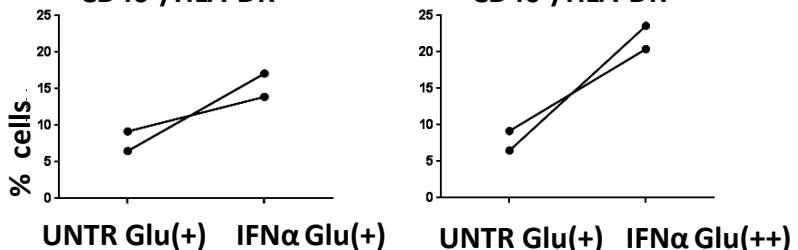
**A. CD86<sup>+</sup>/HLA-DR<sup>+</sup>**



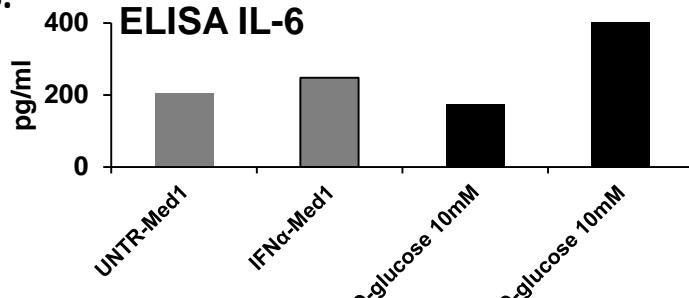
**CD40<sup>+</sup>/CD86<sup>+</sup>**



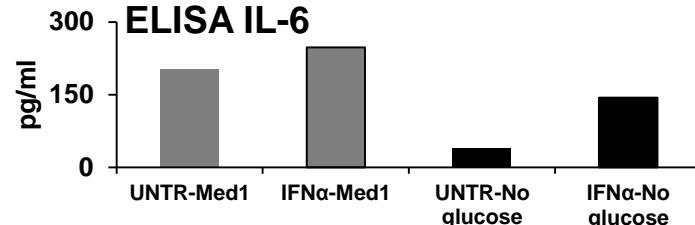
**CD40<sup>+</sup>/HLA-DR<sup>+</sup>**



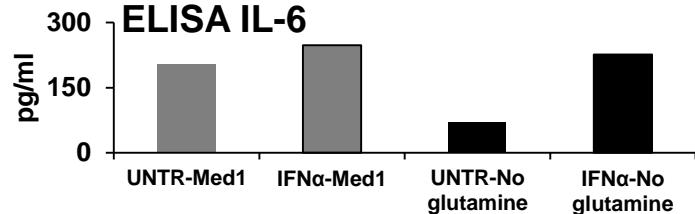
**B.**



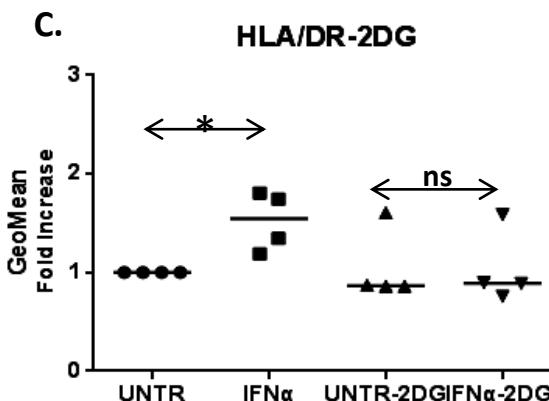
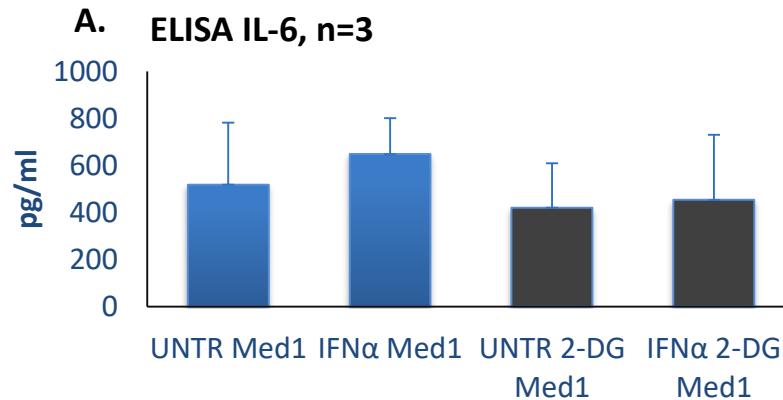
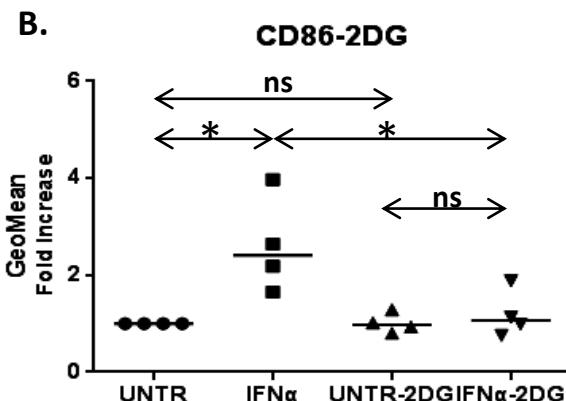
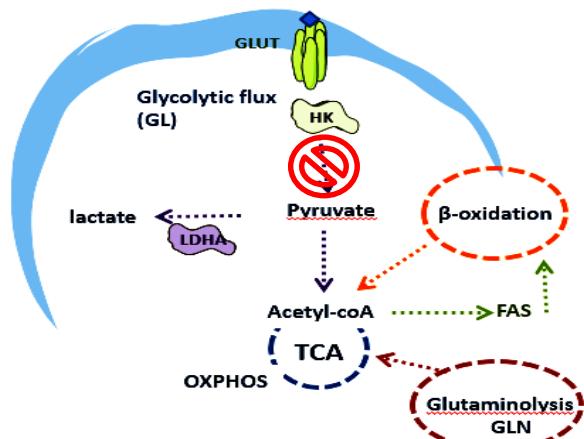
**ELISA IL-6**



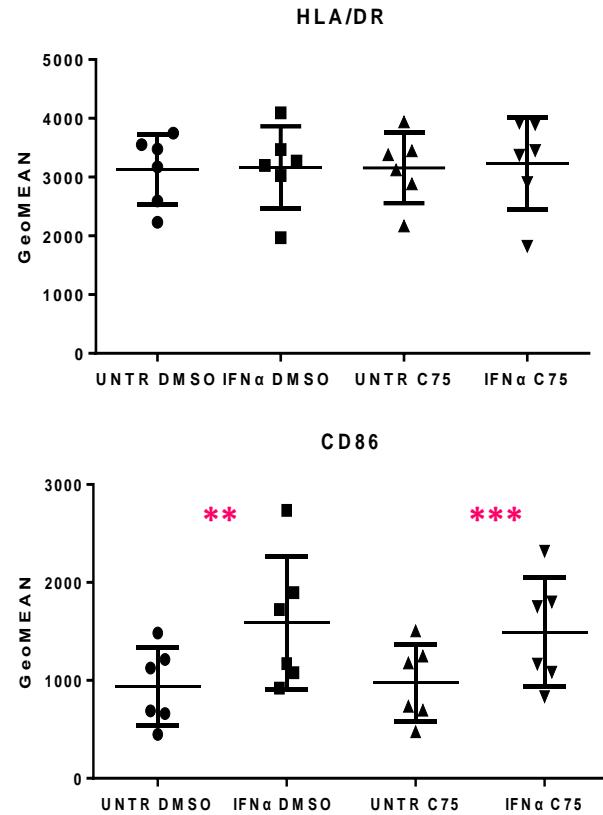
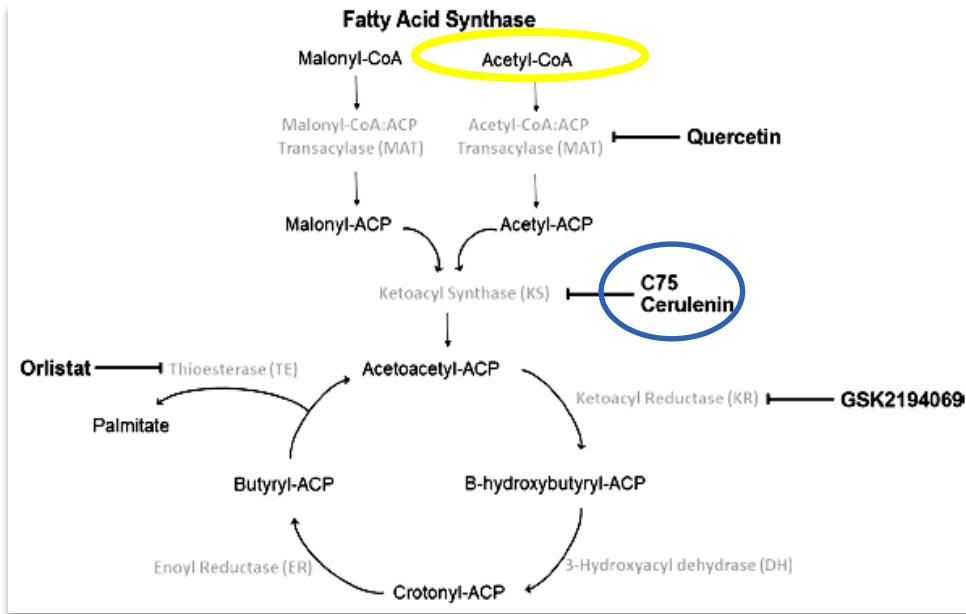
**ELISA IL-6**



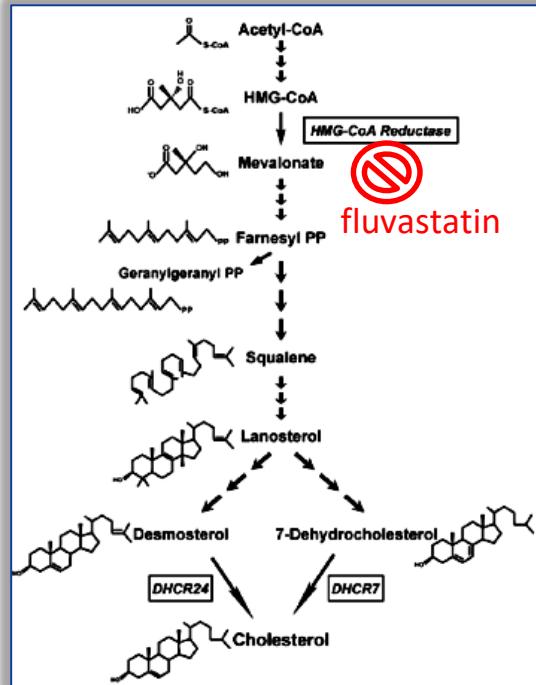
## Results: Hexokinase (HK) inhibition by 2-DG attenuates IFN capacity to activate human CD14<sup>+</sup> PBMCs



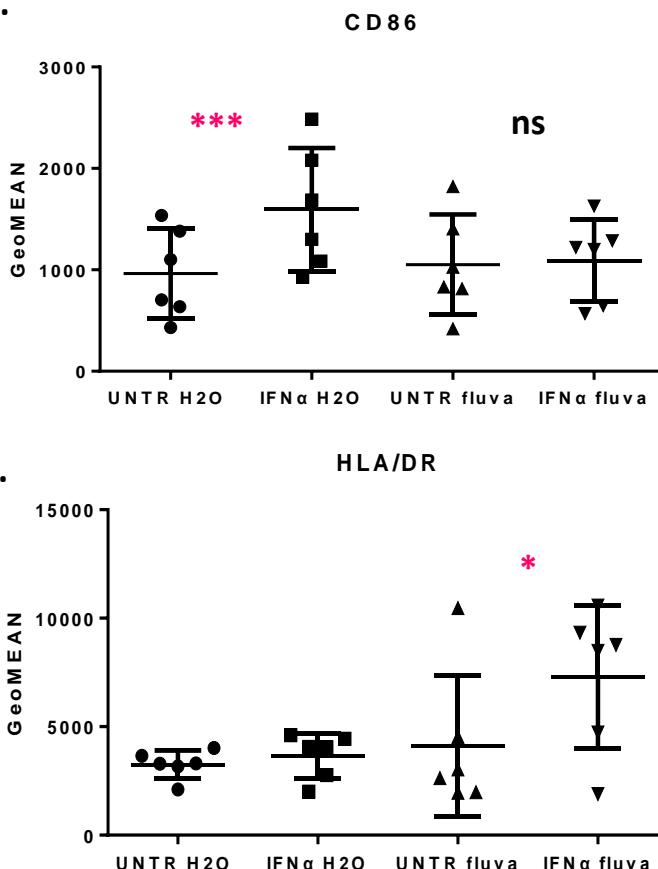
## Results: Inhibition of palmitate synthesis does not affect IFN-mediated activation and AP markers of human CD14<sup>+</sup> PBMCs



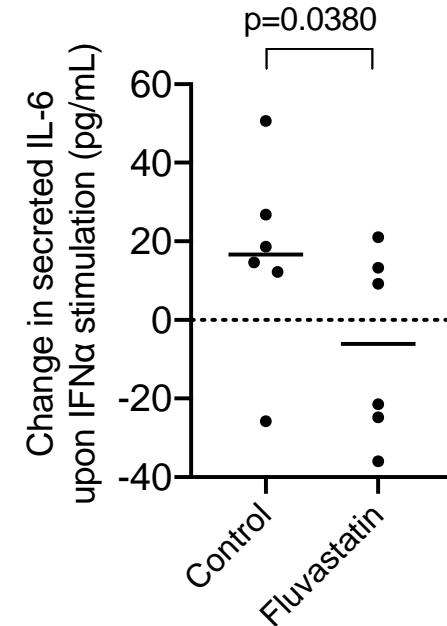
## Results: Inhibition of cholesterol synthesis reduces inflammatory markers in human CD14<sup>+</sup> PBMCs



