Immune cell plasticity in inflammatory arthritis, to be or not to be?

A Mechanisms of autoimmunity

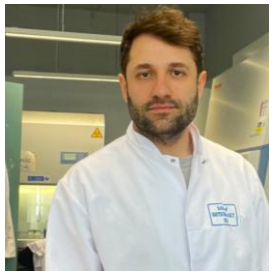
Σχολή Επιστημών Υγείας
Τμήμα Ιατρικής
Πανεπιστήμιο Ιωαννίνων

Επικ. Καθηγητής Αχιλλέας Φλούδας,
Ομάδα Μεταφραστικής Ανοσολογίας
Εργαστήριο Βιολογίας

Translational Immunology Group

Team

Assistant Professor
Achilleas Floudas



PhD Candidate
Roozbeh Senai

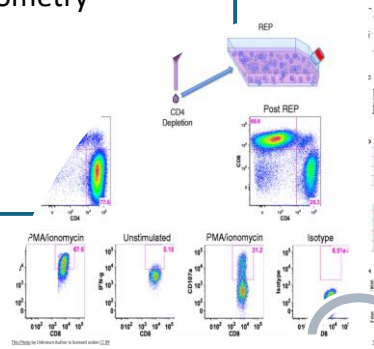
PhD Candidate Daphni
Doulaptsi-Teeuwen

Dr Konstantinos
Panagiotidis

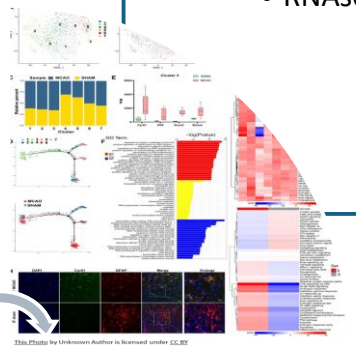
PhD Candidate Karina
Kulakova

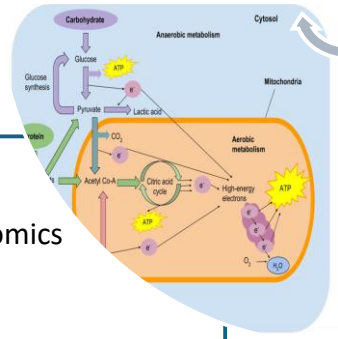
Core methodologies

- Flow cytometry

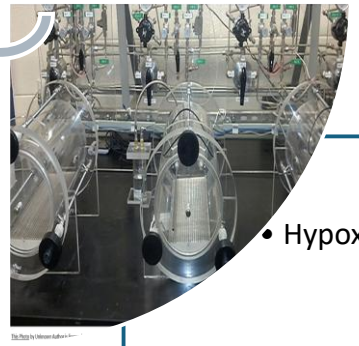


- RNAseq





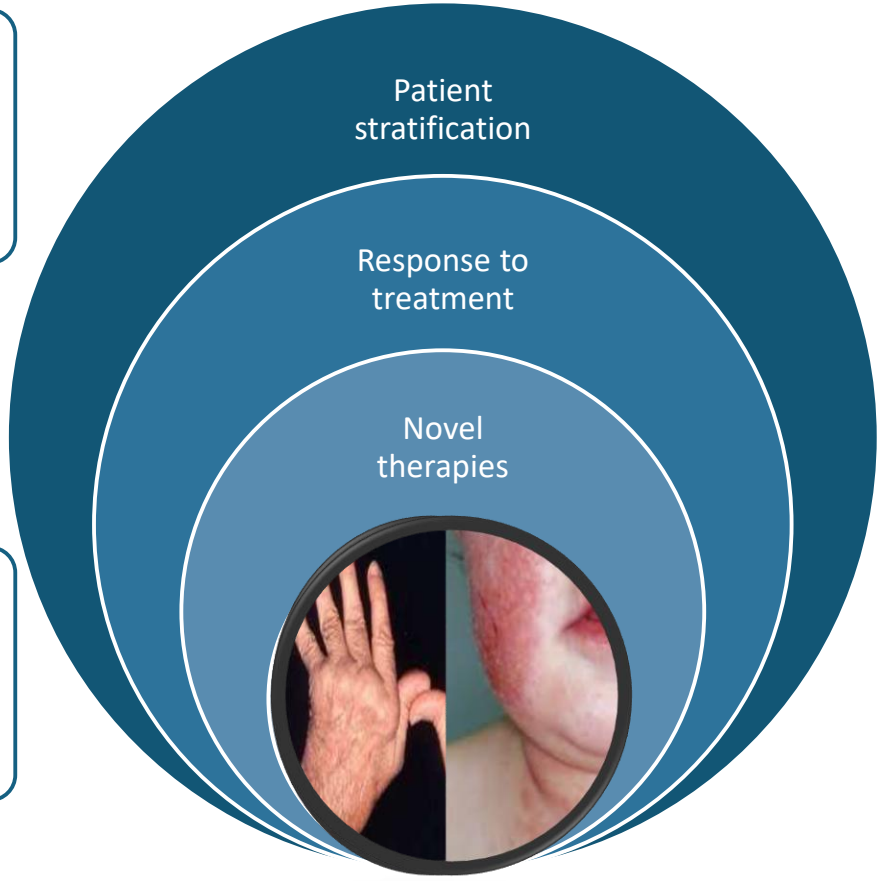
- Metabolomics



- Hypoxia

Outcomes

"Auto-stop"



Autoimmune Diseases

Funders

HRB An Bord Taighde Sláinte

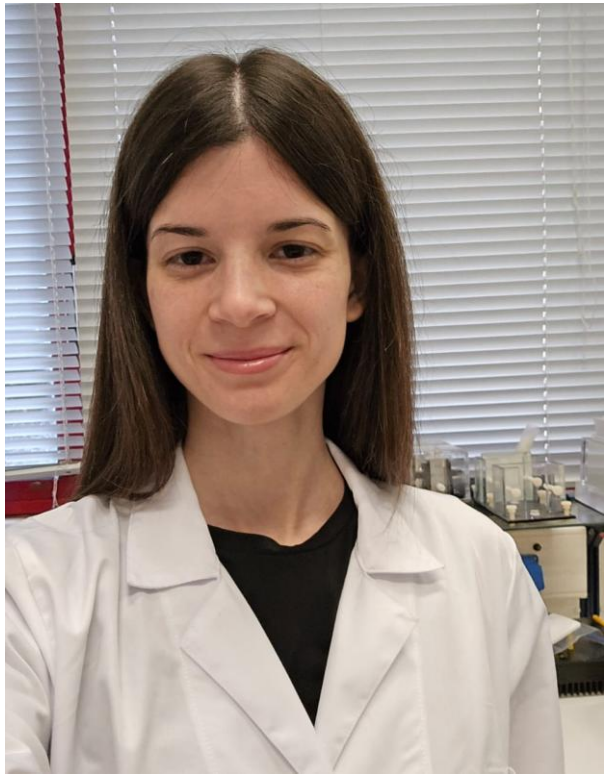
DCU Ollscoil Chathair Bhaile Átha Cliath Dublin City University

Collaborators

Beaumont Hospital
Ospidéal Beaumont

NGH Naas General Hospital

Translational Immunology Group

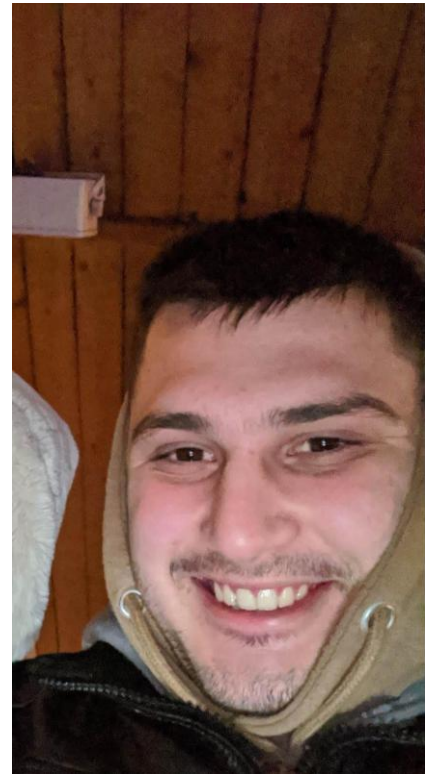


Natalia Papageorgiou

MSc Student - Medicine

George Margetis

BSc Student - BET

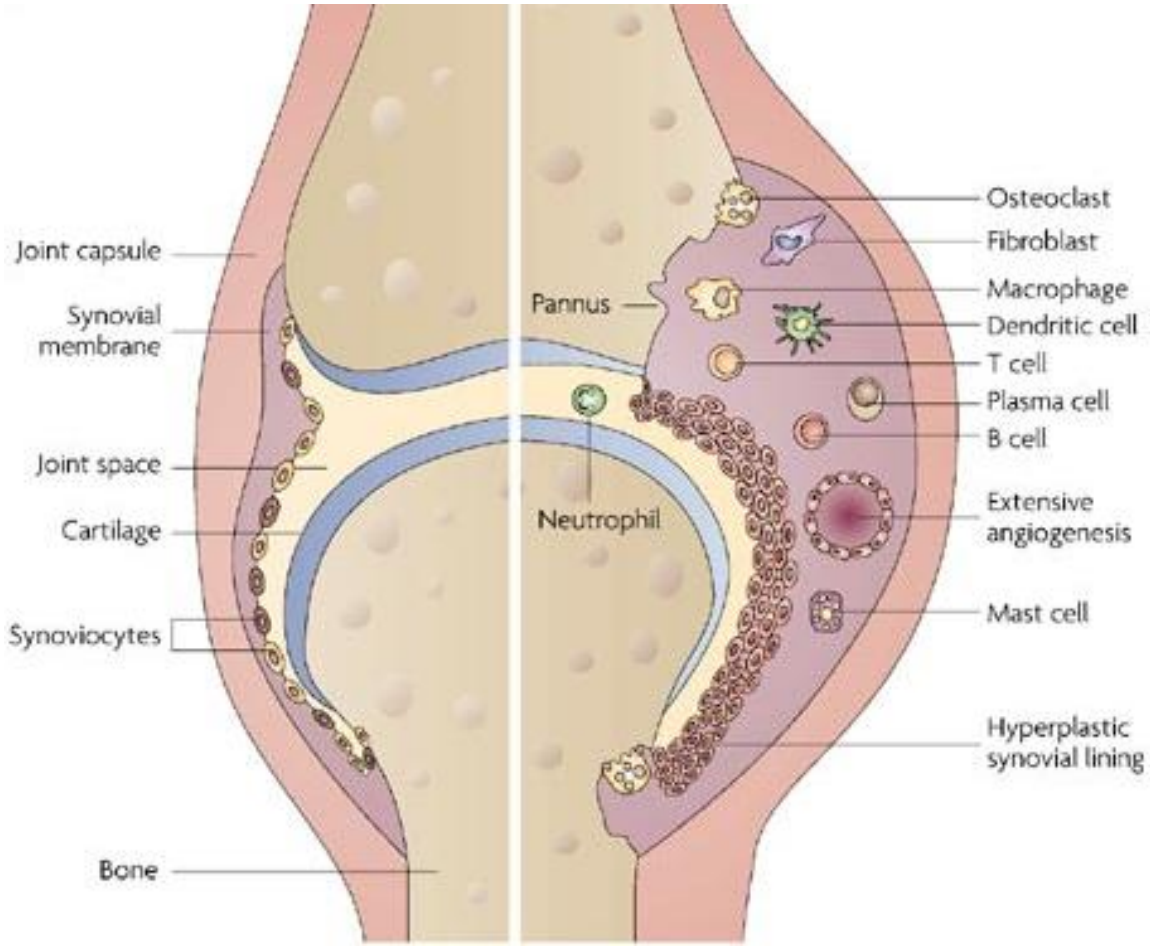


Stefanos Tsavdaridis

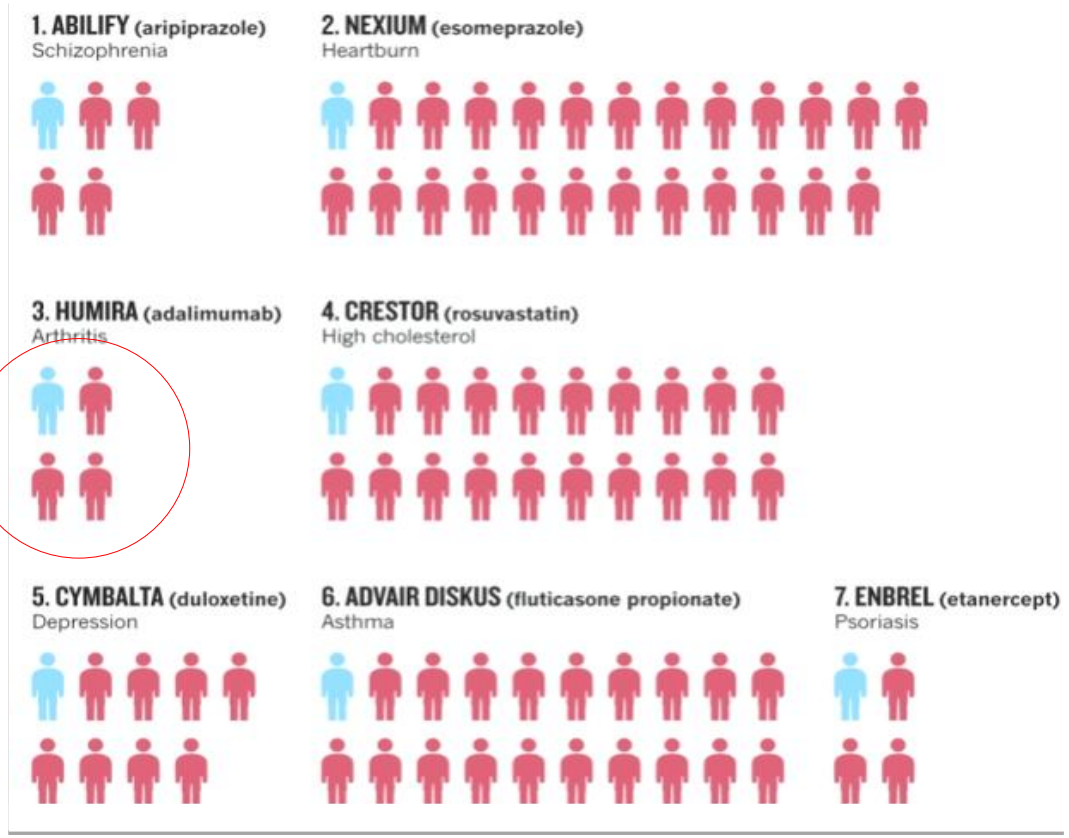
BSc Student - BET

Healthy

Inflamed



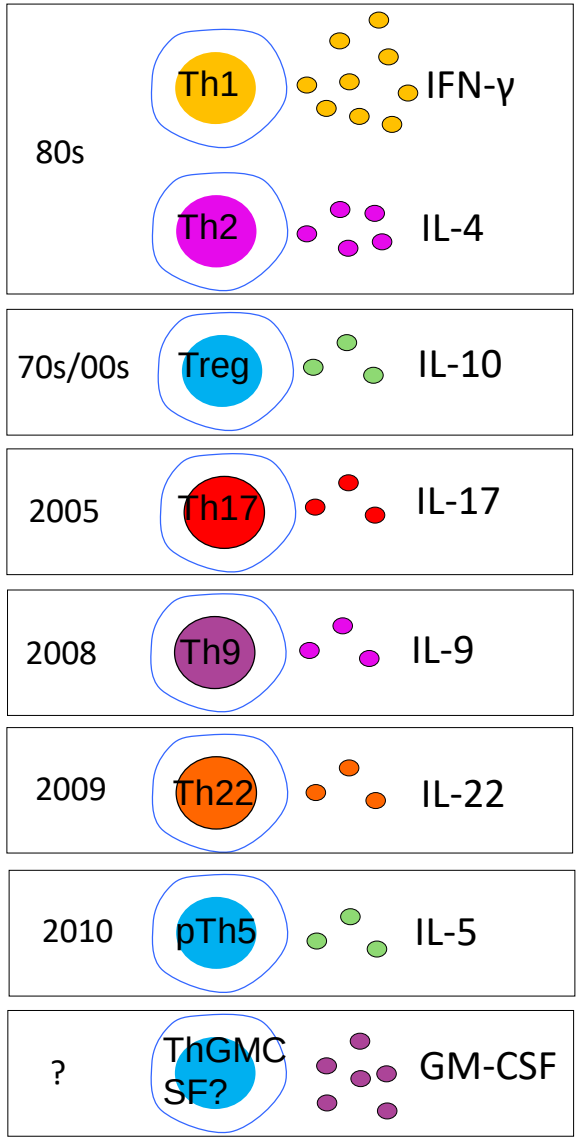
Imprecision of Medicine.

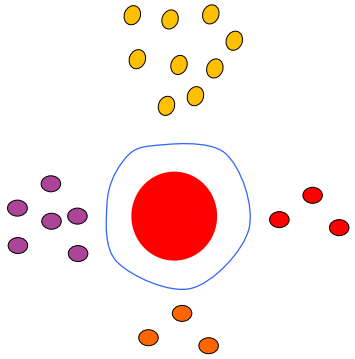


Schork N, Nature 2015

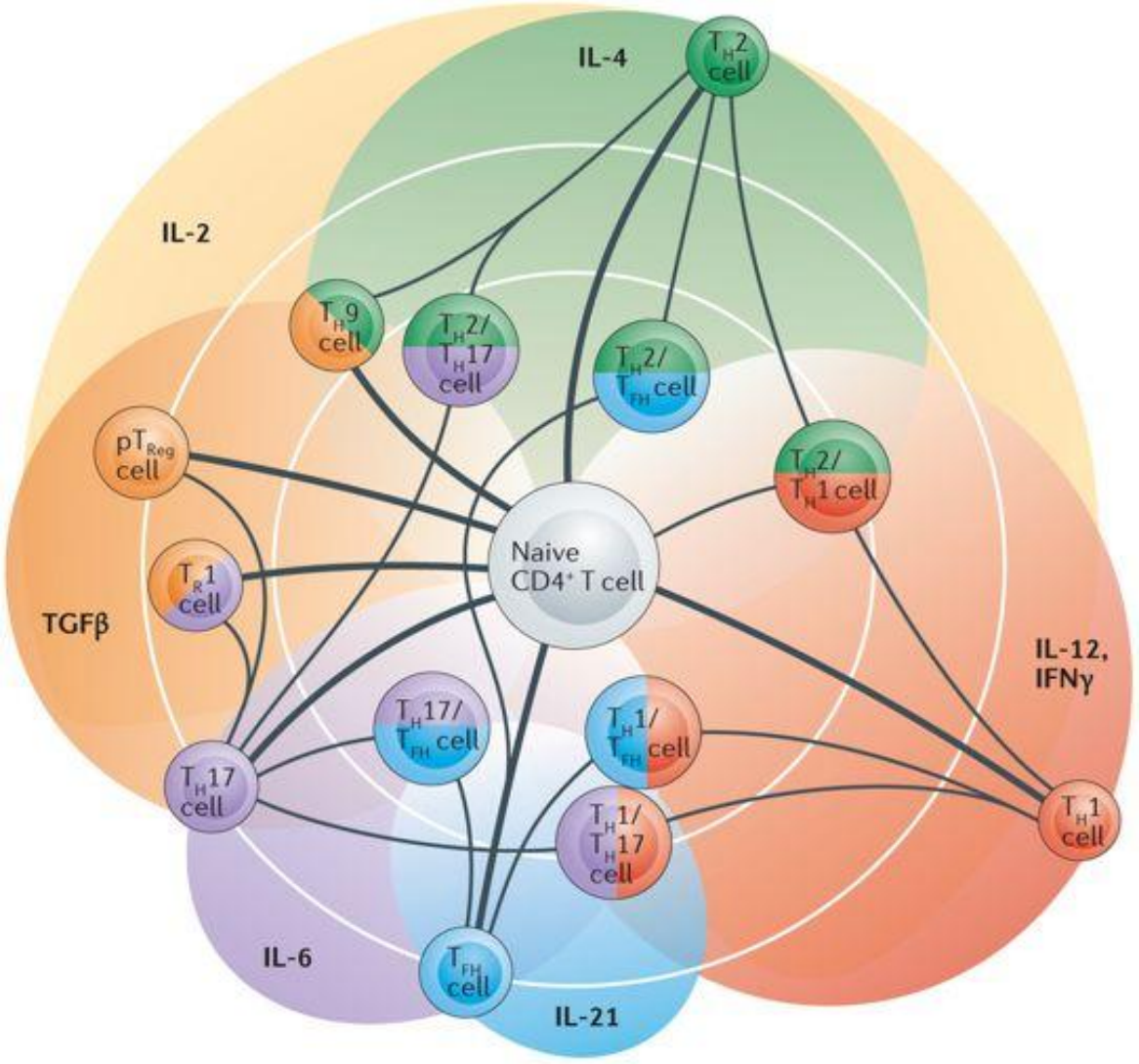
How we view immune cells.

Polyfunctionality



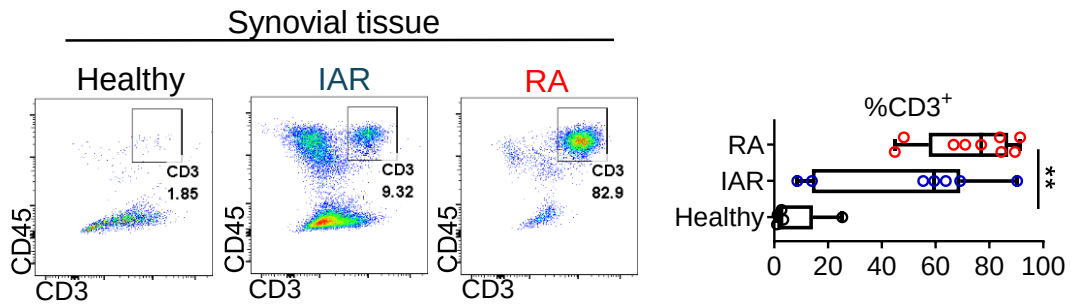


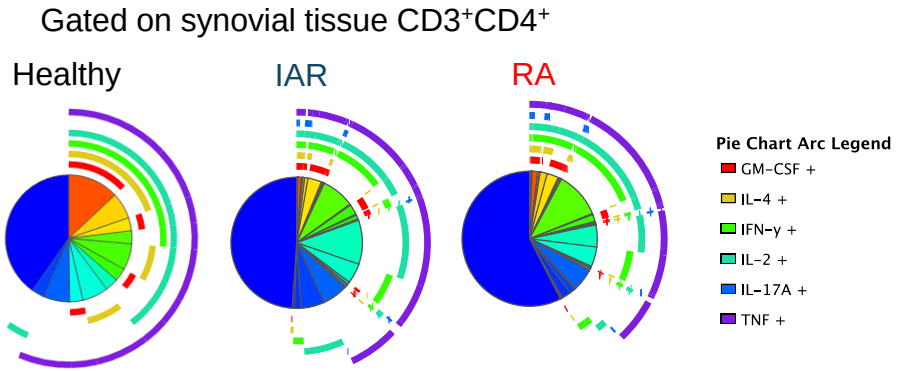
"PolyfunT"

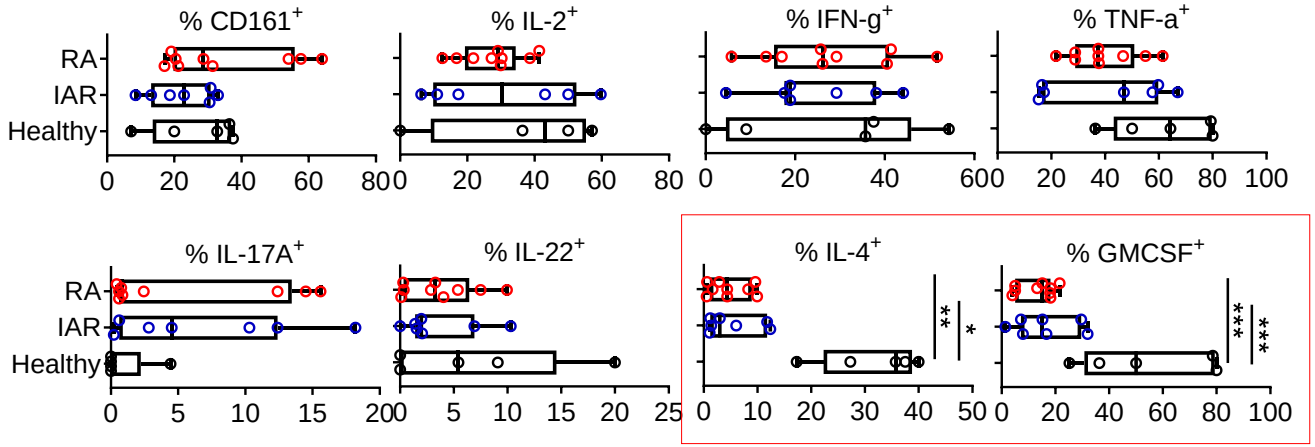


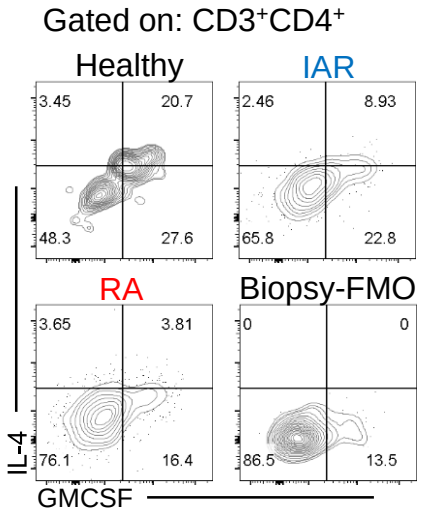
DuPage and Bluestone, Nature Reviews 2016

Synovial poly-T cells predate clinical inflammation in RA.