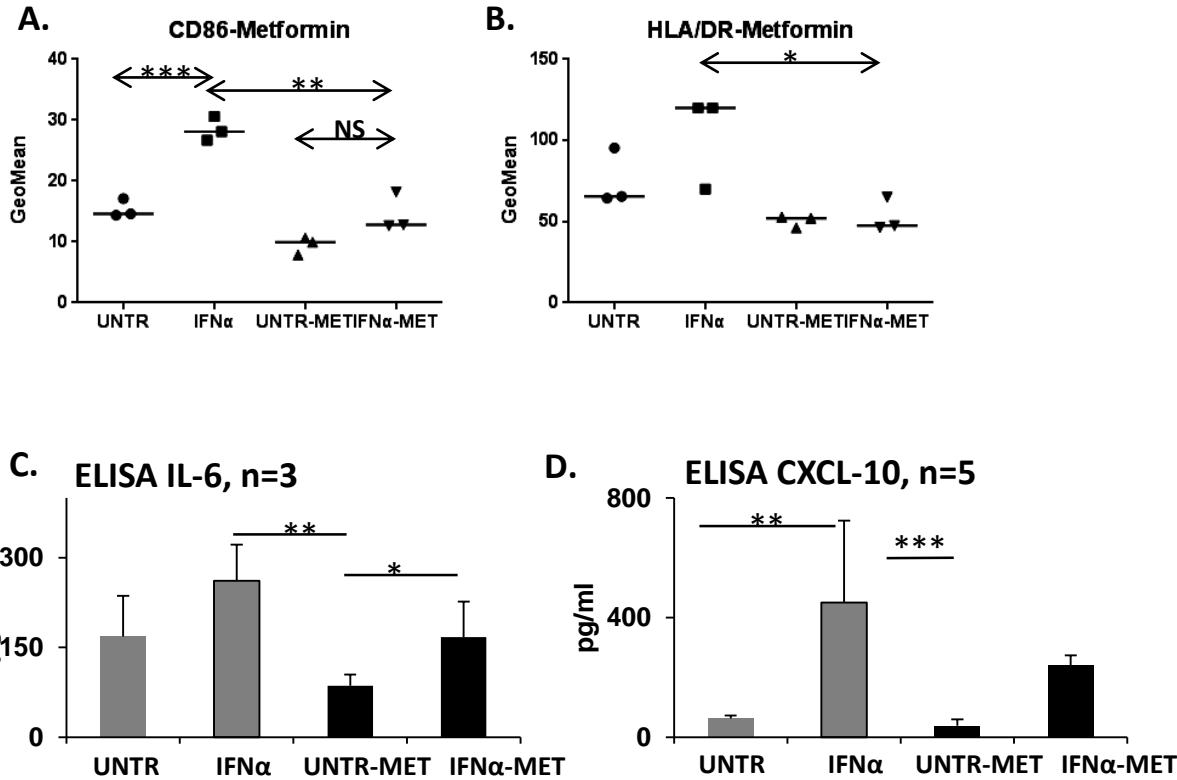
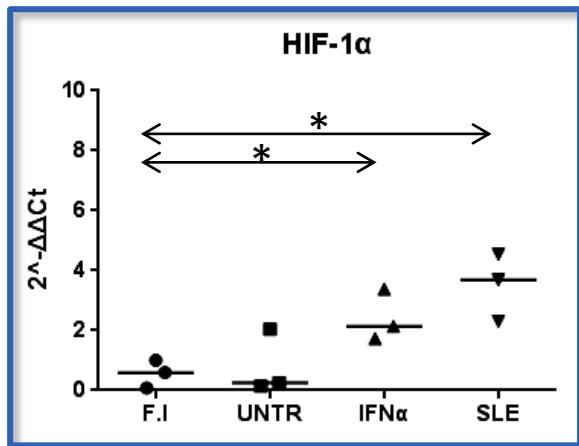
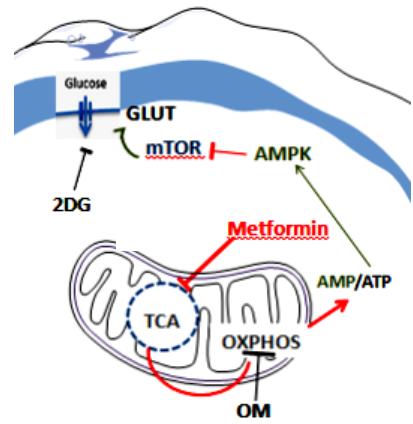
A.



C.



## Results: Inhibition of mTOR pathway activation through AMPK decreases IFN-mediated activation and cytokine production in human CD14<sup>+</sup> PBMCs

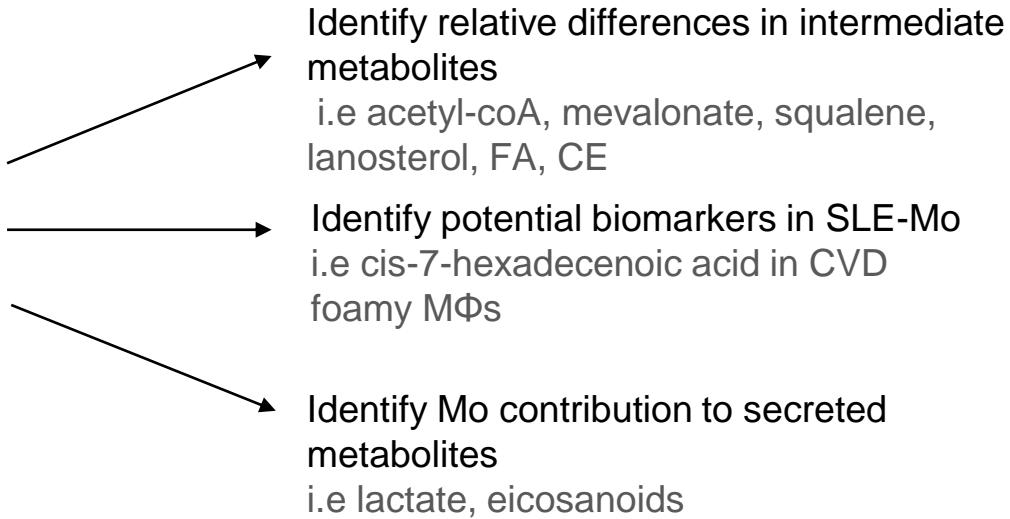


## **Sum of results**

- 1. IFN signature is significantly upregulated in SLE-Mo**
- 2. SLE and healthy IFN-Mo display altered metabolism characterized by increased glycolysis and perturbated lipid metabolism**
- 3. Blockade of upstream glycolysis (2DG) and mTOR (AMPK activation) attenuates inflammation in IFN-activated Mo**
- 4. Inhibition of cholesterol synthesis reduces inflammatory markers in IFN-Mo more effectively compared to FAS inhibition**

## Ongoing experiments: GC-MS in SLE and IFN-Mo CD14<sup>+</sup> PBMCs

- **SLE-Mo vs H-Mo freshly isolated**
- **H-Mo UNTR vs H-Mo IFN $\alpha$  ± 2DG / +SUPs**
- **H-Mo UNTR vs H-Mo IFN $\alpha$  ± Fluvastatin / +SUPs**



## Why is cholesterol metabolism important in immunity?

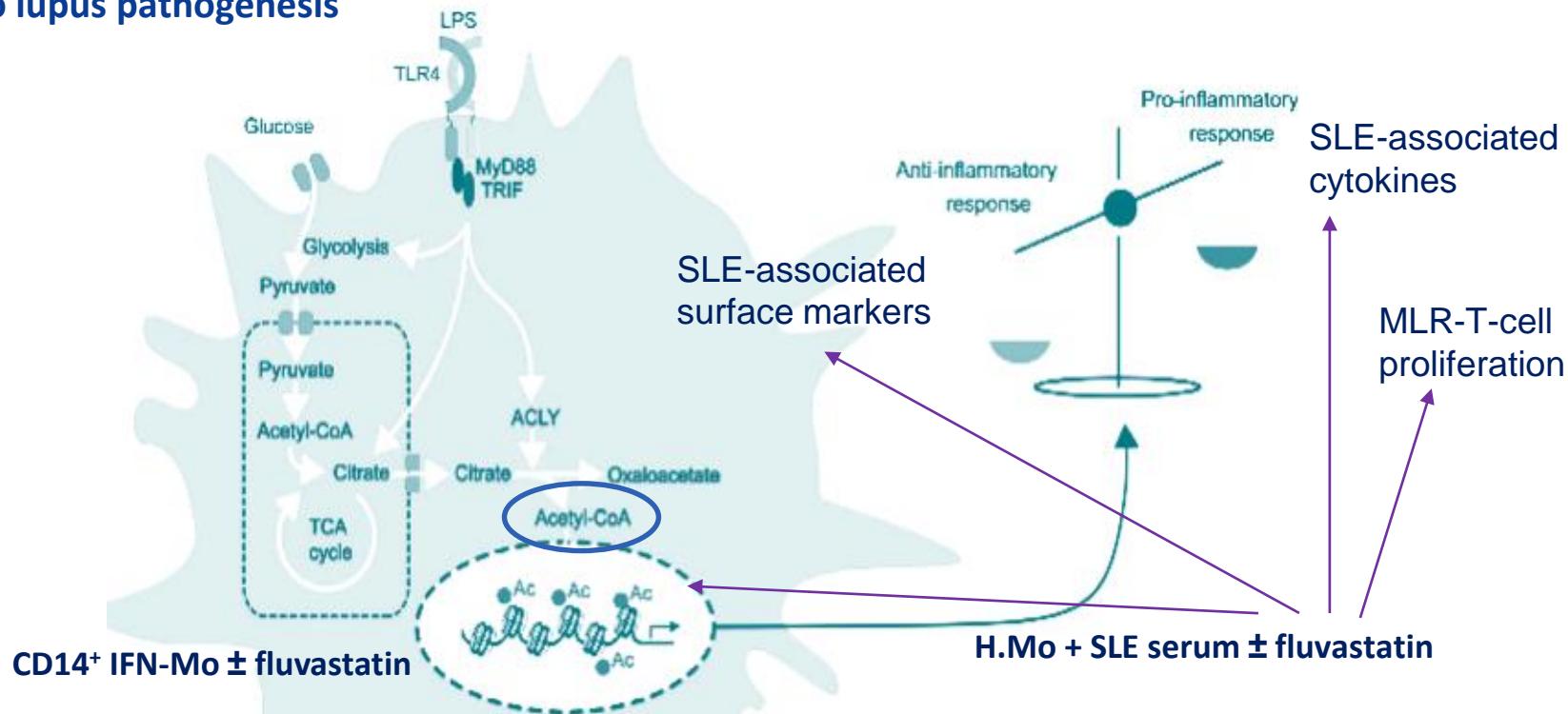
- ❖ Integral component of cell membrane and organelle lipid layers
- ❖ Membrane fluidity and binding of receptors associated with cell signaling
- ❖ Deregulation alters TCR and BCR responses
- ❖ Inhibition blocks T cell proliferation and differentiation
- ❖ Targeting cholesterol esterification potentiates CD8<sup>+</sup> T cell antitumor function
- ❖ Deregulation in MΦs induces AIM2-mediated inflammasome activation
- ❖ Reduction in organelle membranes of MΦs spontaneously engages cGAS-STING activation irrespectively of IFNs

doi: 10.12688/f1000research.15500.1

doi: 10.3389/fimmu.2017.01664

doi: 10.1016/j.cell.2015.11.045

## Ongoing experiments: ChIP-seq in CD14<sup>+</sup> IFN-Mo ± fluvastatin & functional link between IFN-Mo derived data to lupus pathogenesis



- If / how extended are the epigenetic changes?
- What are the GOs of affected genes?
- Do they include cytokine, metabolism genes?

**Laboratory of Rheumatology, Autoimmunity & Inflammation Medical School , Heraklion, Crete, Greece**

**Lab Members**

**Sidiropoulos Prodromos, Professor of Rheumatology**

**Bertsias George, Associate Professor of Rheumatology**

**Papadaki Garyfallia, PhD**

**Georgakis Spiros, PhD**

**Goutakoli Panagiota, MSc**

**Neofotistou Elpida, MSc**

**Nikoleri Dimitra, MSc**

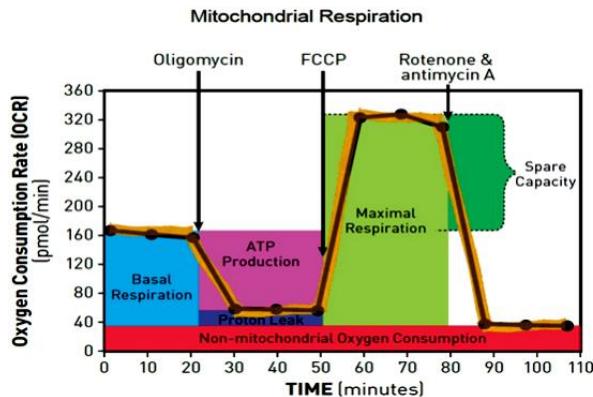
**Kosmara Despoina, MSc**

**Thank you !!!**

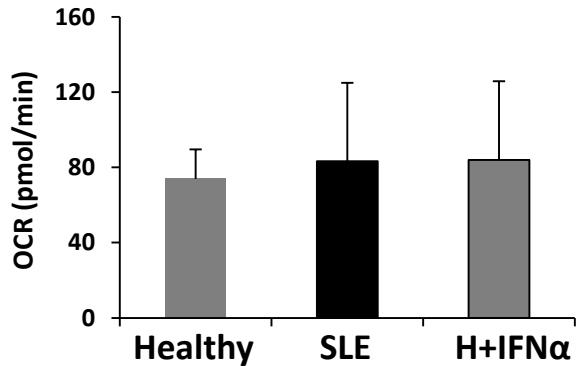


# Results: Seahorse OCR. Basal respiration is not affected by IFN-treatment

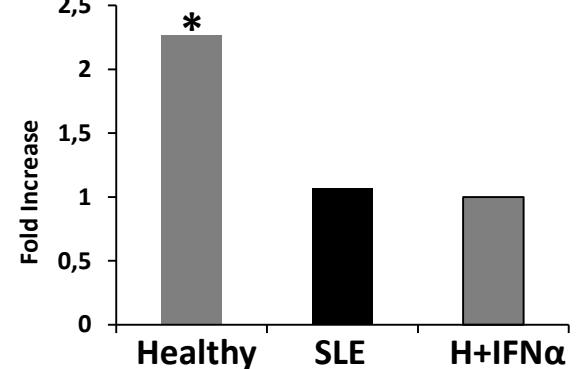
## Seahorse XF Cell Mito Stress Test Profile