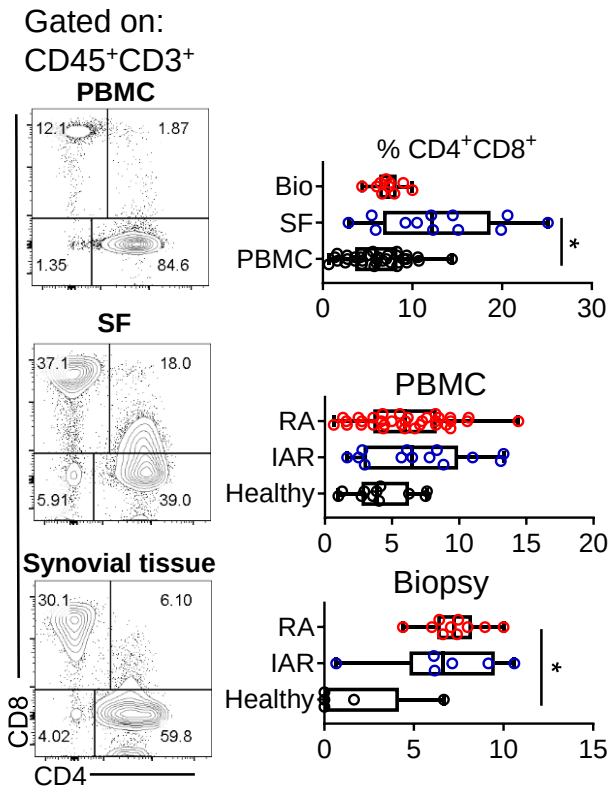


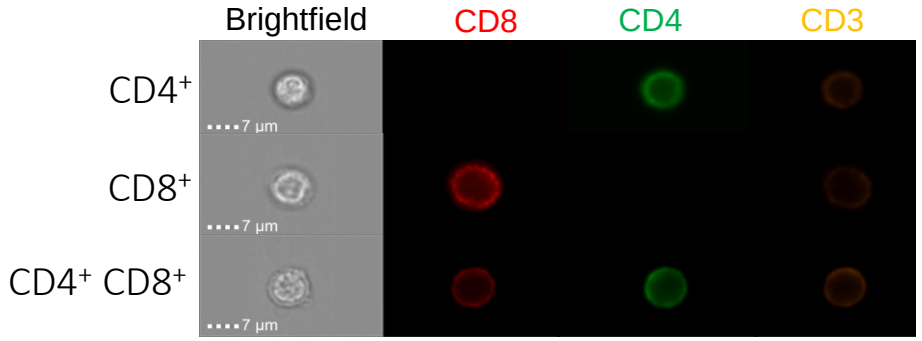


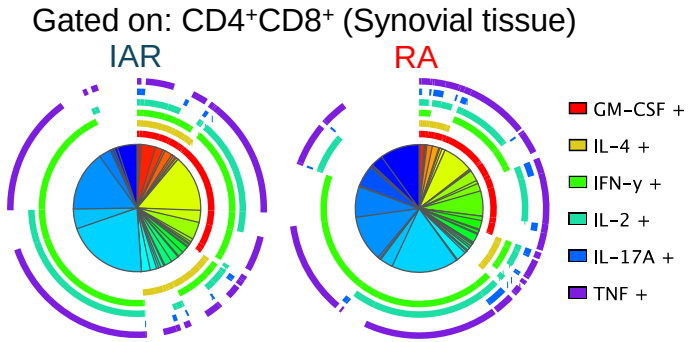


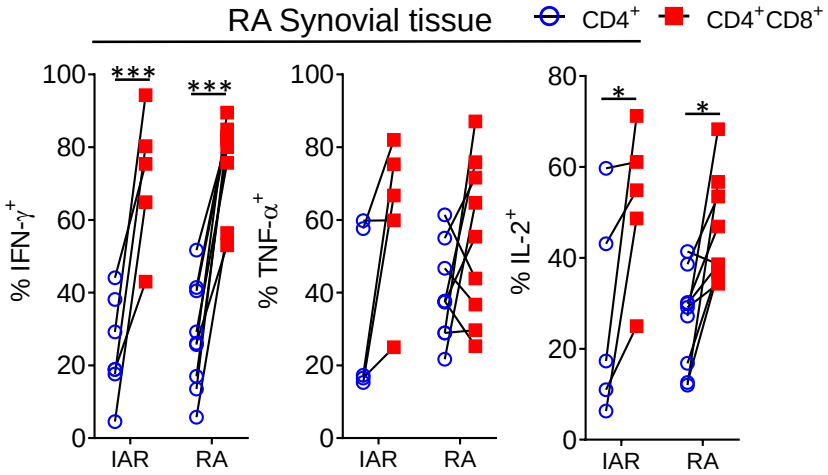
Floudas et al., ARD, 2022a

$CD4^+CD8^+$ T cells accumulate at the site of inflammation in RA.



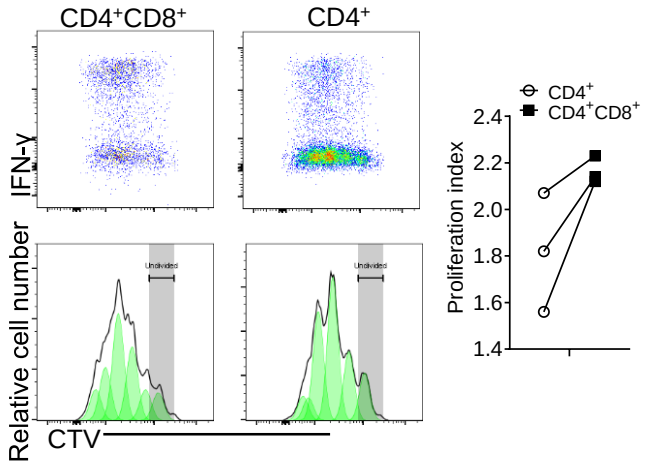


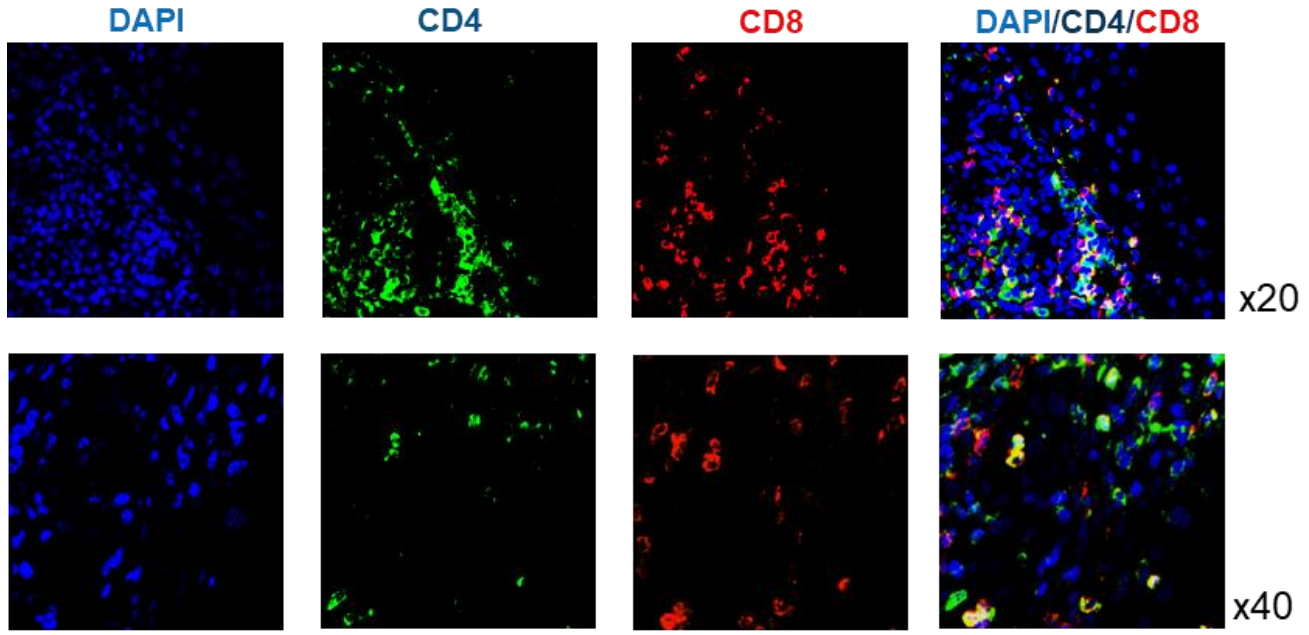




Floudas et al., ARD, 2022a

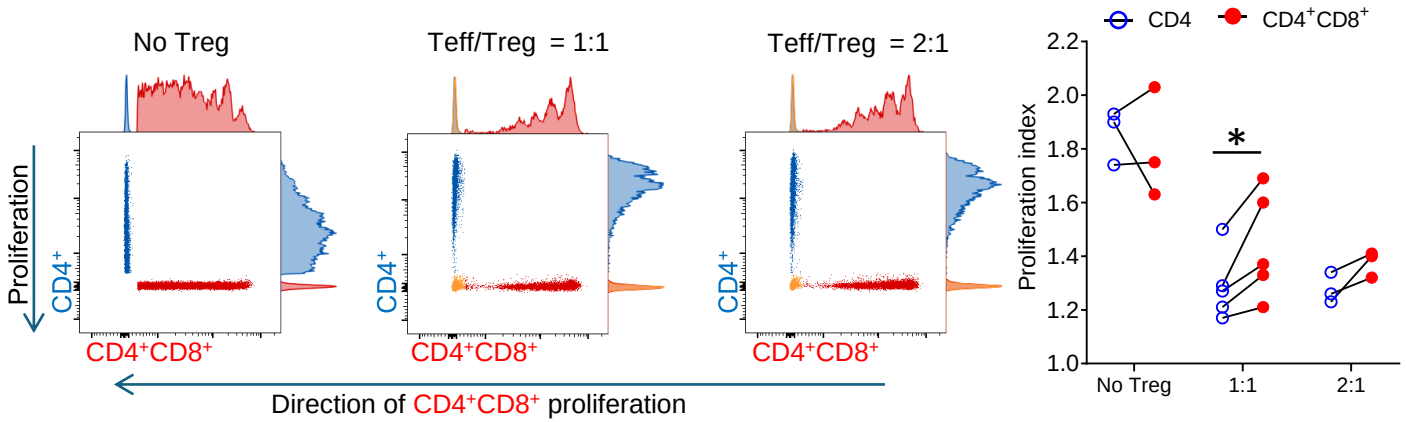
$CD4^+CD8^+$ T cells are detected within ectopic lymphoid organs in RA.





Floudas et al., ARD, 2022a

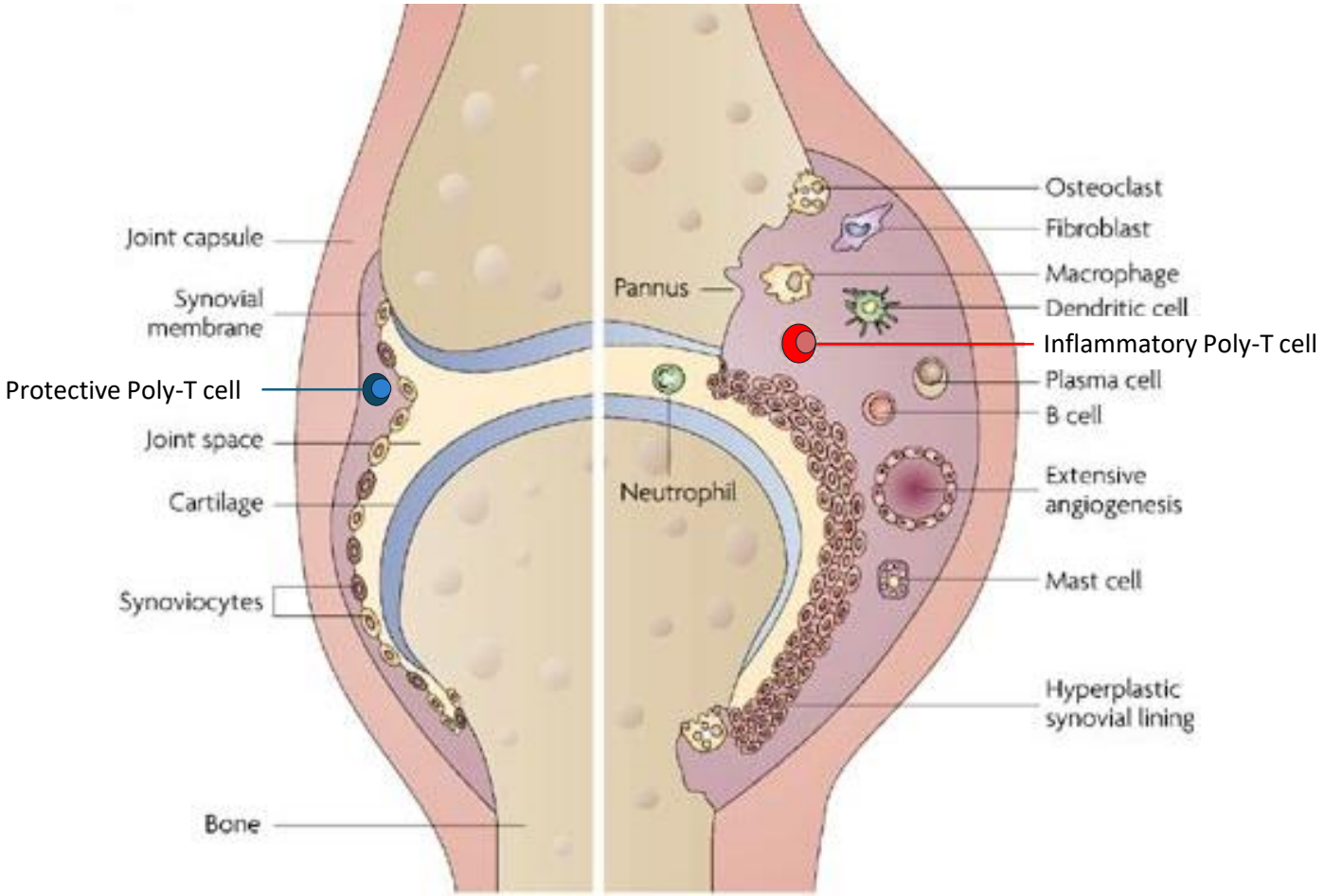
$CD4^+CD8^+$ T cells show signs of resistance to suppression by autologous Treg cells.



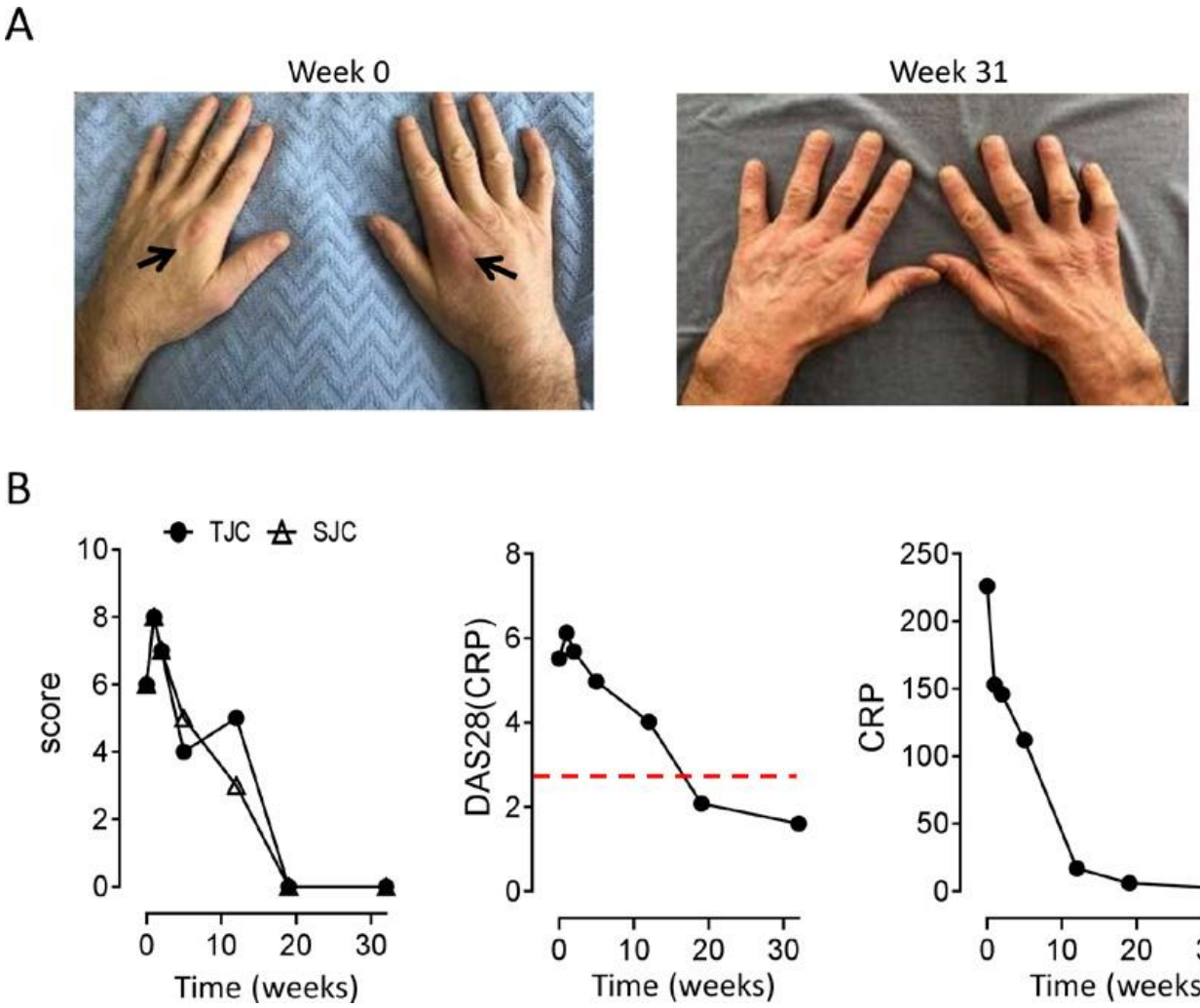
Floudas et al., ARD, 2022a

Healthy

Inflamed

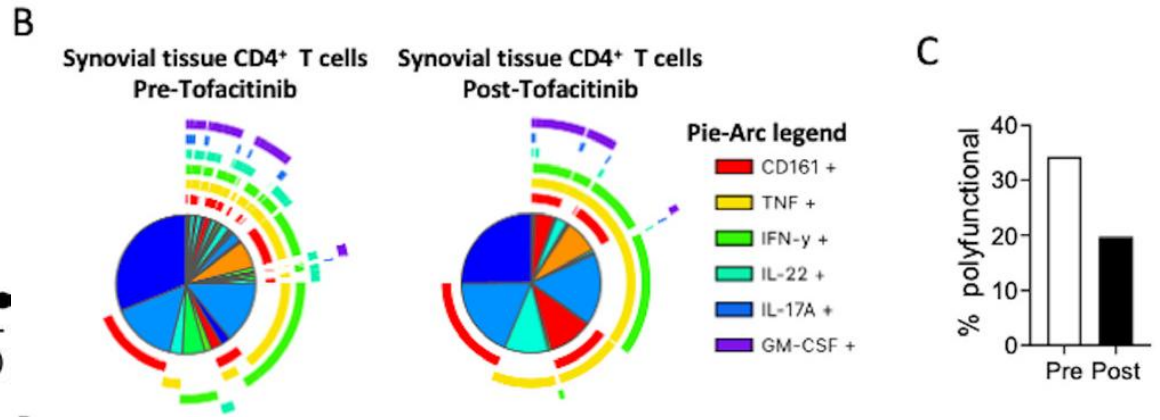


First use of tofacitinib to treat an immune checkpoint inhibitor-induced arthritis.



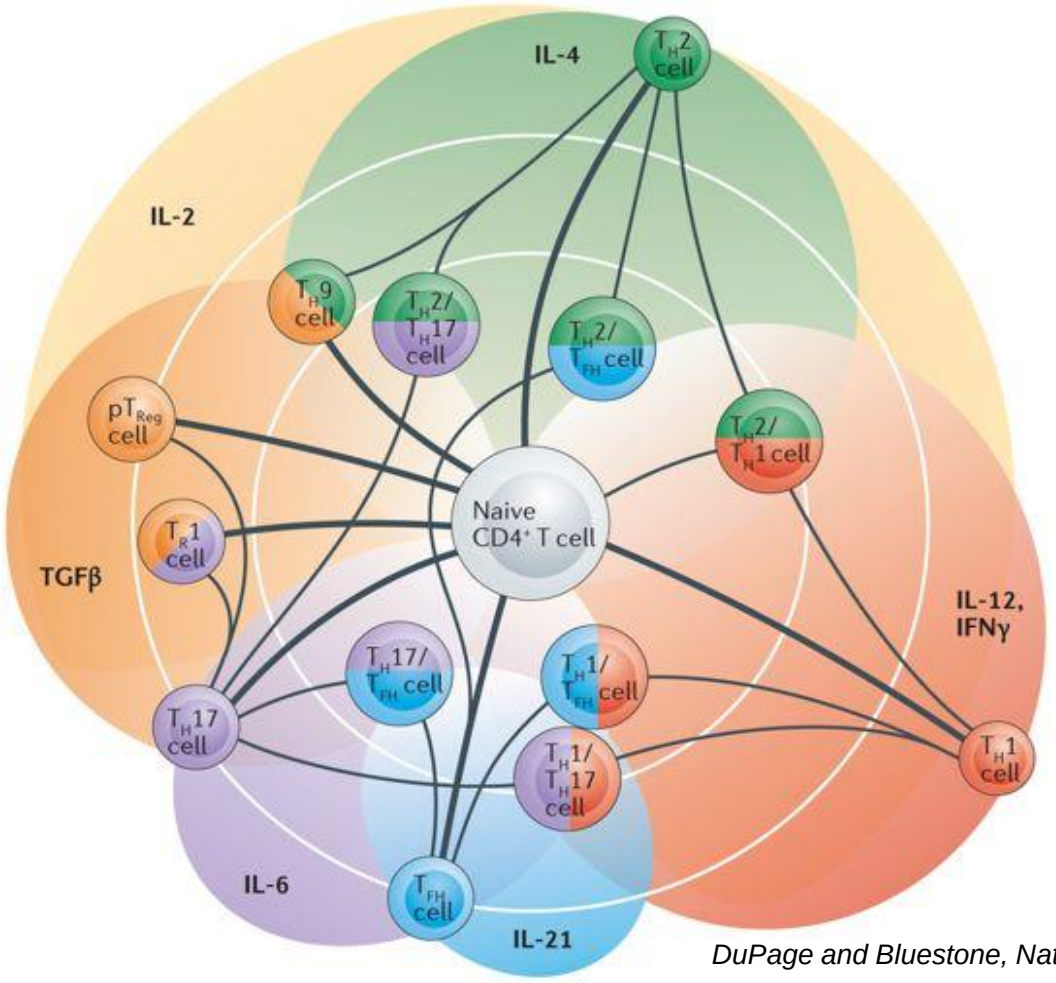
The rheumatology team did a biopsy to my right knee and I was diagnosed with Inflammatory Arthritis. I felt a glimmer of hope knowing I was under a superb team.

I can exercise again, work again and enjoy my life with my wife again. … **they have given me hope and a new lease of life that I cherish so much now**. Thank you to all the amazing medical staff I owe my life to.



Murray, Floudas et al., BMJ case reports 2021

Immune cell plasticity



DuPage and Bluestone, Nature Reviews 2016

Arthritis & Rheumatology
Vol. 72, No. 4, April 2020, pp 677–686
DOI 10.1002/art.41150

American College of Rheumatology
Empowering Rheumatology Professionals

Increased T Cell Plasticity With Dysregulation of Follicular Helper T, Peripheral Helper T, and Treg Cell Responses in Children With Juvenile Idiopathic Arthritis and Down Syndrome–Associated Arthritis

C. Foley,[1] A. Floudas,[2] M. Canavan,[2] M. Biniecka,[3] E. J. MacDermott,[4] D. J. Veale,[3] R. H. Mullan,[5] O. G. Killeen,[4] and U. Fearon[2]

Loss of balance between protective and pro-inflammatory synovial tissue T-cell polyfunctionality predates clinical onset of rheumatoid arthritis

Achilleas Floudas [1], Nuno Neto [2, 2], Carl Orr [3], Mary Canavan [1], Phil Gallagher [4], Conor Hurson [4], Michael G Monaghan [2], Sunil Nagpar [5], Ronan H Mullan [6], Douglas J Veale [3], Ursula Fearon [1]

Rheumatoid arthritis

OPEN ACCESS

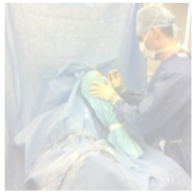
TRANSLATIONAL SCIENCE

Distinct stromal and immune cell interactions shape the pathogenesis of rheumatoid and psoriatic arthritis

Achilleas Floudas ,[1,2] Conor M Smith ,[3] Orla Tynan,[1,2] Nuno Neto,[4] Vinod Krishna,[5] Sarah M Wade ,[1,2] Megan Hanlon,[1,2] Clare Cunningham,[1,2] Viviana Marzaioli,[1,2] Mary Canavan,[1,2] Jean M Fletcher,[3] Ronan H Mullan,[6] Suzanne Cole,[5] Ling-Yang Hao,[5] Michael G Monaghan ,[4] Sunil Nagpal ,[5] Douglas J Veale .[2] Ursula Fearon [1,2]

Methodology

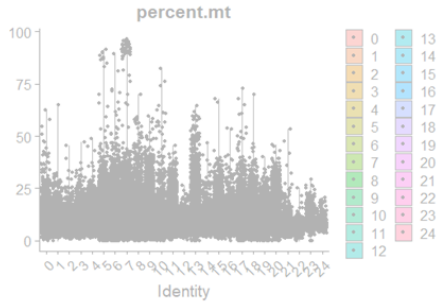
Bioinformatic



Generation of
single cell suspension

Doublet exclusion
DoubletFinder, v 2.0.3

Dead cell exclusion (n = 178.000)
(Mitochondrial cut off ~25%)

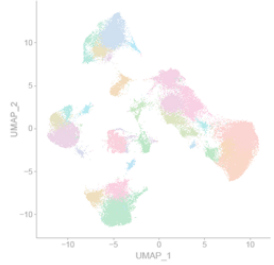


Data normalisation
(SCtransform, v 0.3.2)

Determination of
dimensionality

Data integration
(Harmony, v 1.0)

Harmony



Cluster annotation
(manual)

+

Cluster annotation
(automated)

Data imputation
(Rmagic, v2.0.3)

Further analysis

List of packages used
- PathfindR (v 1.6.1)
- ggplot2 (v 3.3.2)
- ReactomeGSA (v 1.2.4)
- scCATCH (v 2.1)
- nichenetr (v1.0)
- Dorothea (v1.01)
- Monocle3 (v0.2.3)

Functional

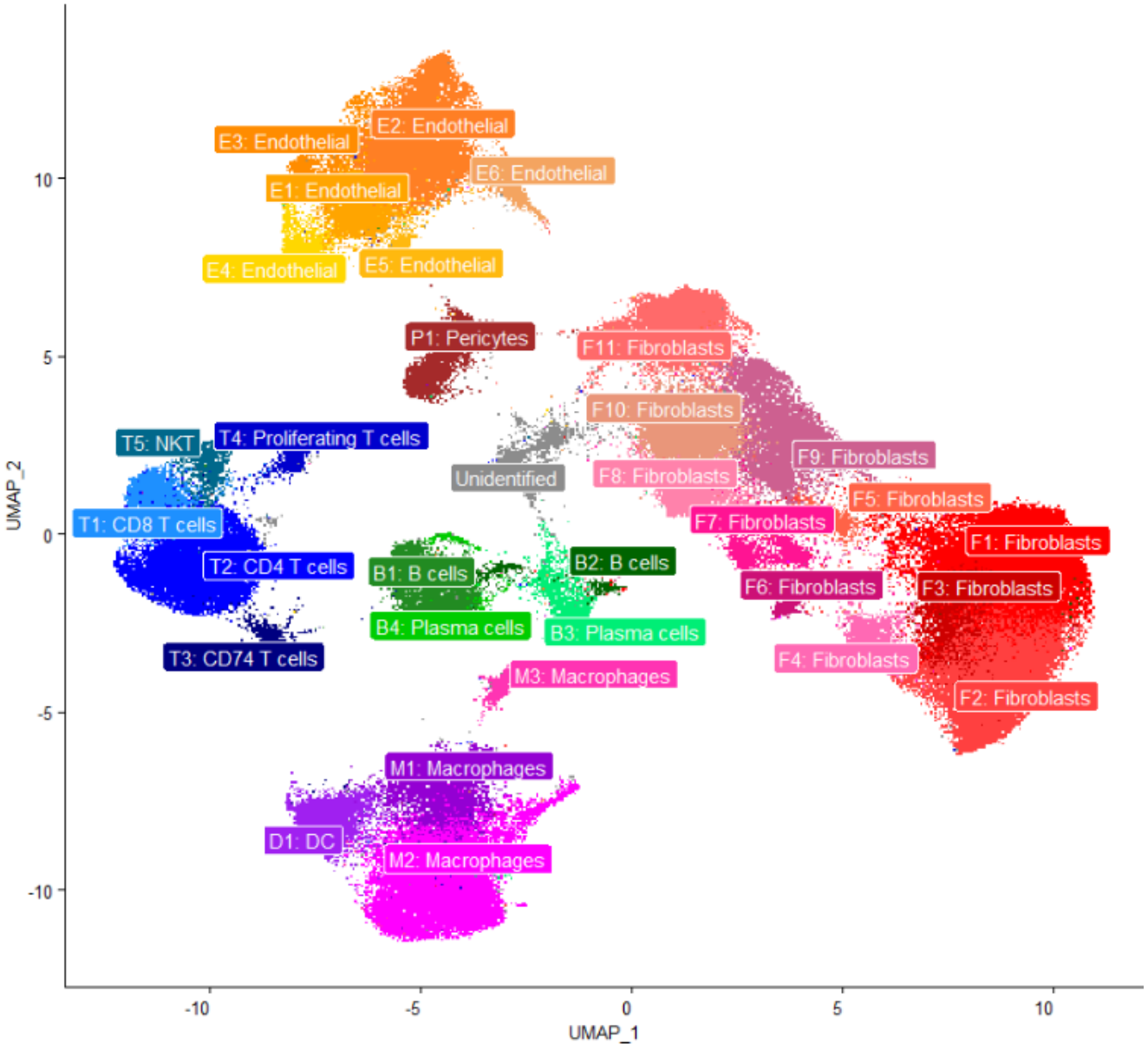
Seahorse
Metabolic characterisation of RA patient synovial tissue fibroblasts following treatment with IL-1β and TGF-β.