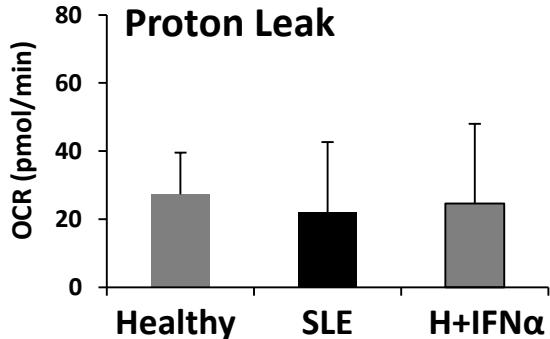
## Basal Respiration



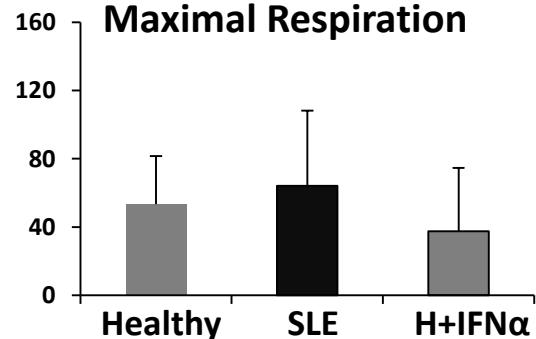
## Basal respiration/Glycolysis



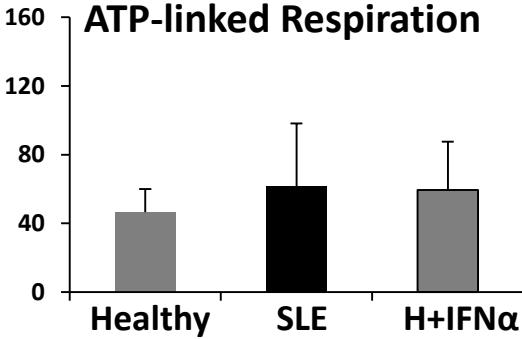
## Proton Leak



## Maximal Respiration

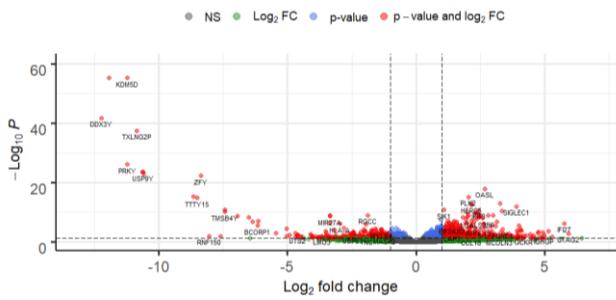


## ATP-linked Respiration



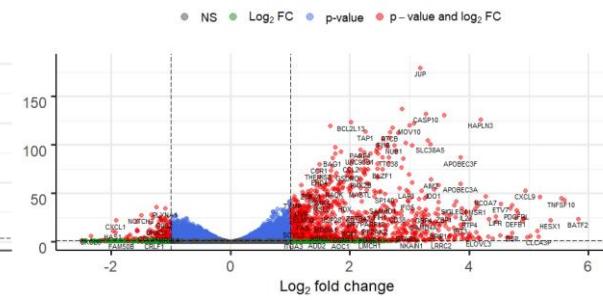
**Results: Differential gene expression analysis yielded an increased number of DEGs in all depicted comparisons**

# Patient vs Healthy



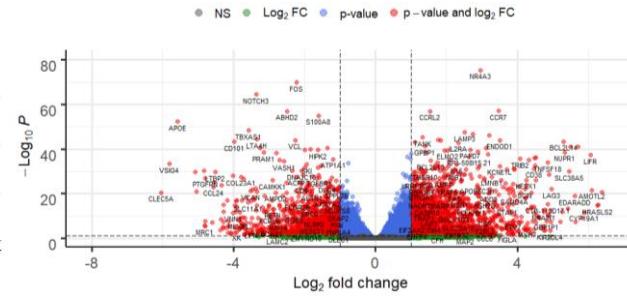
UP	1029
DOWN	625
Total	20824
p<0.05	

## H<sub>2</sub>IFNa vs H<sub>2</sub>UNTR 4h



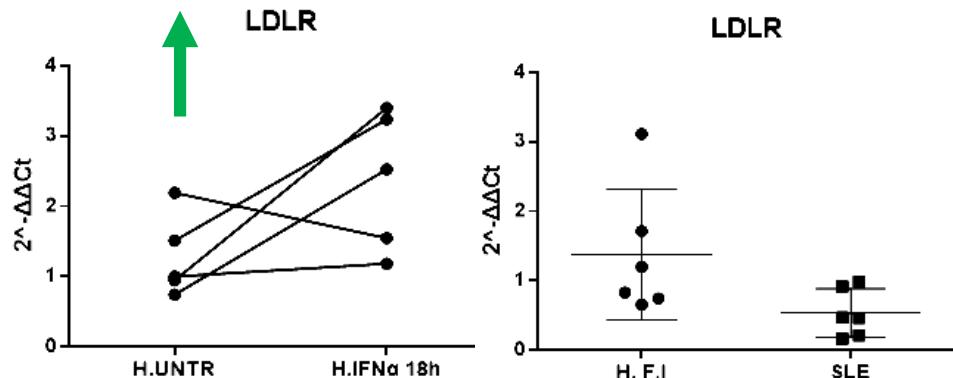
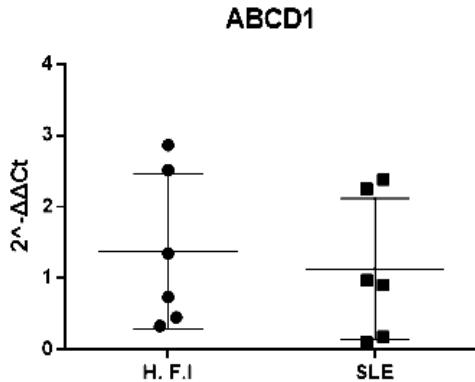
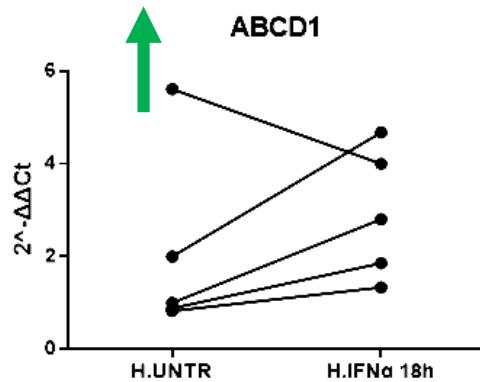
UP	3405
DOWN	3335
Total	23675
p<0.05	

## H\_IFNa vs H\_UNTR 18h

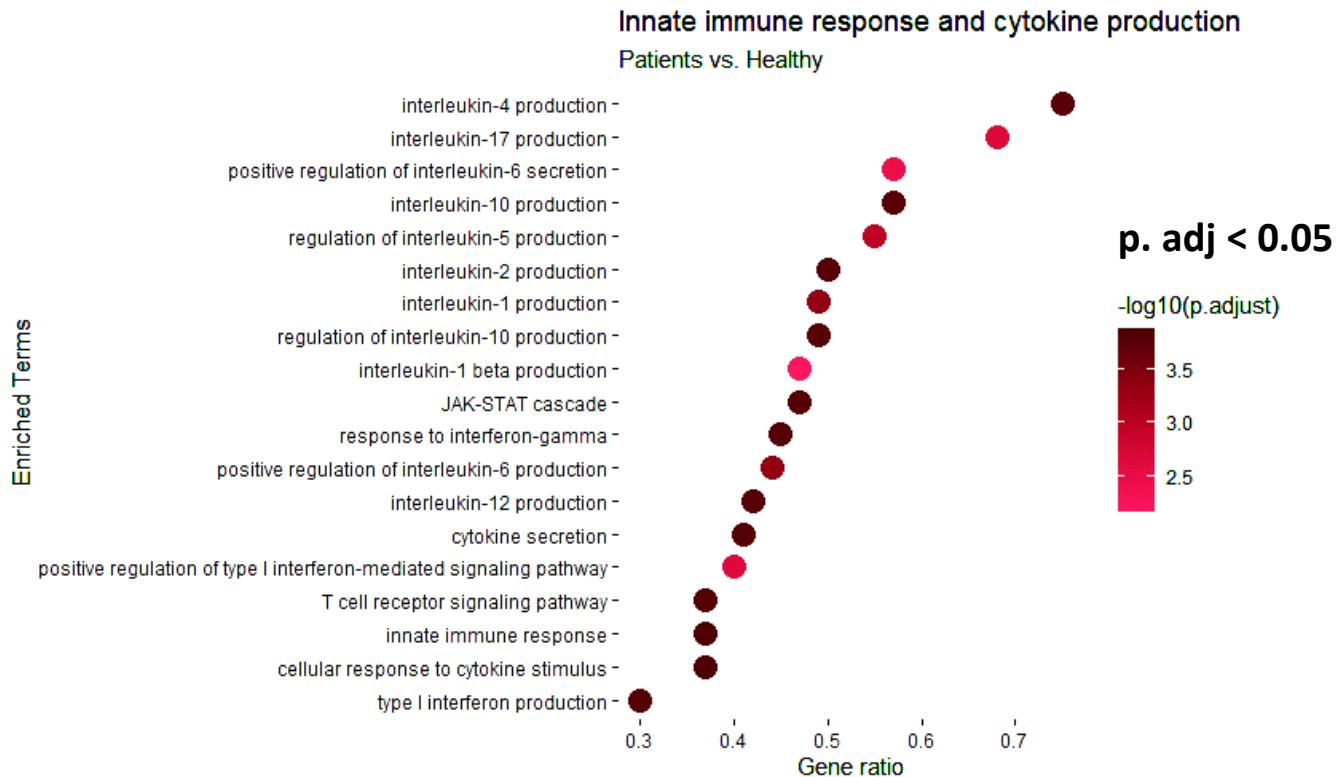


UP	4147
DOWN	4133
Total	20029
p<0.05	

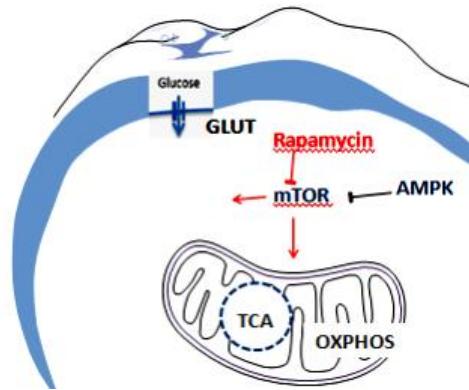
## Results: Gene transcription trend verification between DEG analysis and qPCR in a new donor cohort



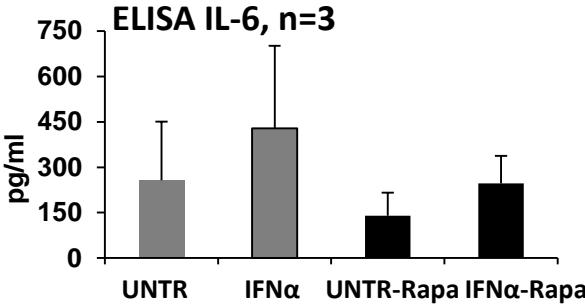
## Results: Innate immune response is among the major deregulated pathways in SLE-Mo, H-Mo (4h) & H-Mo (18h)



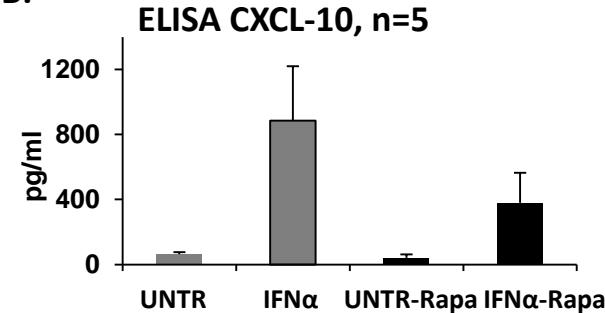
## Results: Inhibition of mTOR pathway activation reduces pro-inflammatory cytokine production in human CD14<sup>+</sup> PBMCs



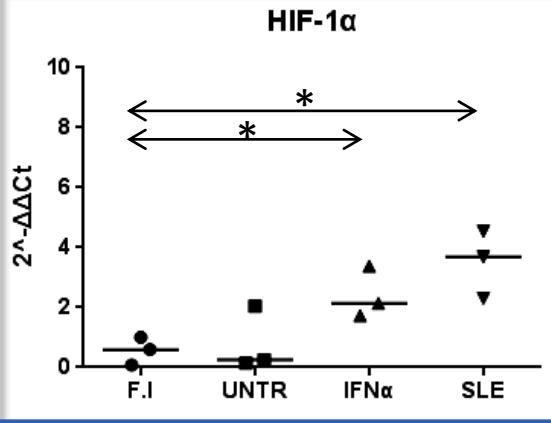
A.



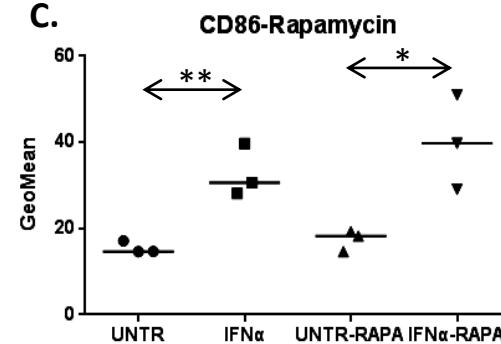
B.



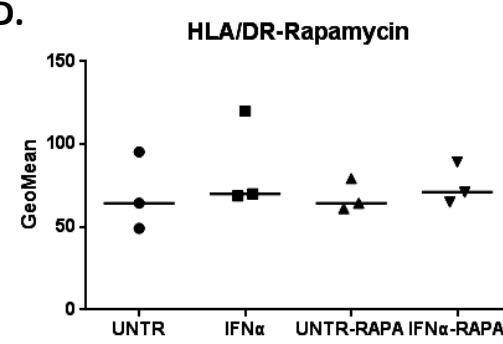
HIF-1 $\alpha$



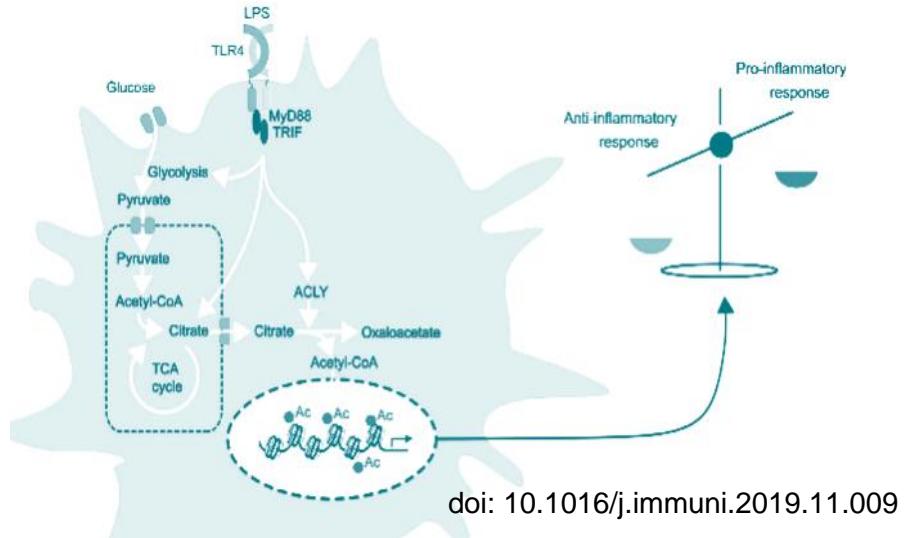
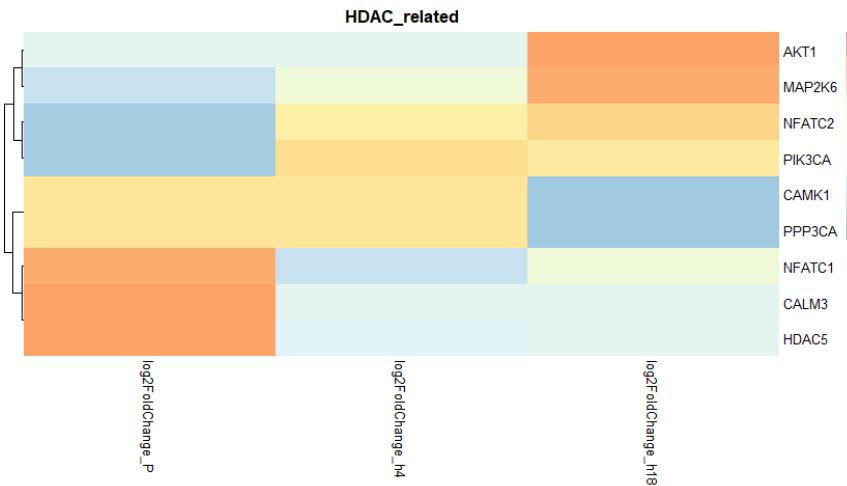
C.