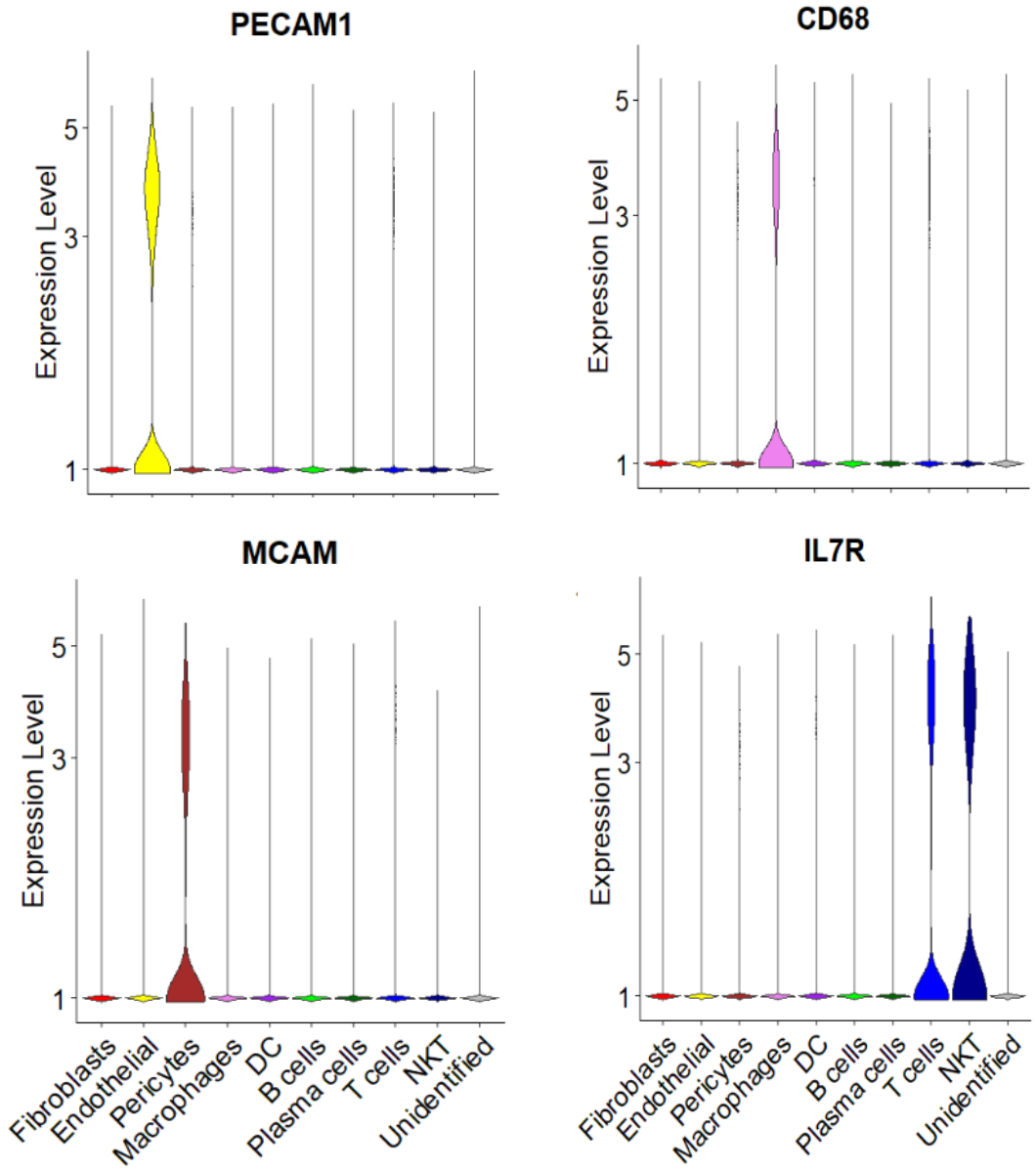
Flow cytometry
Flow cytometric analysis of synovial tissue fibroblast activation and adhesion markers.

ELISA
Detection of fibroblast IL-6 in response to IL-1β and TGF-β. .

Microscopy
TMRN staining of fibroblast mitochondria and assessment of mitochondrial size and aspect ratio

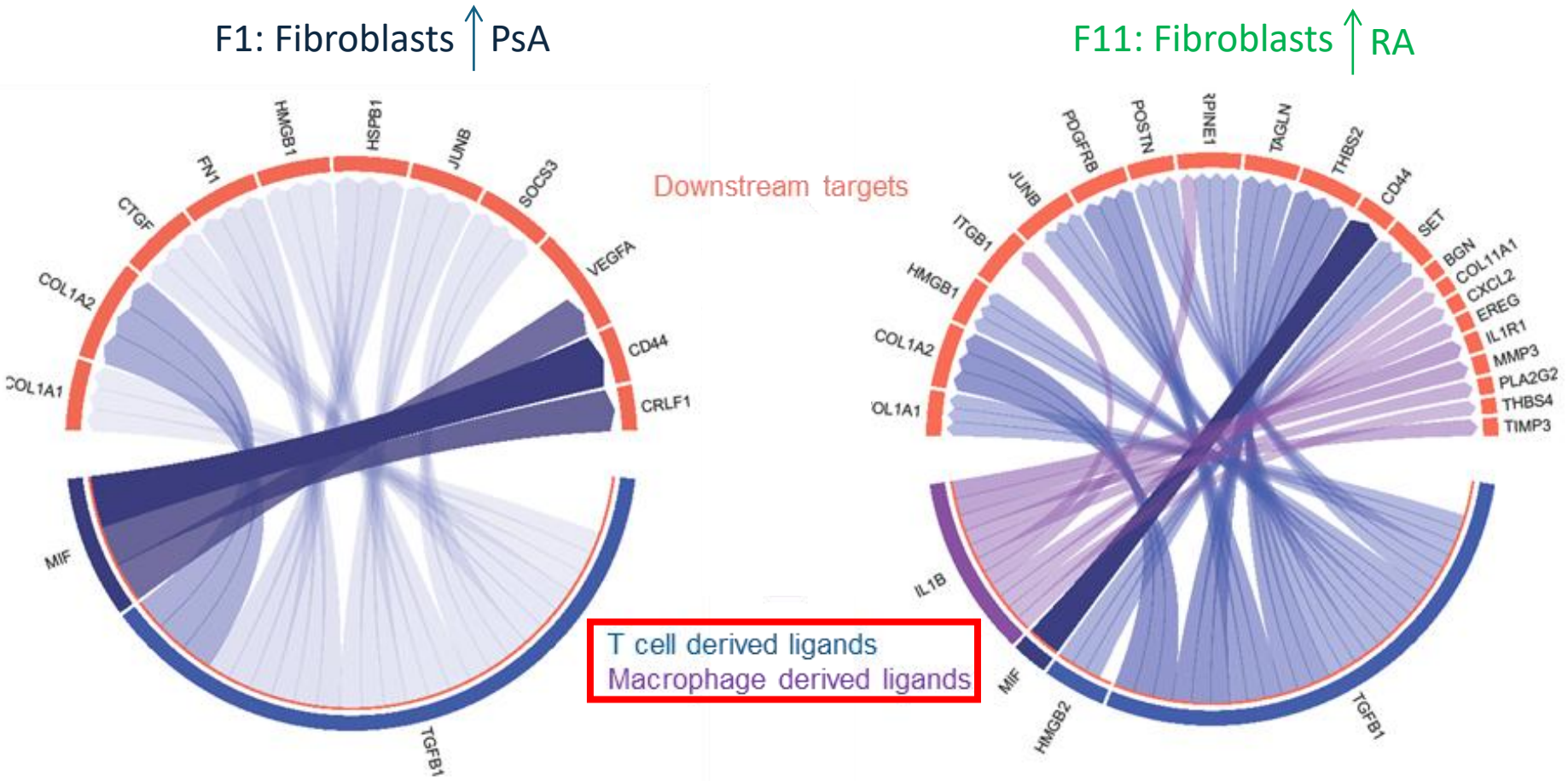
Cellular landscape of RA and PsA





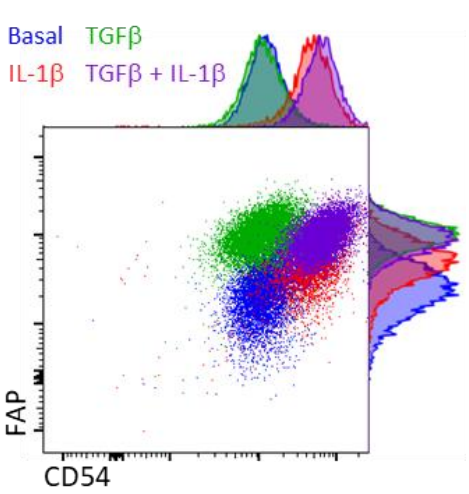
Floudas et al., ARD, 2022b

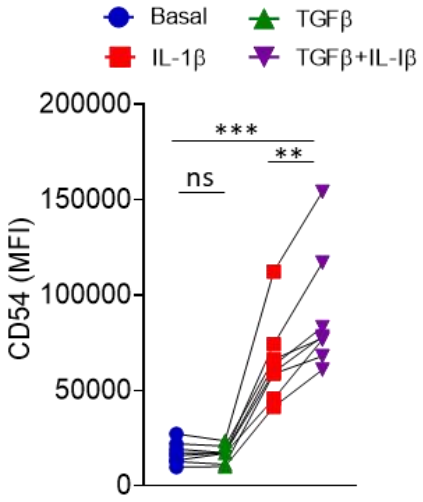
Synovial T cell and macrophage derived ligands drive the transcriptional profile of enriched in RA FAP^{+}THY1^{+} fibroblasts

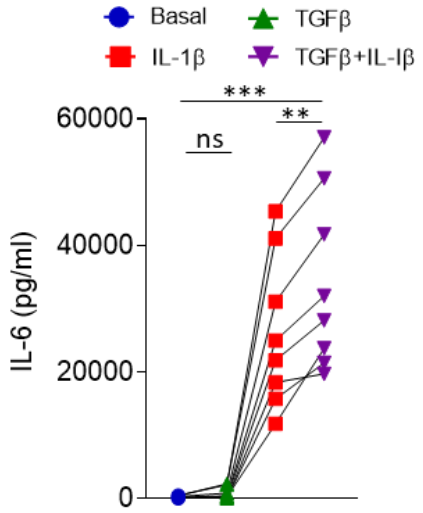


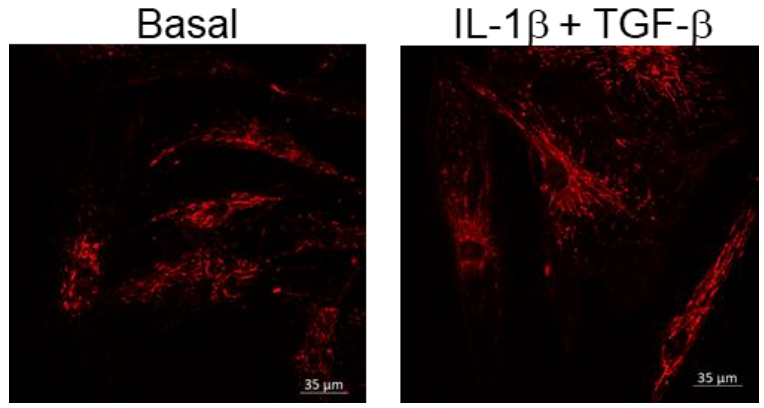
Floudas et al., ARD, 2022b

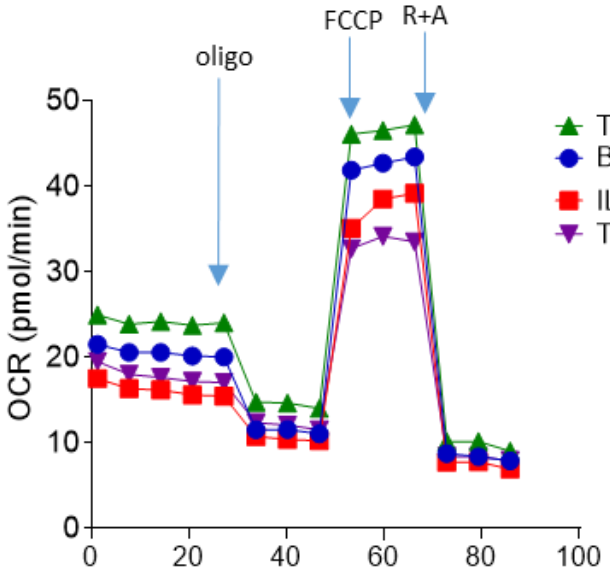
IL-1β and TGF-β synergy drives the activation and metabolism of synovial fibroblasts