D.



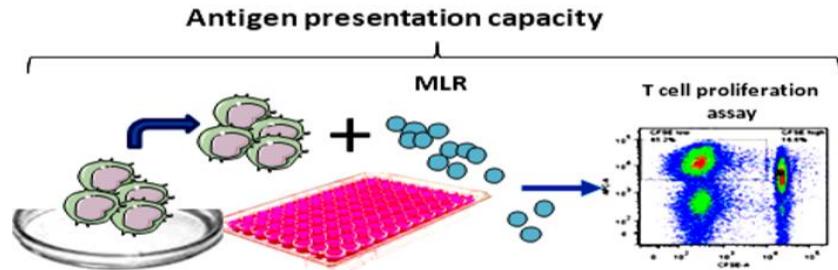
## Ongoing experiments: ChIP-seq in CD14<sup>+</sup> IFN-Mo ± fluvastatin



- If / how extended are the epigenetic changes?
- What are the GOs of affected genes?
- Do they include cytokine, metabolism genes?

## Future experiments: Functional link between IFN-Mo derived data to lupus pathogenesis

MLR: use H.Mo με SLE serum ± fluvastatin and measure T cell proliferation



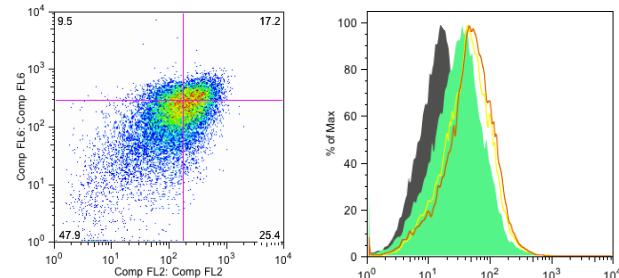
Other functional assays : use H.Mo με SLE serum ± fluvastatin and measure:

- 1) cytokines found upregulated in SLE RNA-seq
- 2) Surface markers upregulated in SLE RNA-seq CD40, siglec1
- 3) Gene expression affected by epigenetic alterations due to fluvastatin treatment

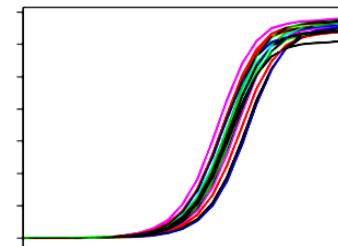
### 1. ELISA



### 2. Flow cytometry

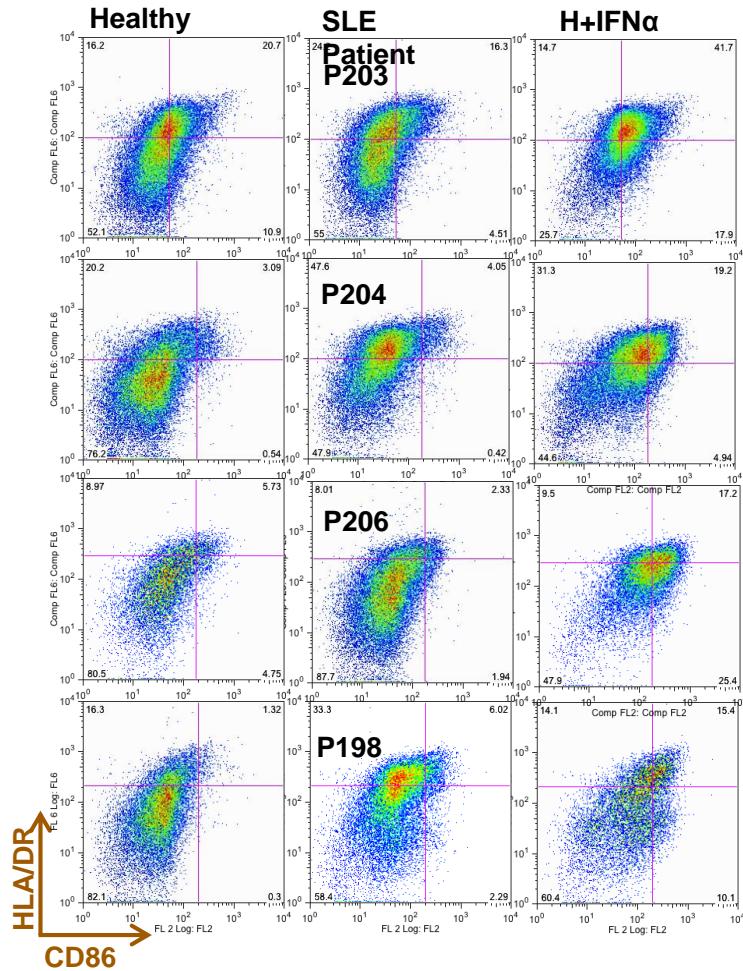


### 3. qPCR

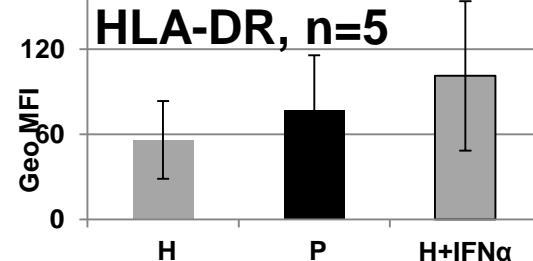
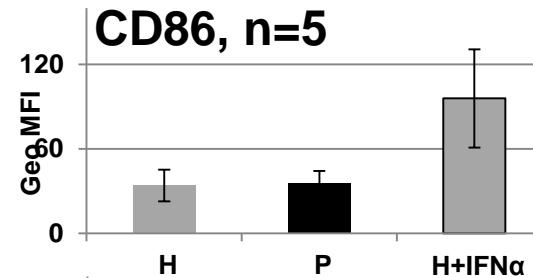


Supplementary Figs.

# Seahorse Patient Profile

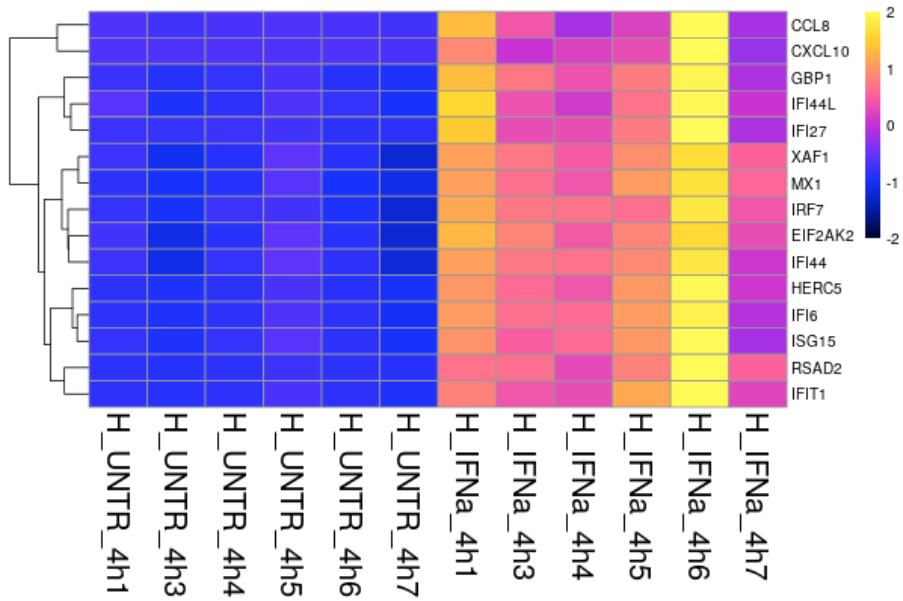


SLE Patient	Disease state
P202	Remission
P203	Moderate active, ds(+)
P204	Moderate active, ds(-), obese
P205	Moderate active
P206	Moderate active, obese
P198	Remission, ds(+)

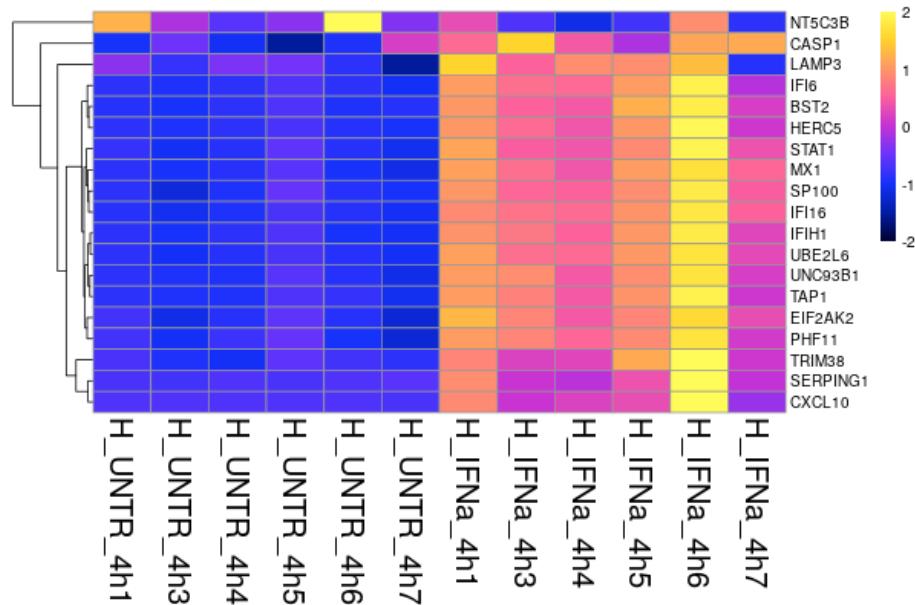


## 4h sample set - IFN signature scoring

Signature 1 - El-Sherbiny et al - Scientific Reports - 2018

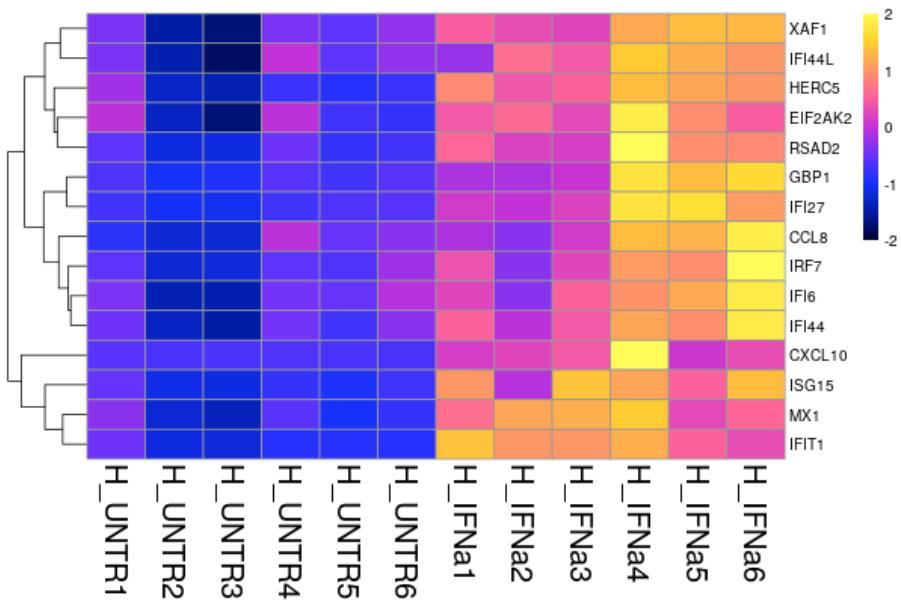


Signature 2 - El-Sherbiny et al - Scientific Reports - 2018

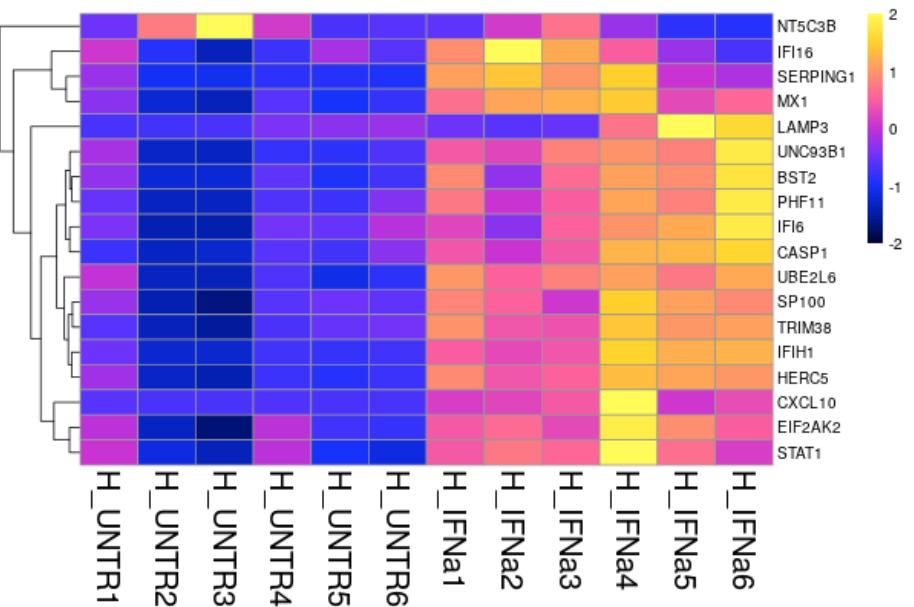


## 18h sample set - IFN signature scoring

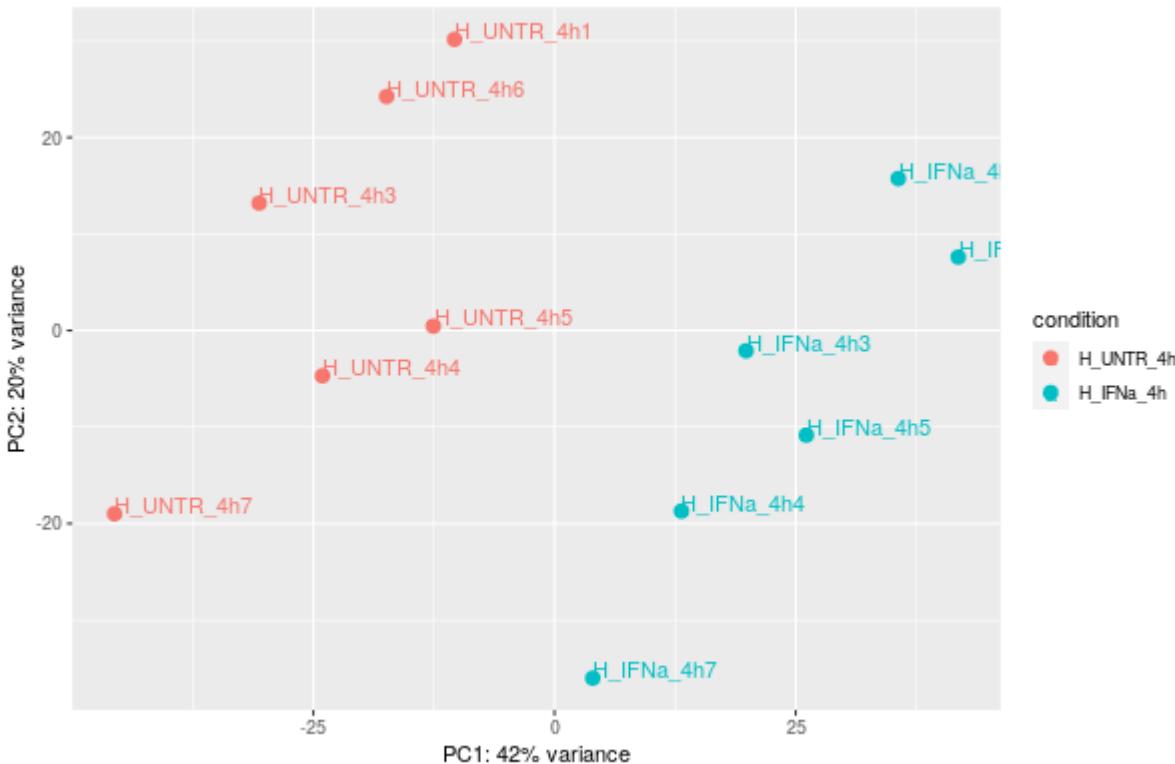
Signature 1 - El-Sherbiny et al - Scientific Reports - 2018



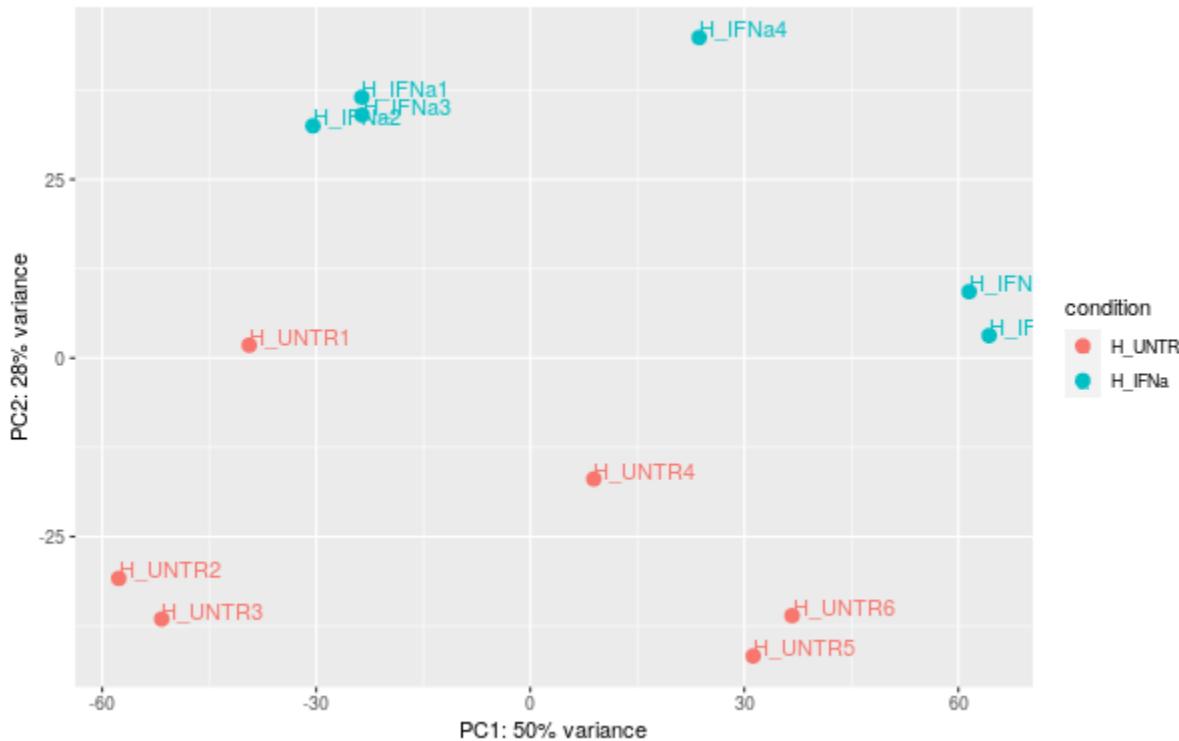
Signature 2 - El-Sherbiny et al - Scientific Reports - 2018



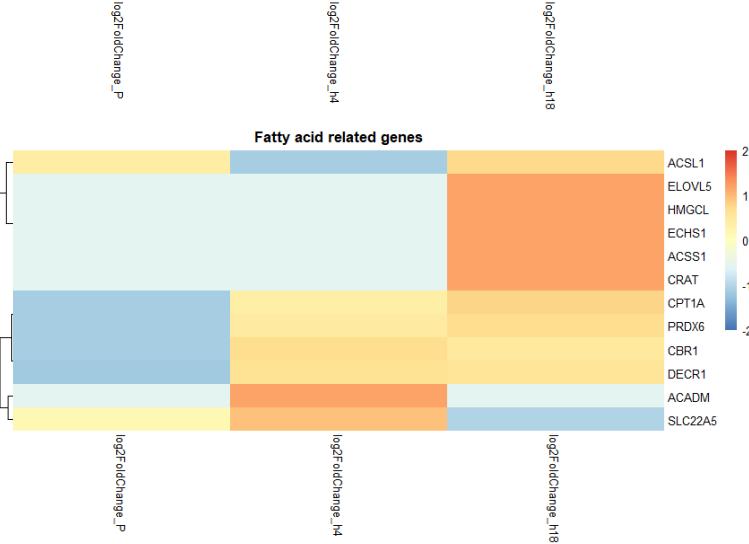
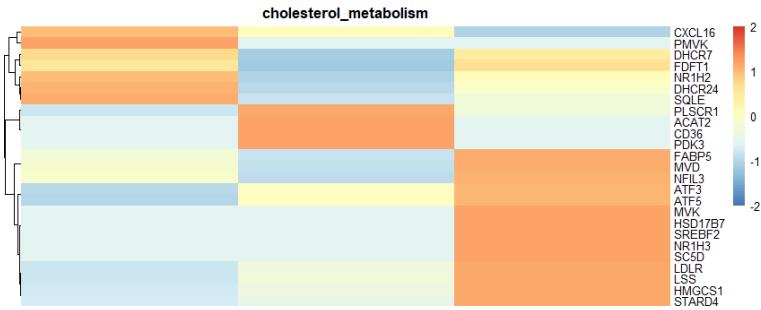
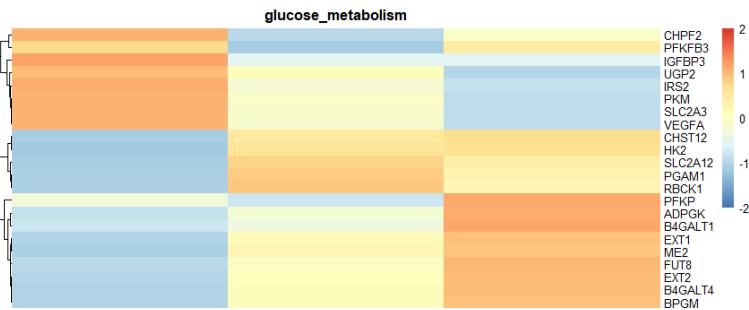
## 4h sample set - PCA



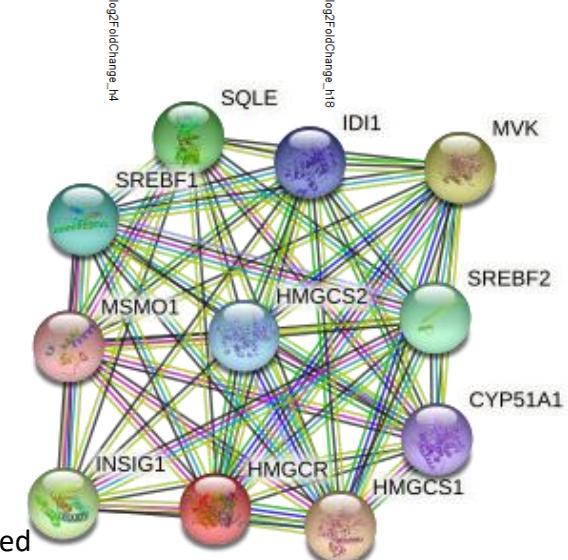
## 18h sample set - PCA



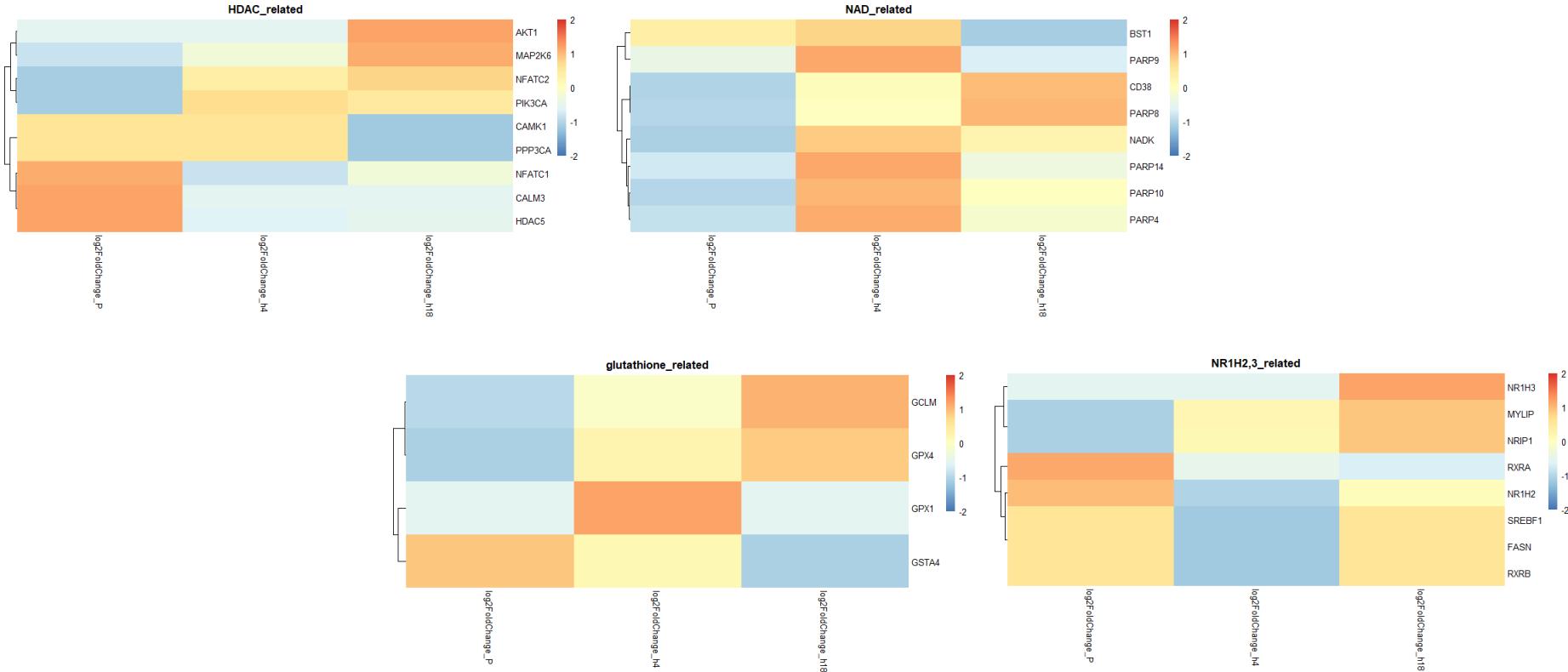
## Results: Genes belonging to the same metabolic pathways may differ in compared data sets



String.database derived

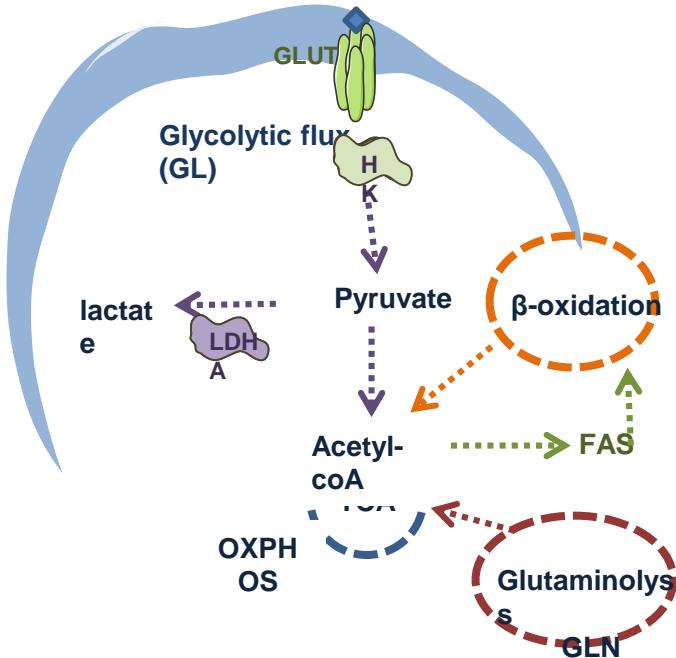


# Other related pathways shared by SLE-Mo, H-Mo(4h, 18h IFN)

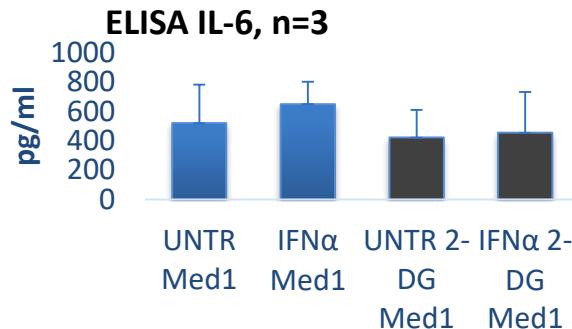
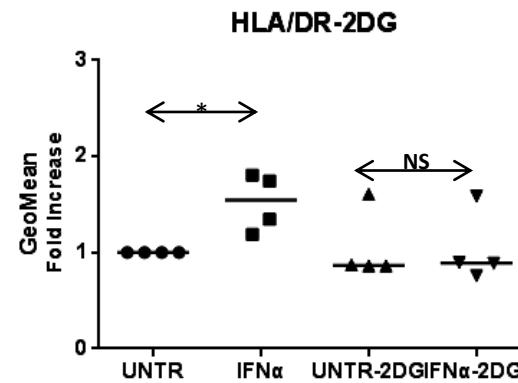
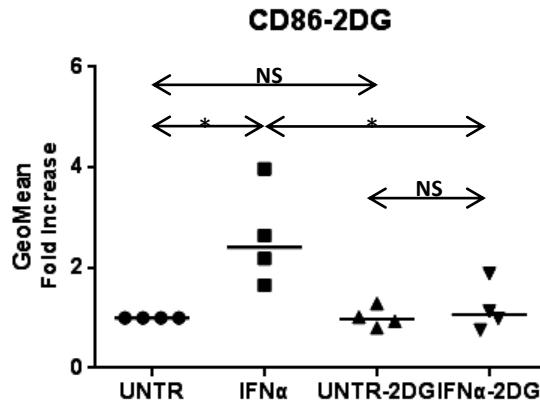