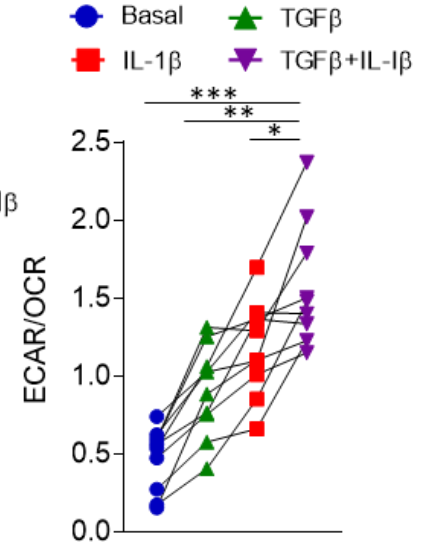


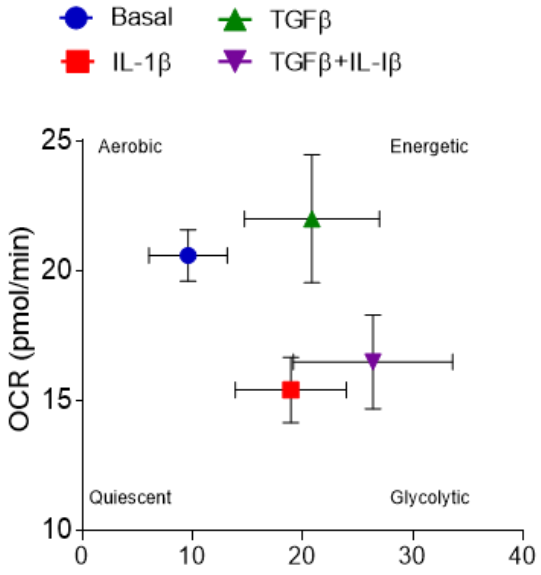


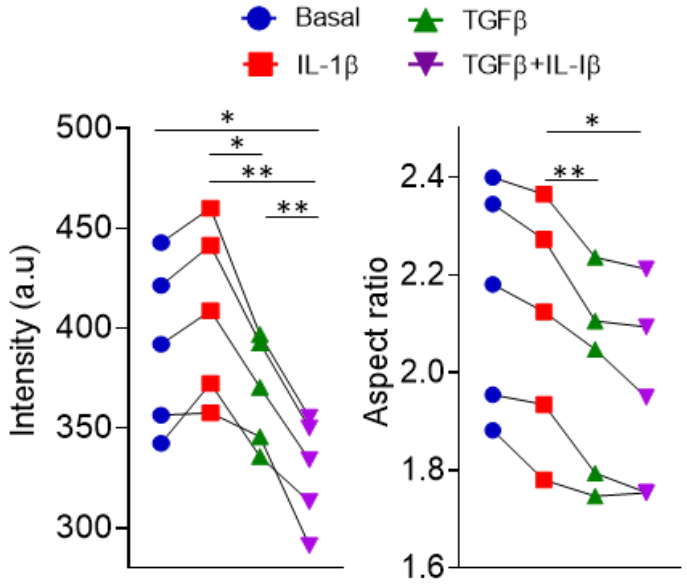










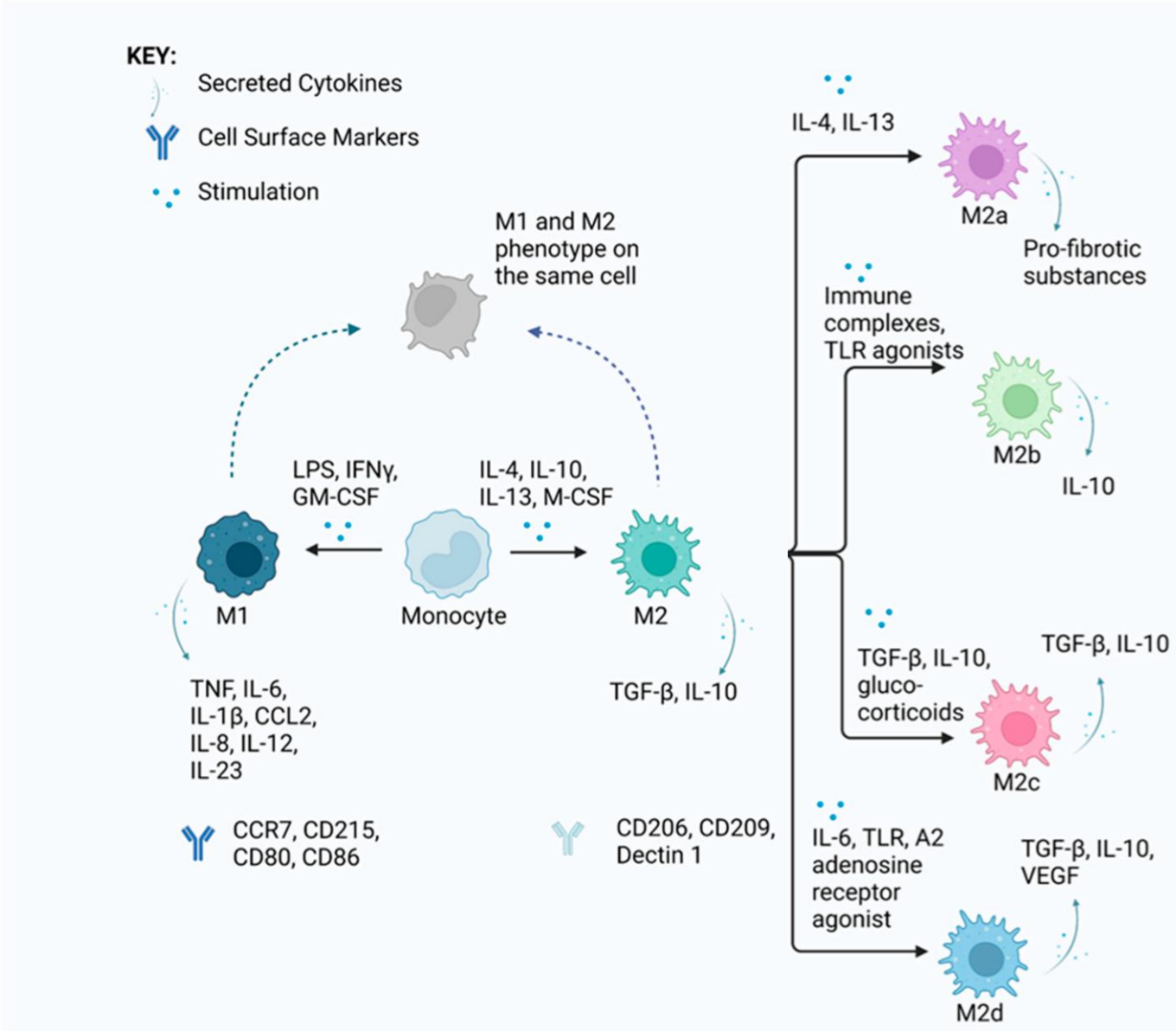


Floudas et al., ARD, 2022b

PhD Candidate Karina Kulakova

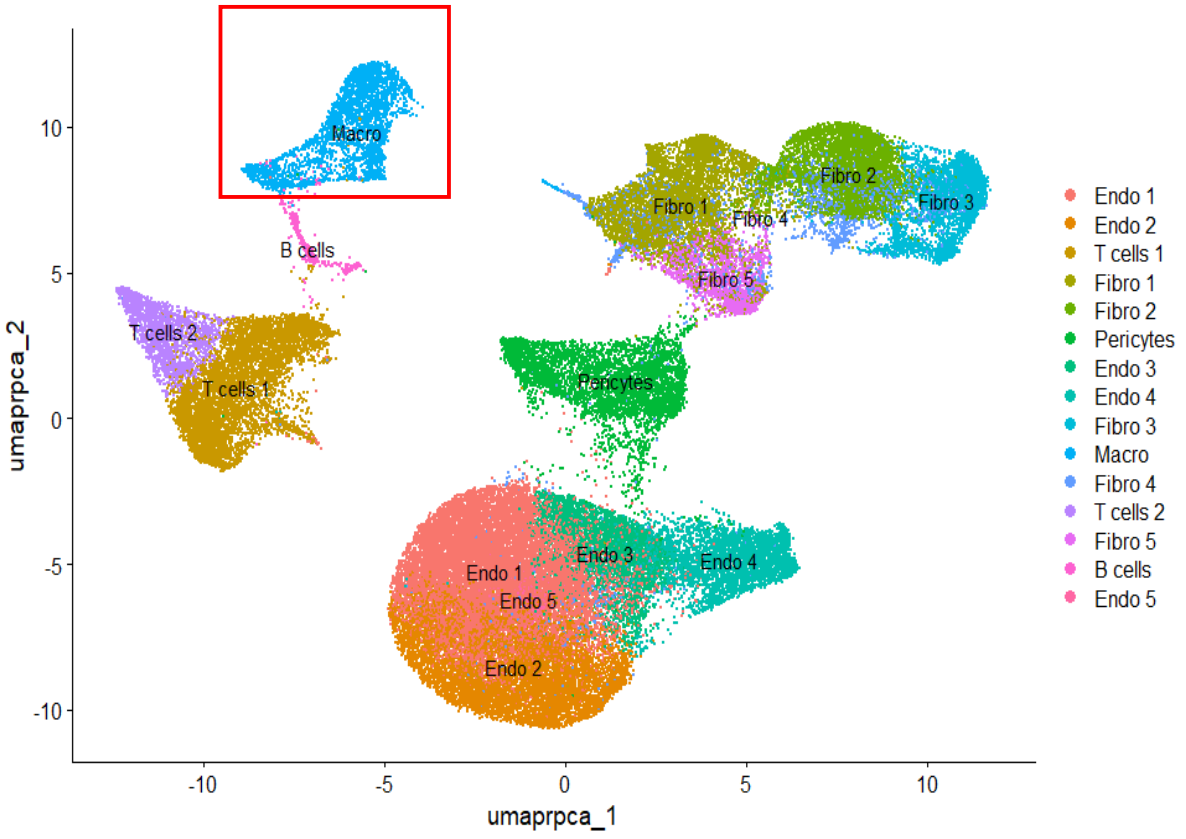
Dysregulated Macrophage Plasticity Promotes Progression of Inflammation in Patients with Rheumatoid Arthritis.

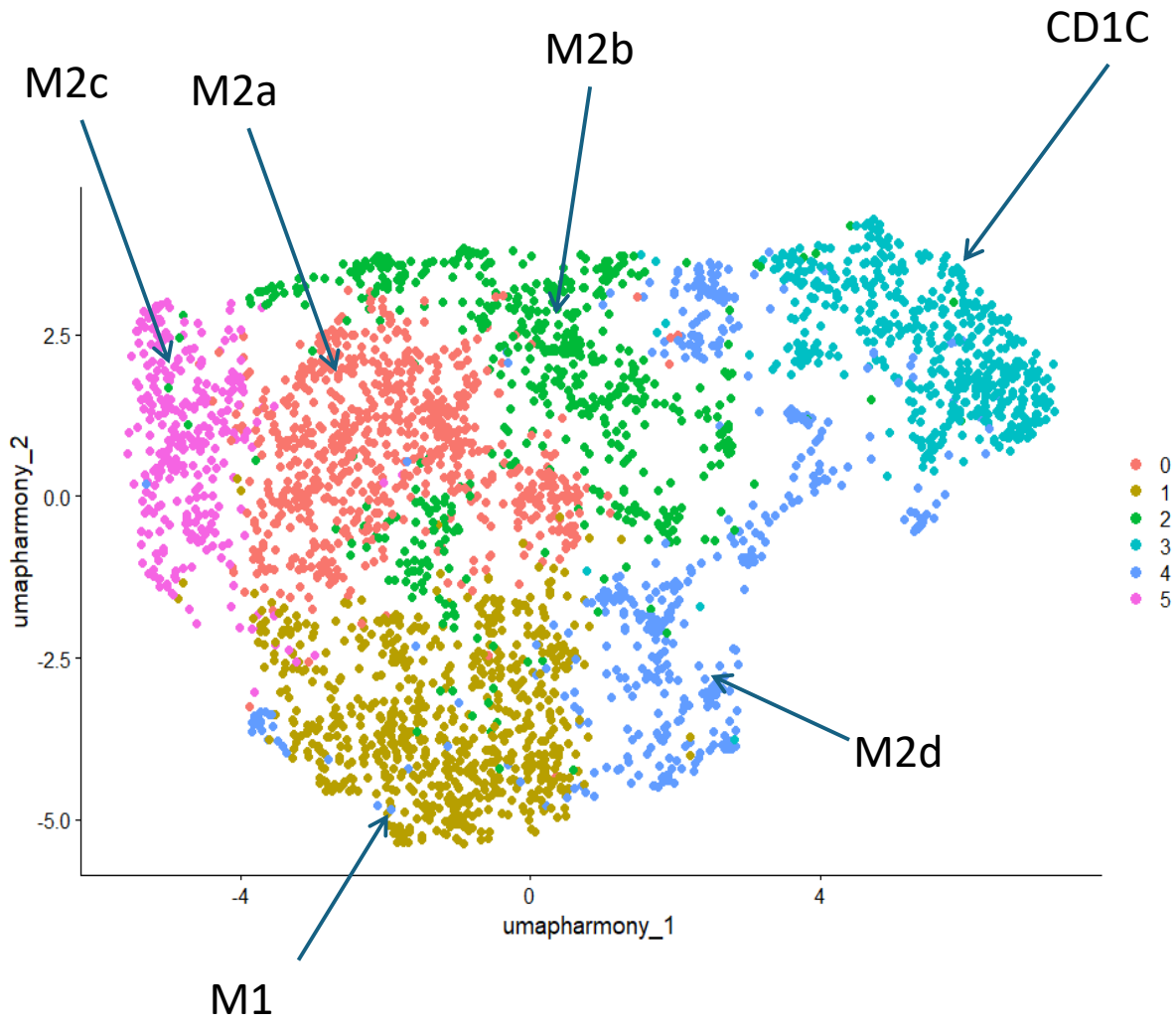
- CD68+ cells correlate with synovial inflammation.
- MertK macrophages correlate with remission.
- CX3CR1 Macrophages form a protective barrier in healthy joints.



Kulakova et al., Cells, 2024

scRNAseq of patient with RA and HC synovial tissue biopsies.