## Results synopsis WP3: IFN $\alpha$ regulates monocyte activation and cytokine secretion by altering metabolic pathways

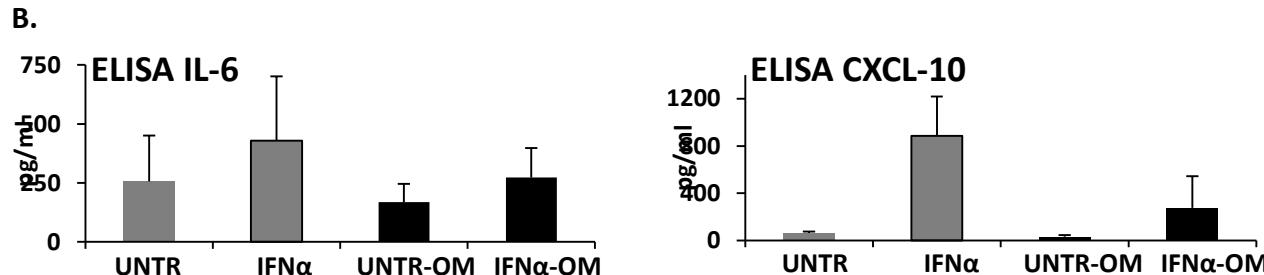
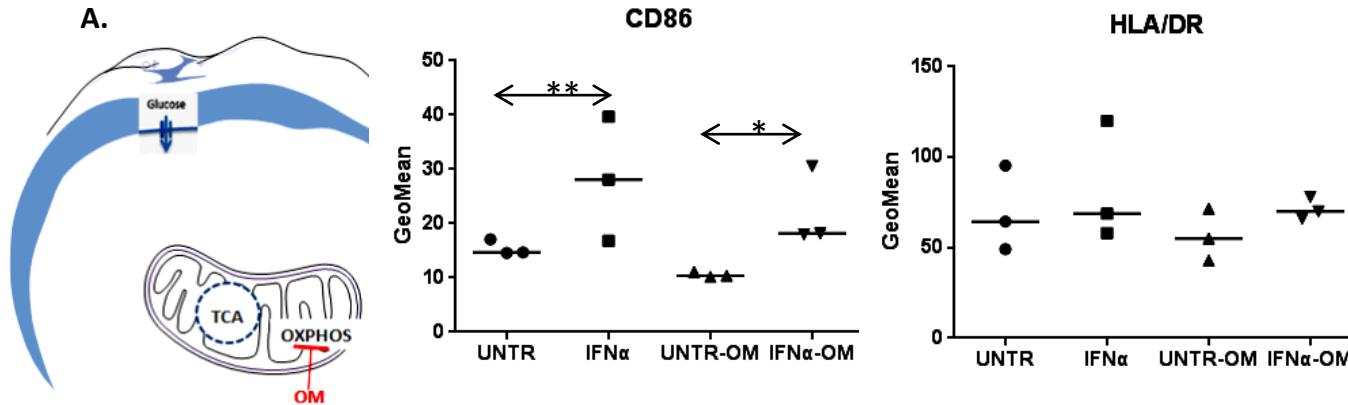
		+IFN $\alpha$			
Metabolic Screening		FACS		ELISA	
Modulator	Target	CD86	HLA-DR	IP-10	IL6
2-DG	GL	Down	Down	Not Tested	Down
Rapa	mTOR/G L	Down	No effect	Down	Down
Metf	OXPHOS	Down	Down	Down	Down
OM	OXPHOS	Down	No effect	Down	Down
UK5099	GL/OXP H	No effect	No effect	Not tested	No effect
Etomoxir	FAO	No effect	No effect	Not Tested	No effect
TOFA	FAS	No effect	Down	Not tested	UP
LC	FAO	No effect	No effect	Not tested	No effect
GLN(-)		No effect	No effect	Not Tested	No effect



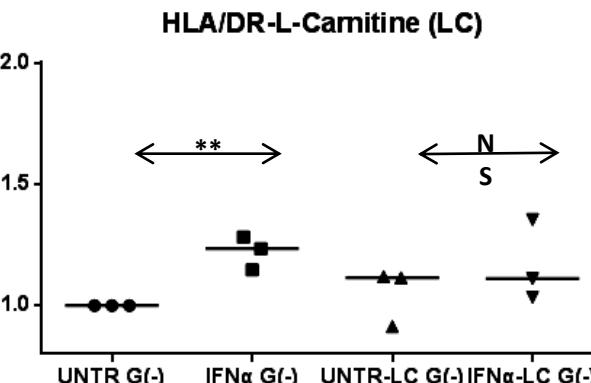
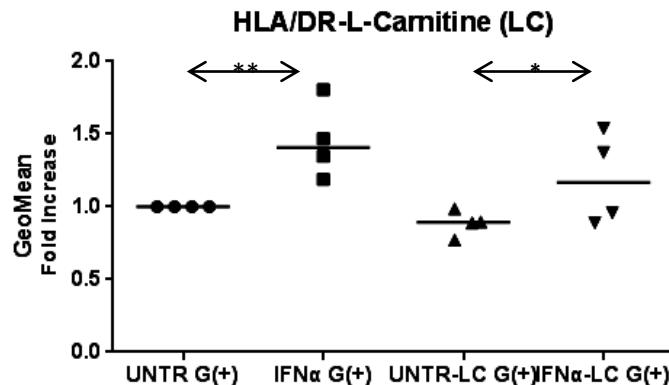
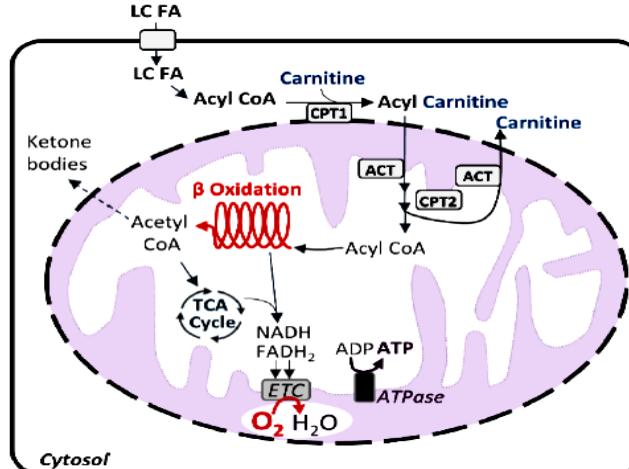
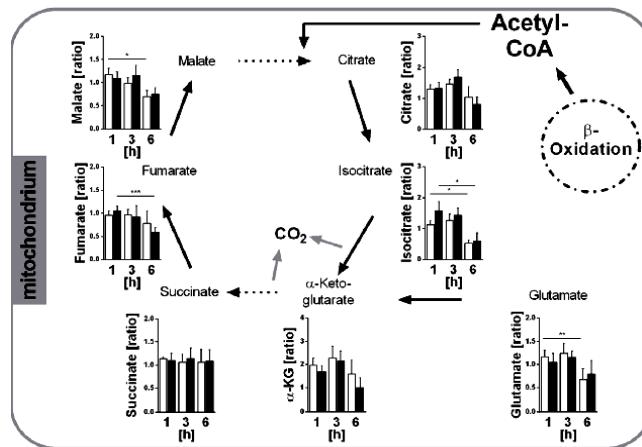
## Hexokinase (HK) inhibition by 2-DG attenuates IFN capacity to activate human CD14<sup>+</sup> PBMCs



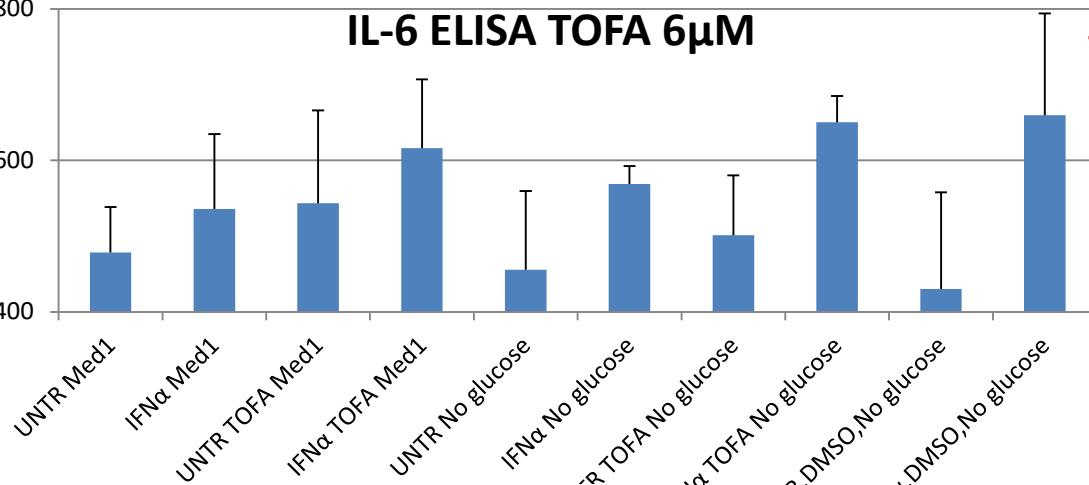
## OligoMycin A (OM-ATPase inhibitor) lowers IFN-cytokine secretion in human monocytes



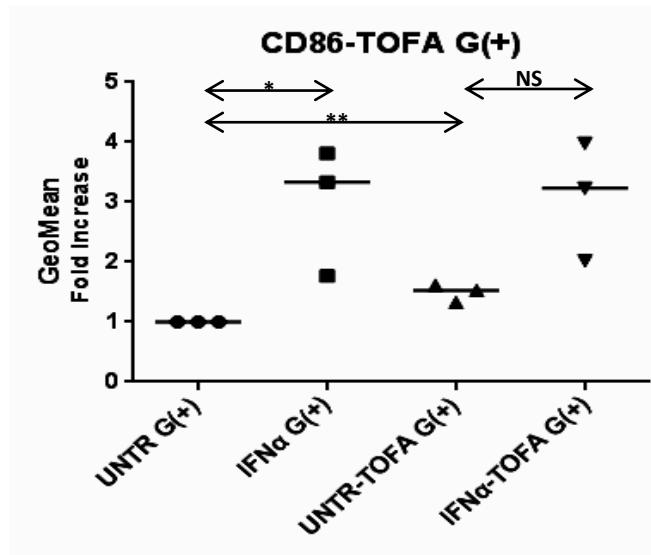
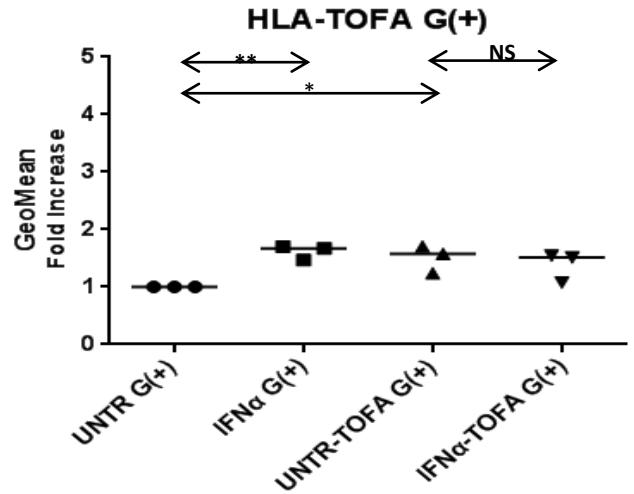
# Implications for FAO-mediated inhibition of HLA/DR production under glucose deprivation conditions



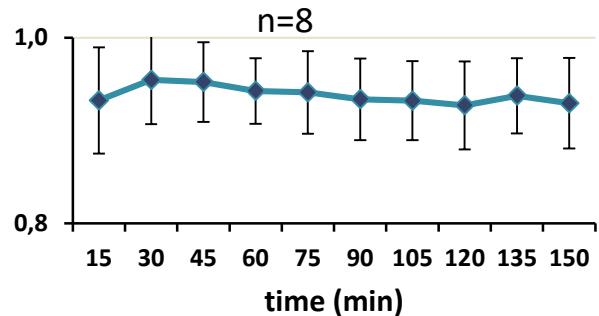
## IL-6 ELISA TOFA 6 $\mu$ M



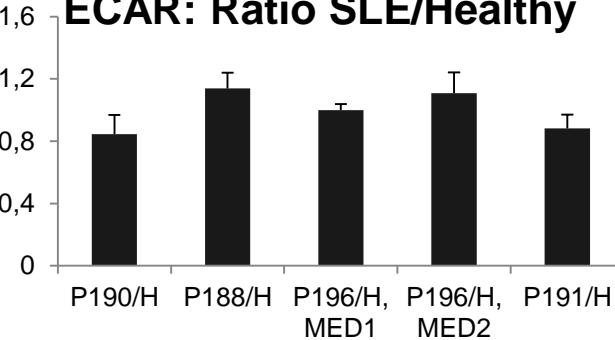
TOFA (irreversible acetyl-coA inhibitor) results:  
need repeat, possible DMSO effect