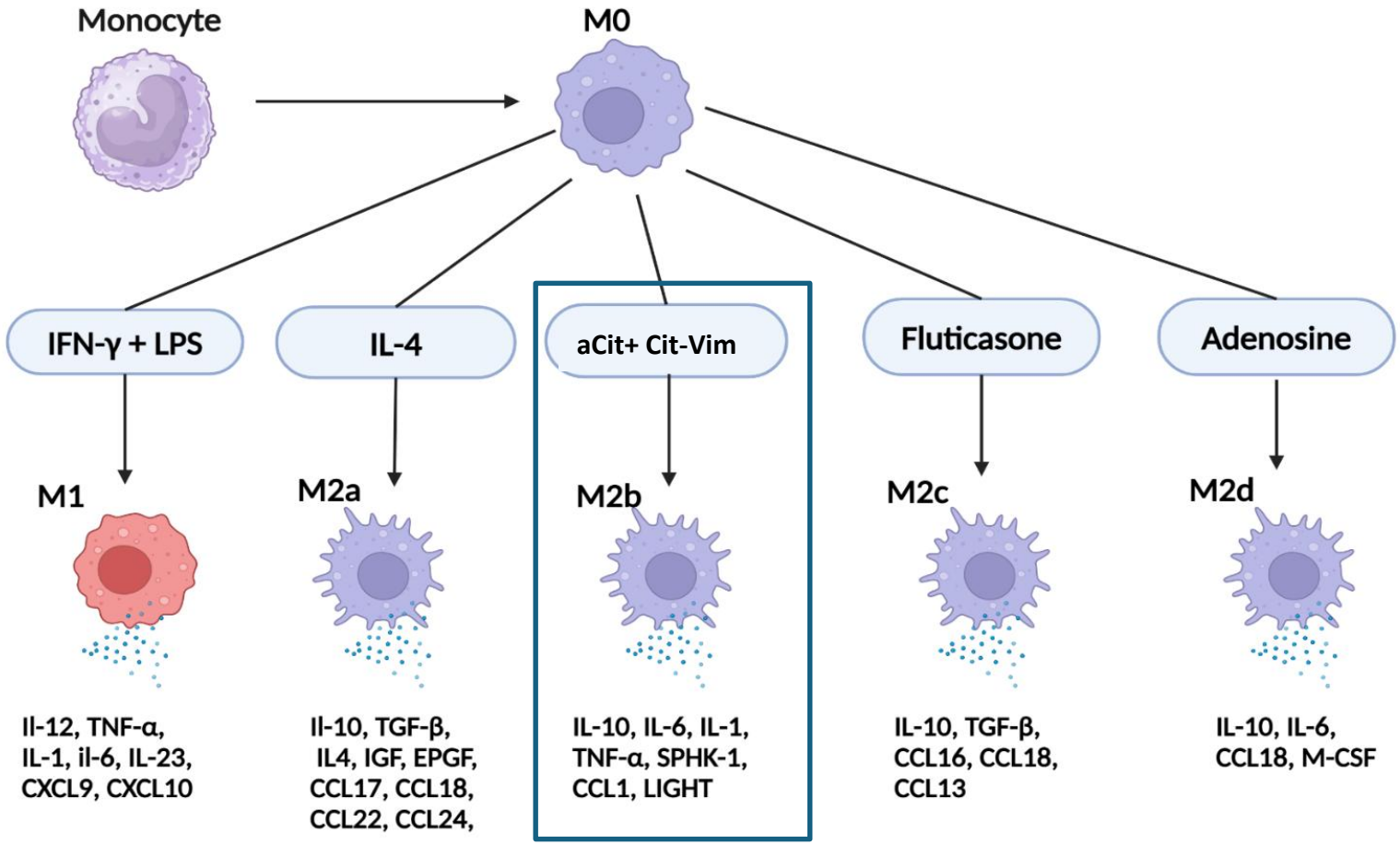




Kulakova et al., in preparation

In vitro macrophage polarisation.