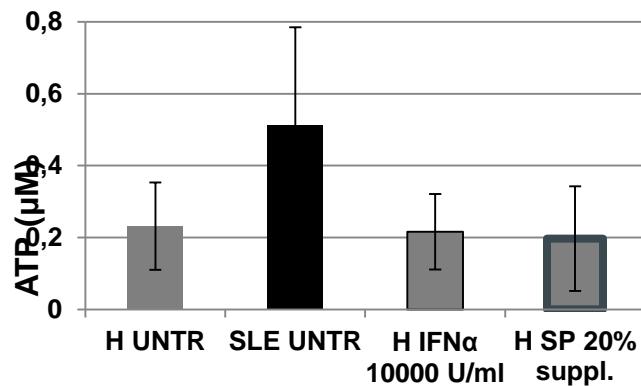
### OCR:Ratio SLE/Healthy



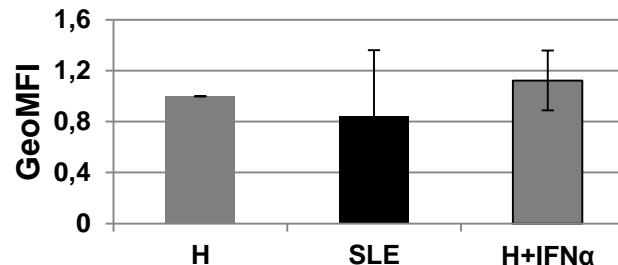
### ECAR: Ratio SLE/Healthy



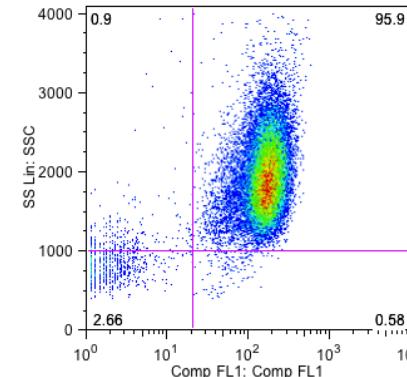
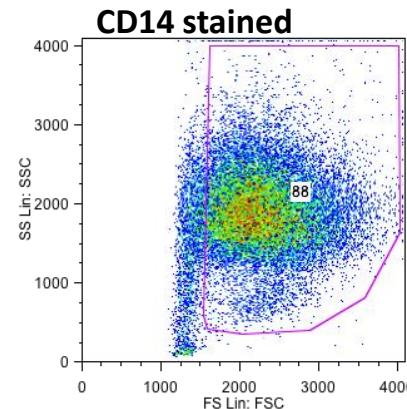
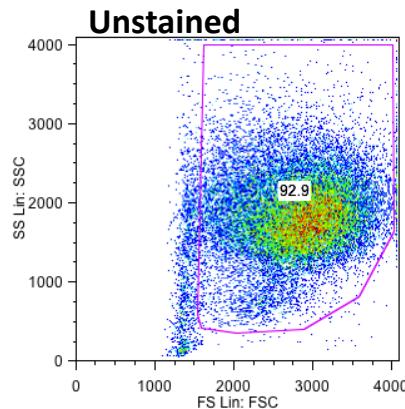
### ATP concentration, n=4, P186-P189



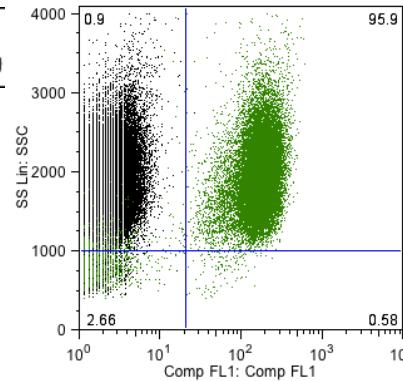
### Total ROS SLE(n=4), H(n=5) Amplex red?



## Methods and Results: CD14<sup>+</sup> Monocyte purity directly after MACs bead isolation

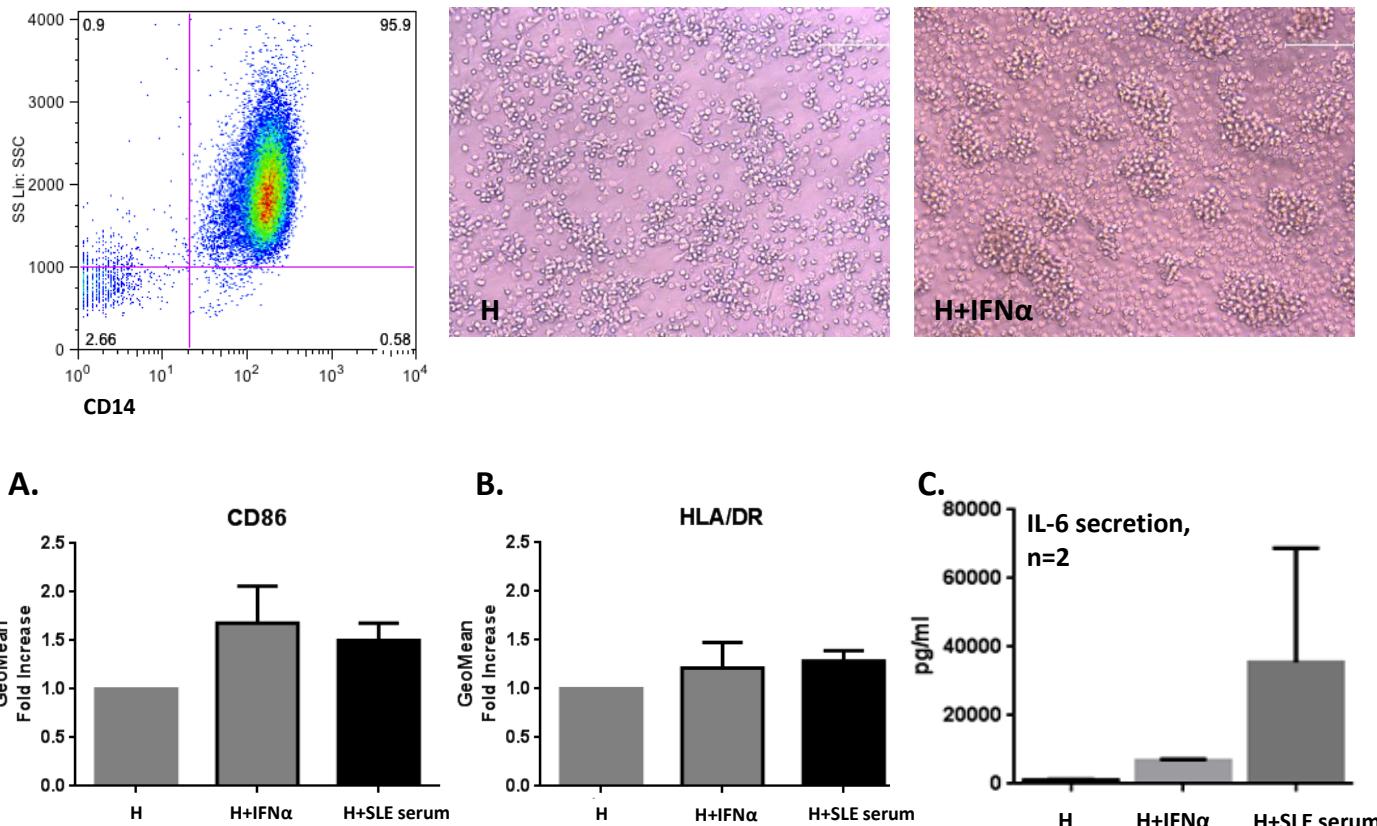


<span style="background-color: green; display: inline-block; width: 15px; height: 15px;"></span>	H1 CD14.fcs	88
<span style="background-color: black; display: inline-block; width: 15px; height: 15px;"></span>	H1 CD14 UNST.fcs	92.9

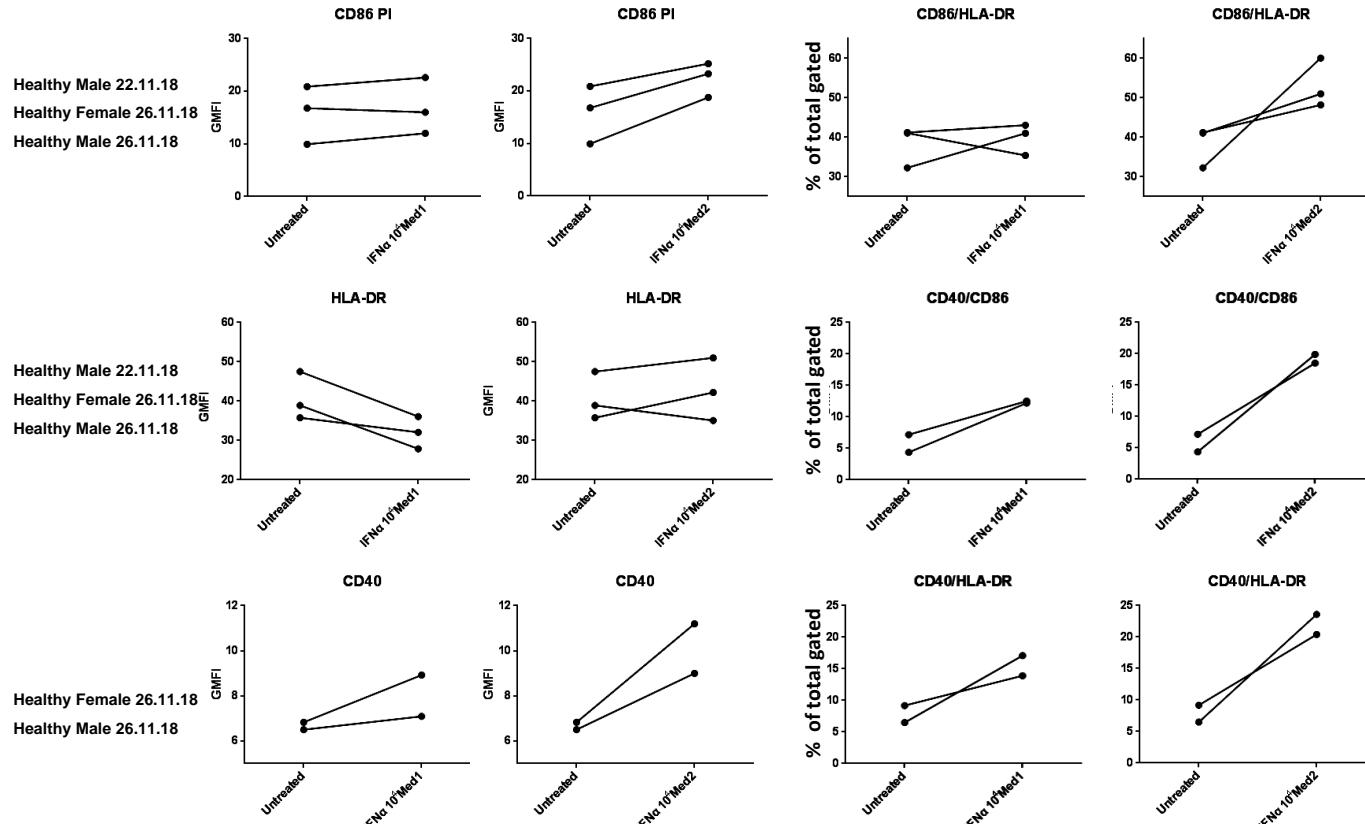


**CD14<sup>+</sup> Monocyte purity is  
96.13%  $\pm$  2.15% directly  
after bead isolation, n=3**

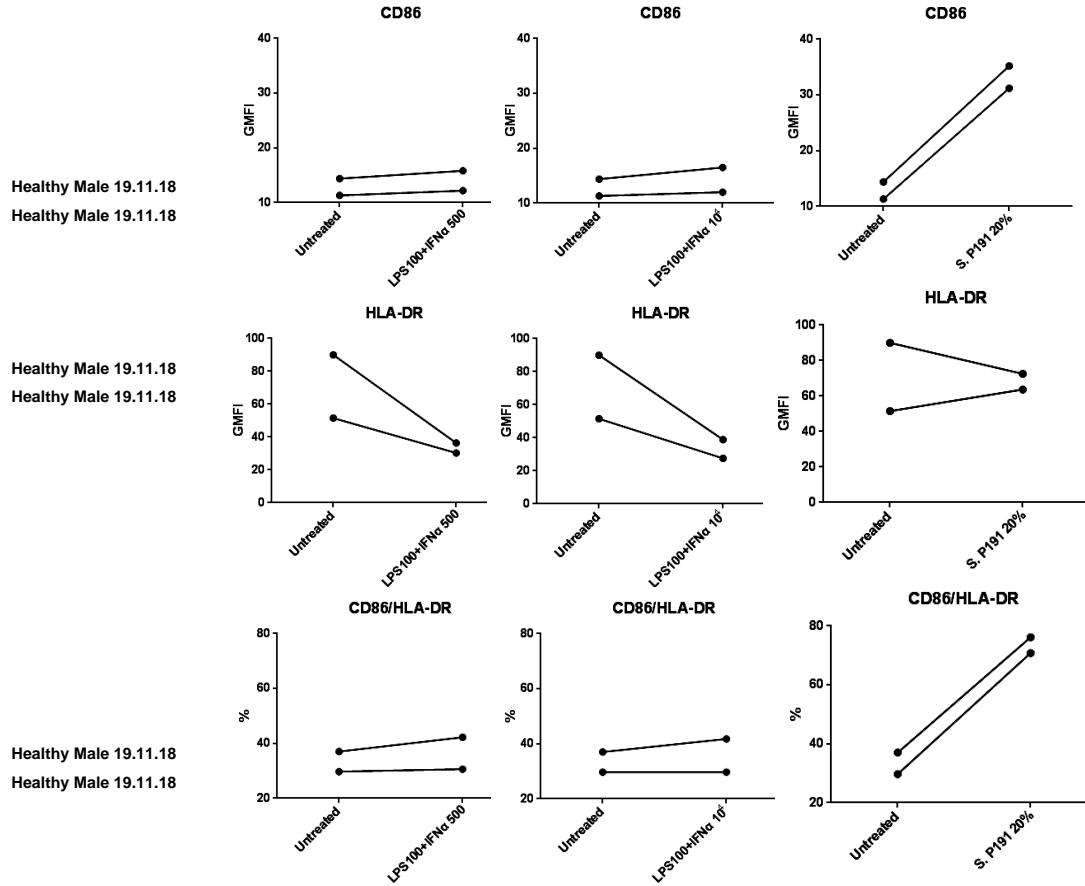
## IFN $\alpha$ effect on Human Monocytes



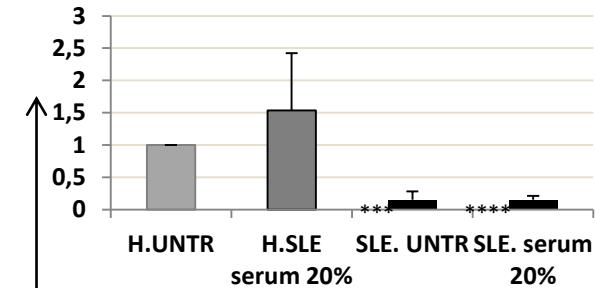
## IFN $\alpha$ effect on monocyte activation markers, with or without glucose supplementation



## Combined effect of LPS+IFN $\alpha$ or patient serum on monocyte activation markers



## Methods and Results: TNF $\alpha$ secretion is positively regulated by TLR4 signaling in cultured CD14 $^{+}$ Monocytes. IFNR signaling downregulates TNF $\alpha$ secretion



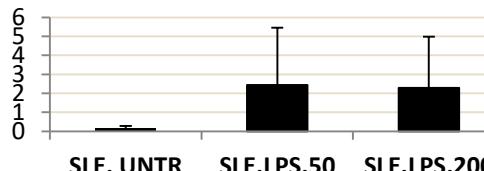
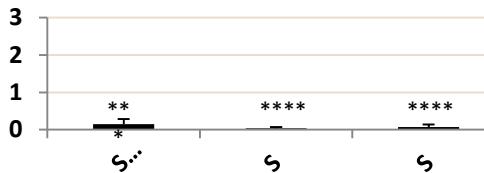
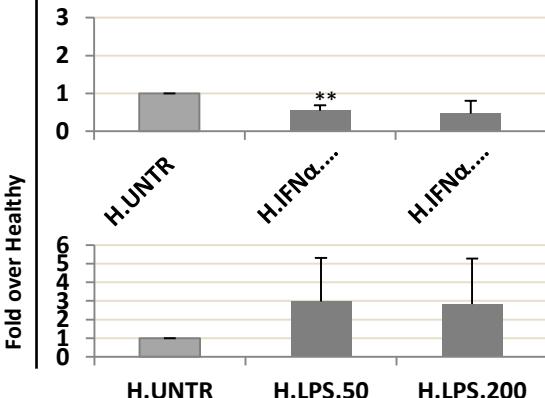
- Serum had no significant effect on TNF $\alpha$  production
- SLEs display reduced TNF $\alpha$  secretion
- IFN $\alpha$  downregulated TNF $\alpha$  secretion

N=3

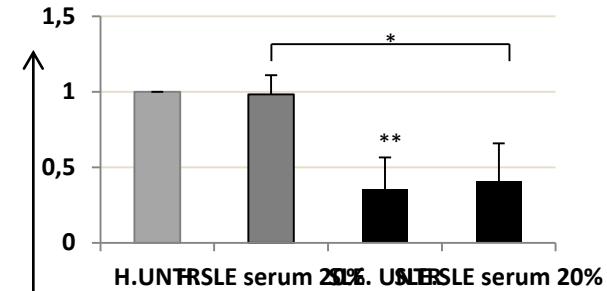
Normalized values to Healthy Untreated

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001

Under HCQ, AZA, MTX



## Methods and Results: IL-6 secretion is positively regulated by TLR4 signaling in cultured CD14<sup>+</sup> Monocytes.

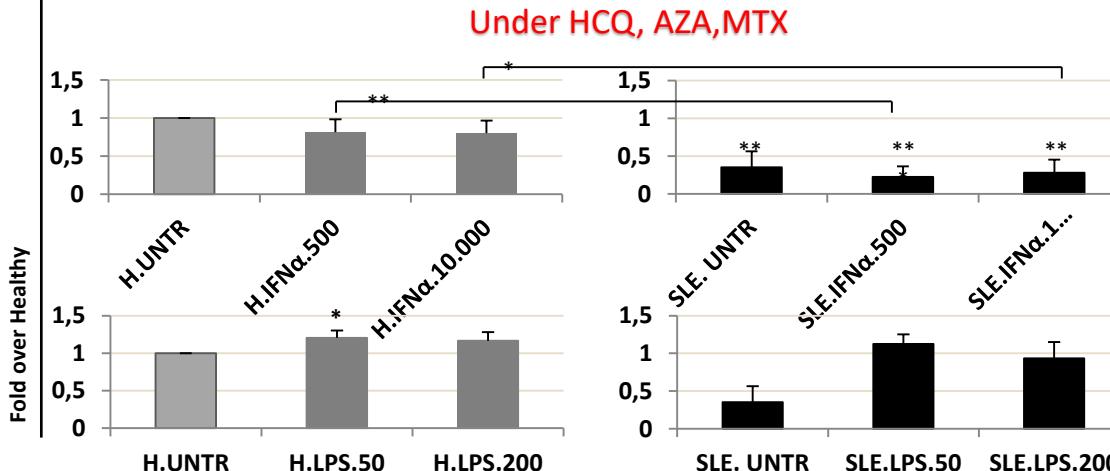


- Serum had no significant effect on IL-6 production
- SLEs display reduced IL-6 secretion compared to Healthy controls

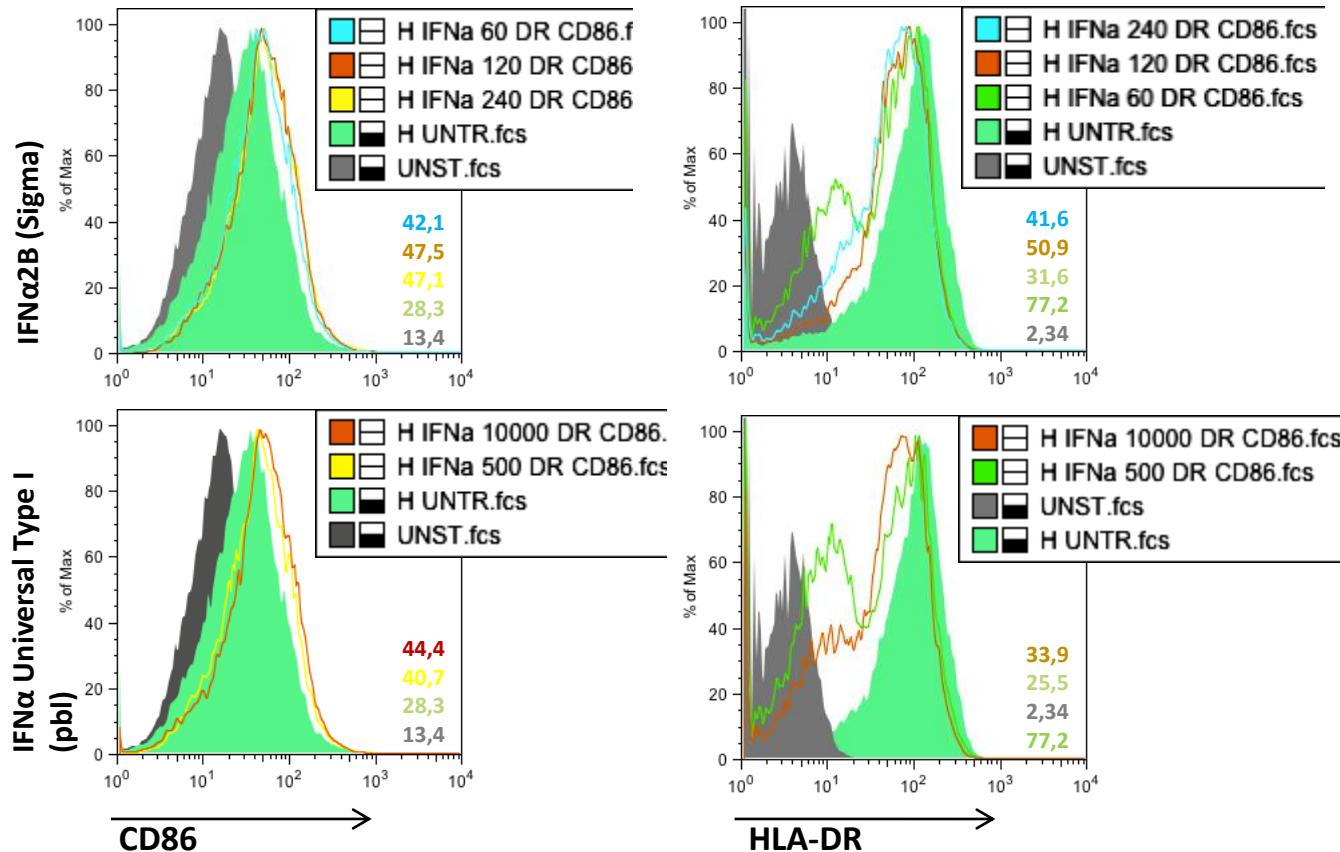
N=3

Normalized values to Healthy Untreated

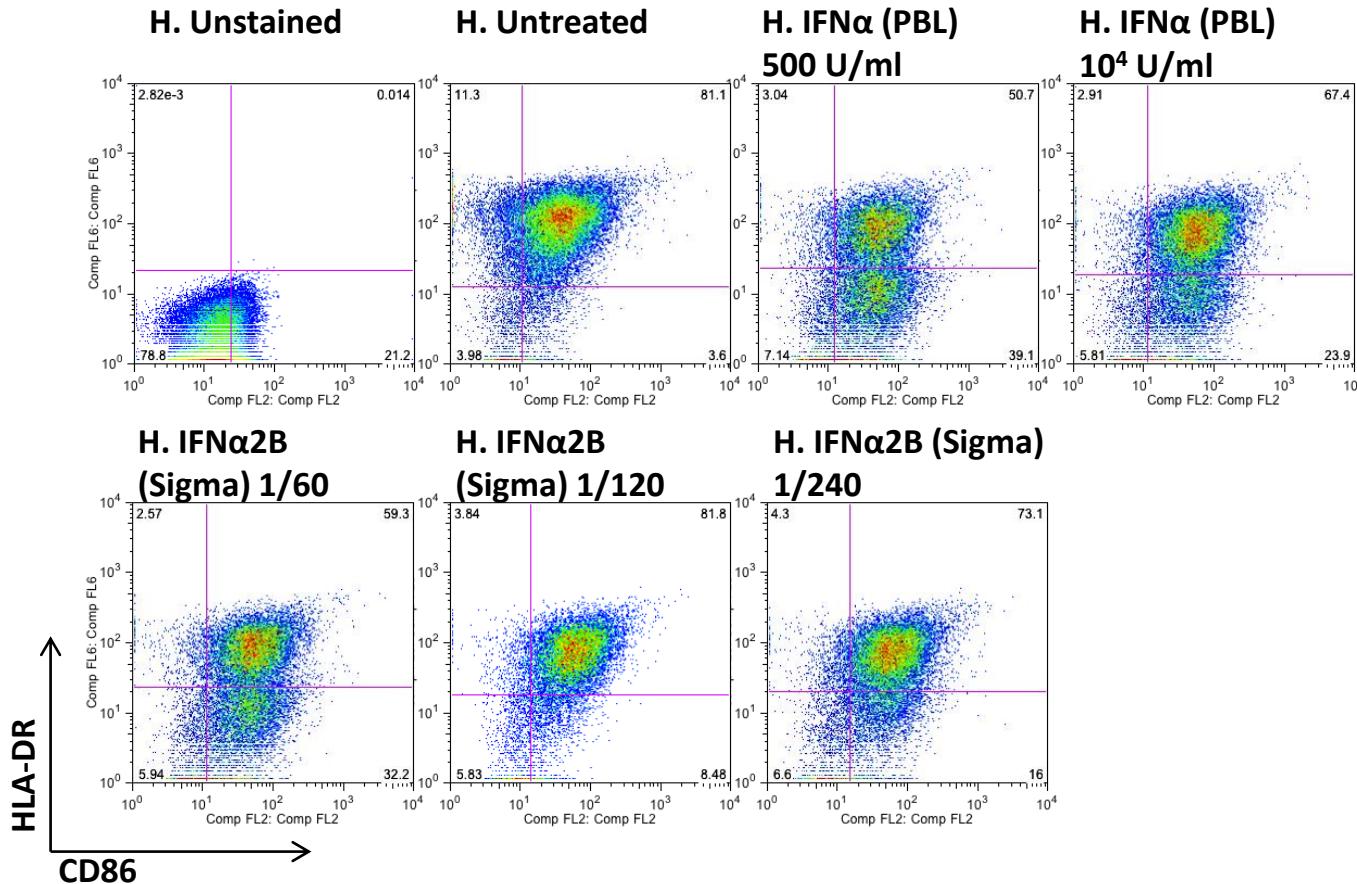
\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001



## Methods and Results: CD86 is induced in IFN treated Mo (FACS)

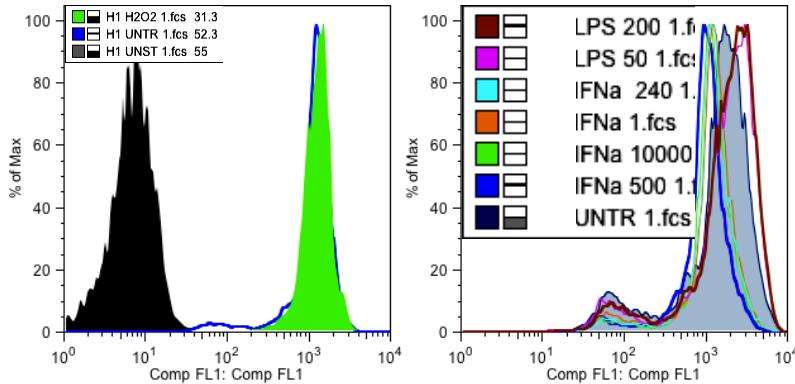


## Methods and Results: CD86 is induced in IFN treated Mo (FACS)

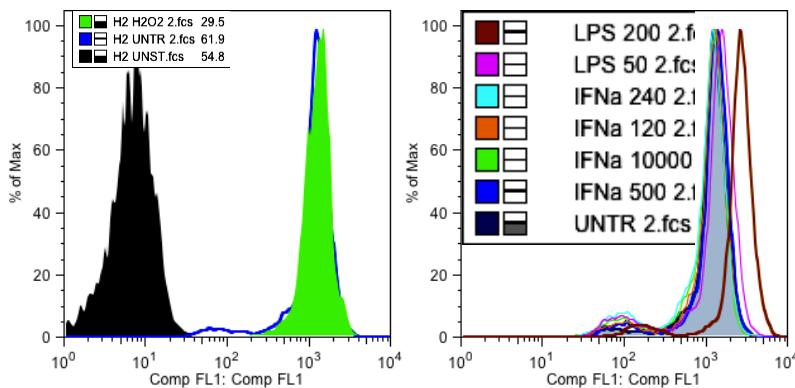


## Methods and Results: No difference in ROS production (FACS), N=2

### A. Healthy Donor 1



### B. Healthy Donor 2



Sample ID	Geom. Mean H1	Geom. Mean H2
FBS Cntrl	6,56	6,82
Untr	<b>1276</b>	<b>1090</b>
H <sub>2</sub> O <sub>2</sub> 24.000 μM	1296	1391
IFN $\alpha$ 500 U/ml (PBL)	924	1092
IFN $\alpha$ 10000 (PBL)	1097	943
IFN $\alpha$ 2B SIGMA 1/120	1098	1036
IFN $\alpha$ 2B SIGMA 1/240	1145	883
LPS 50 ng/ml	<b>1625</b>	<b>1174</b>
LPS 200 ng/ml	<b>1647</b>	<b>2264</b>

## Methods and Results: No difference in either TNF $\alpha$ or IL-6 secretion in CD14 $^{+}$ Monocytes stimulated with different interferons