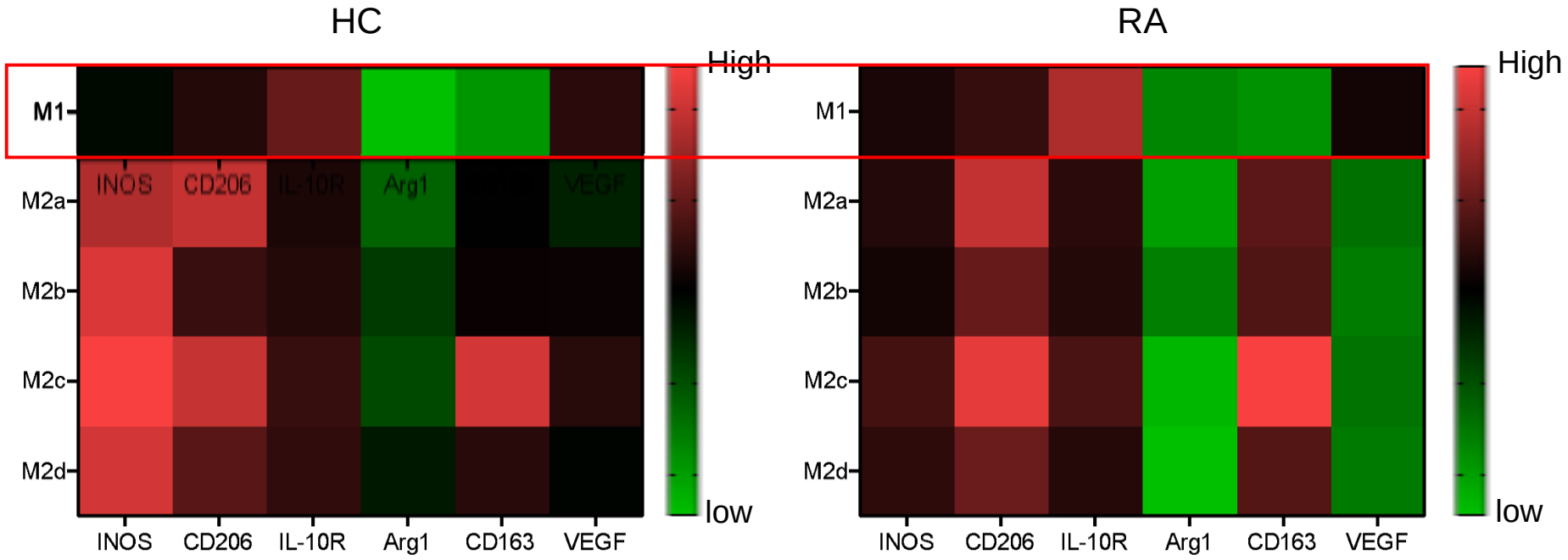
PhD Candidate Karina Kulakova



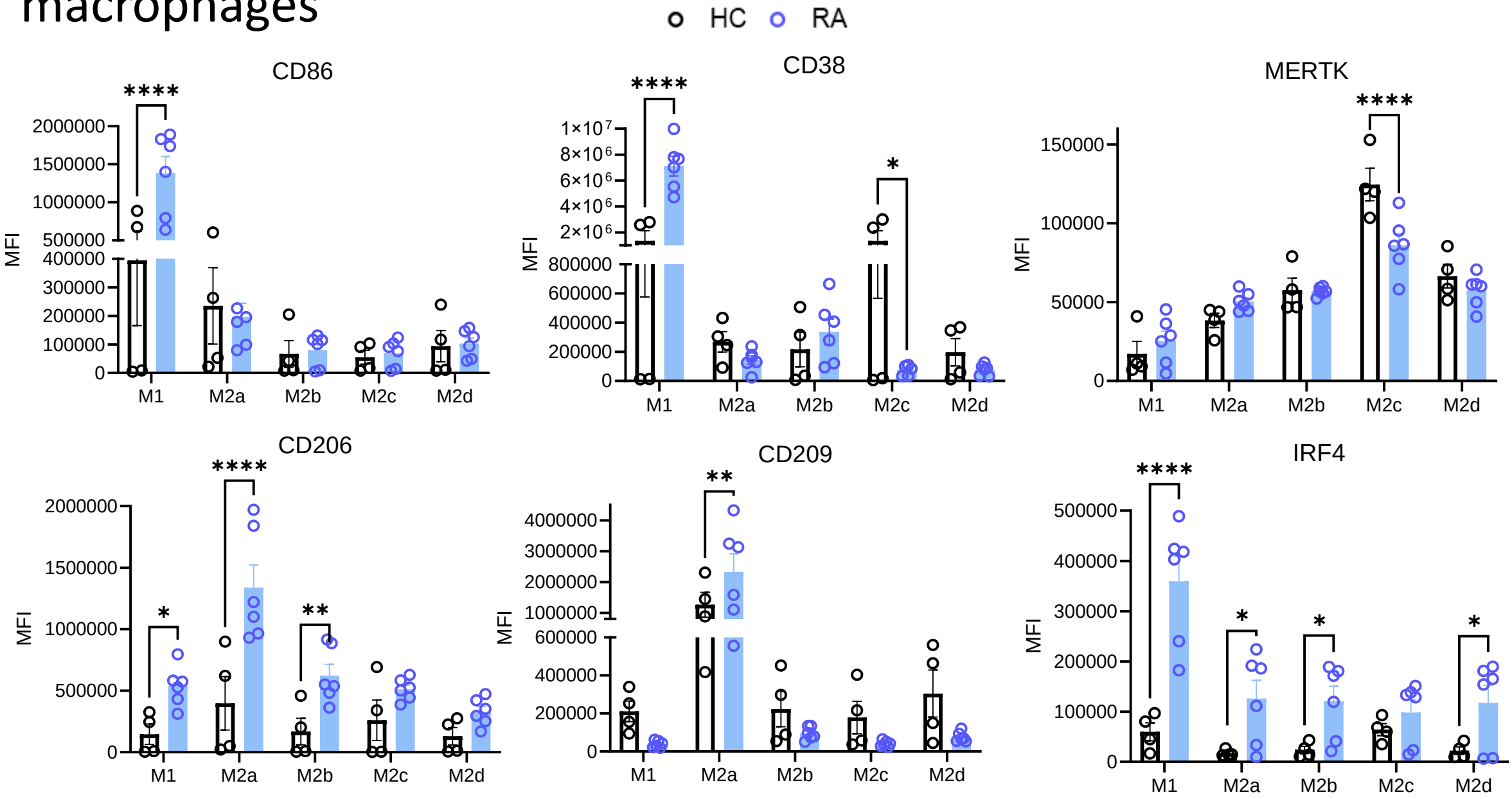
Kulakova et al., in preparation

HC and patient with RA Macrophage Polarisation Profile

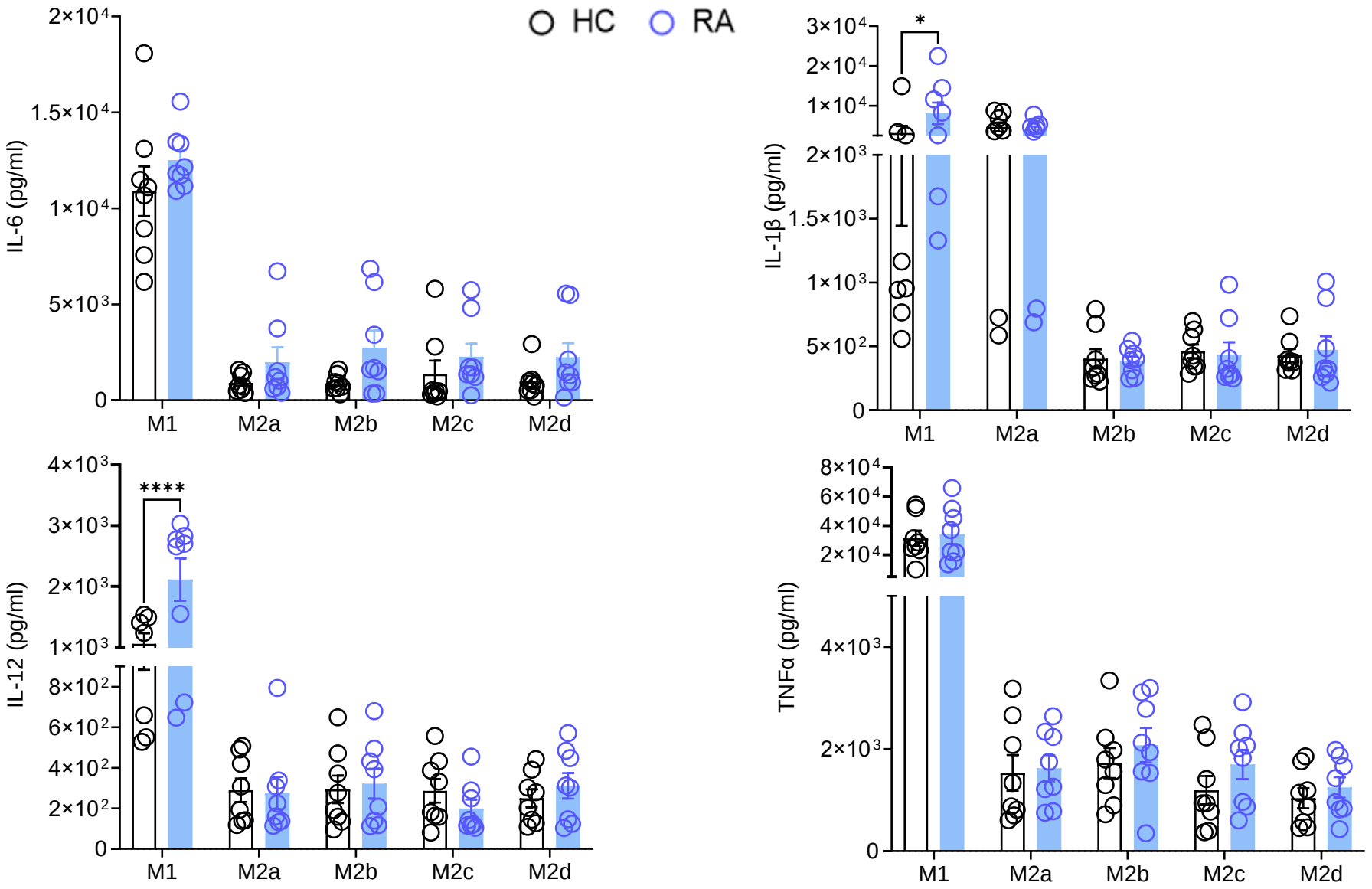
PhD Candidate Karina Kulakova



Increased IRF4 and costimulatory capacity by patient with RA macrophages



Inflammatory profile of macrophage subsets



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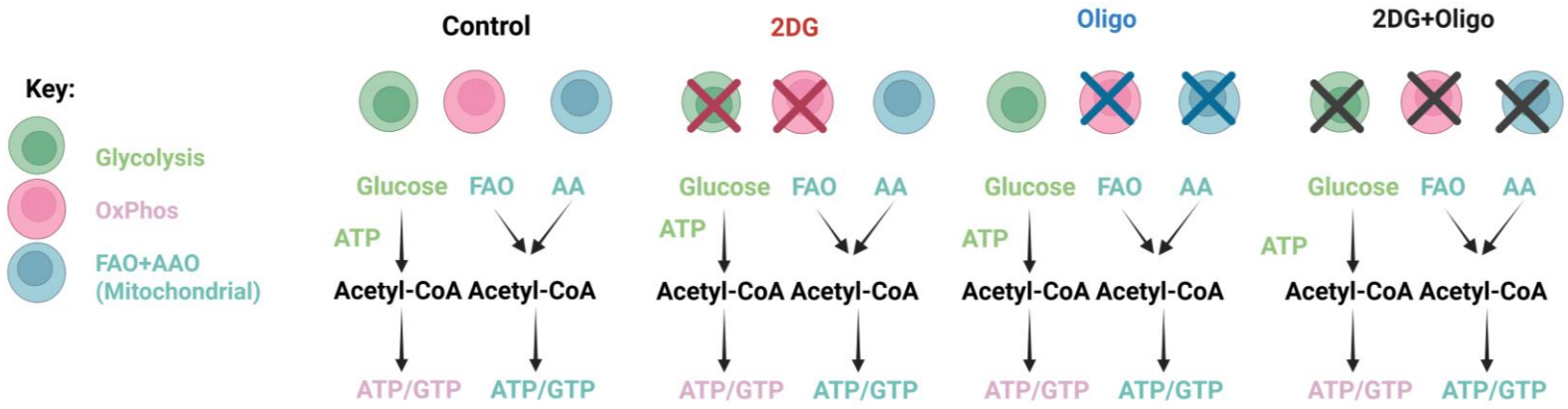
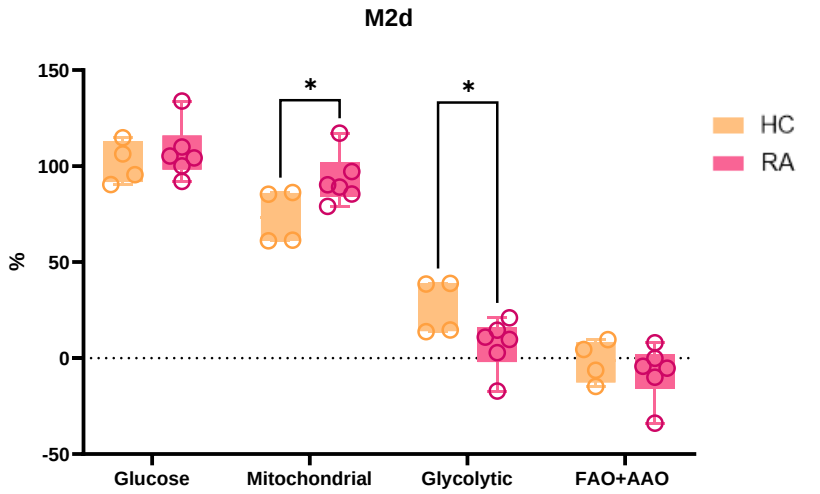
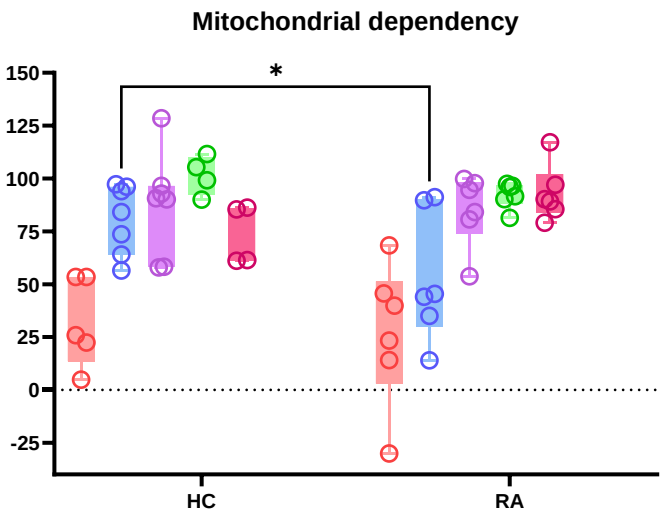
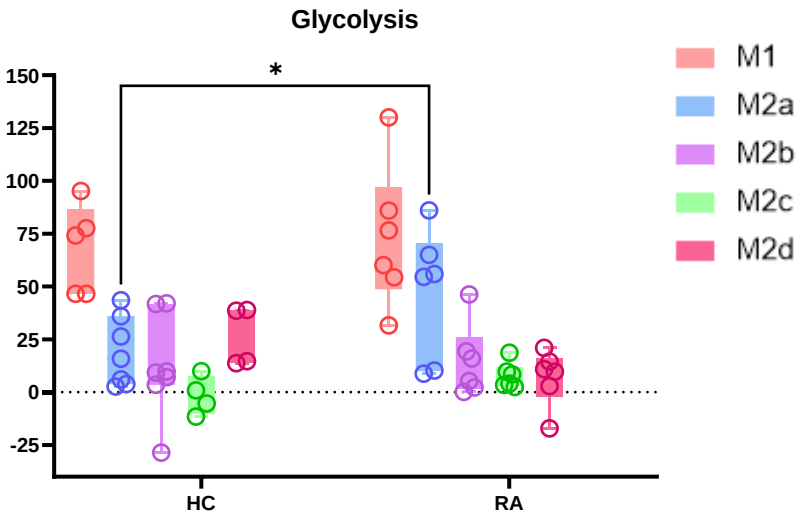
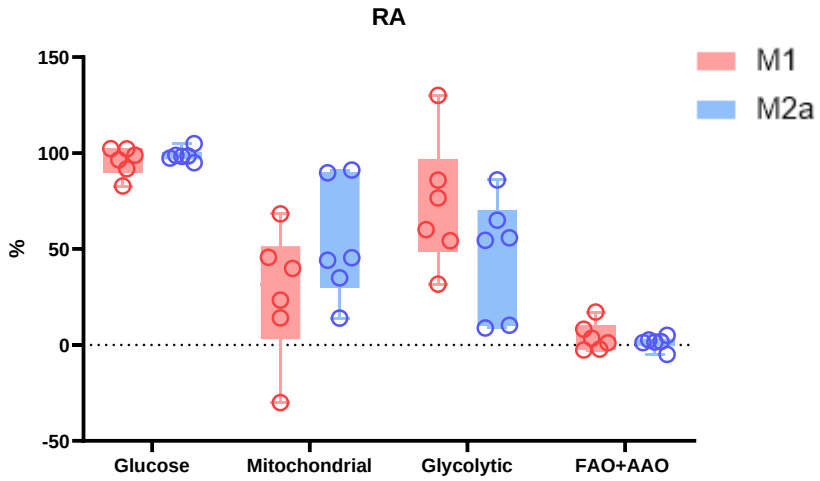
Altered Metabolic Profile of Macrophage Subsets











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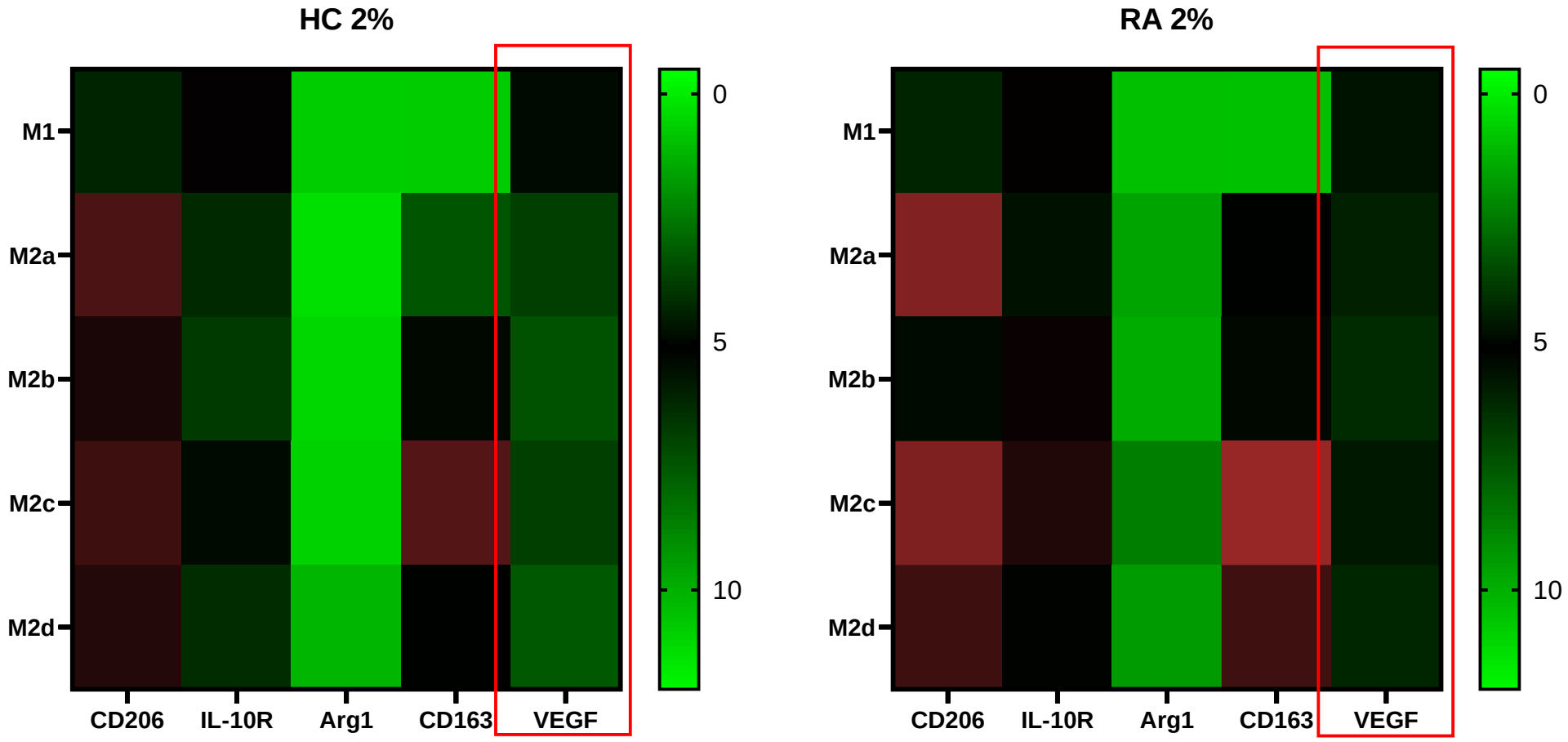
Increased VEGF expression by patient with RA macrophages under hypoxic conditions



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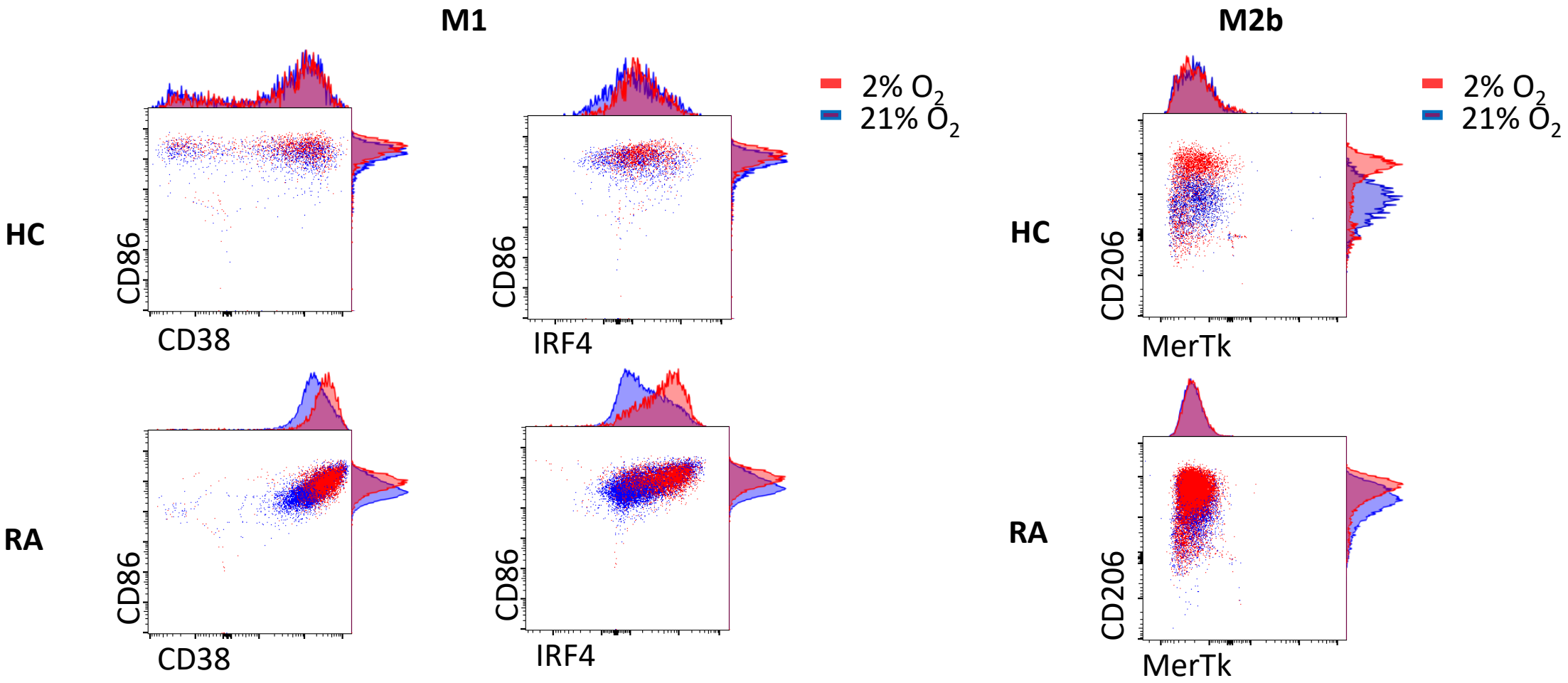
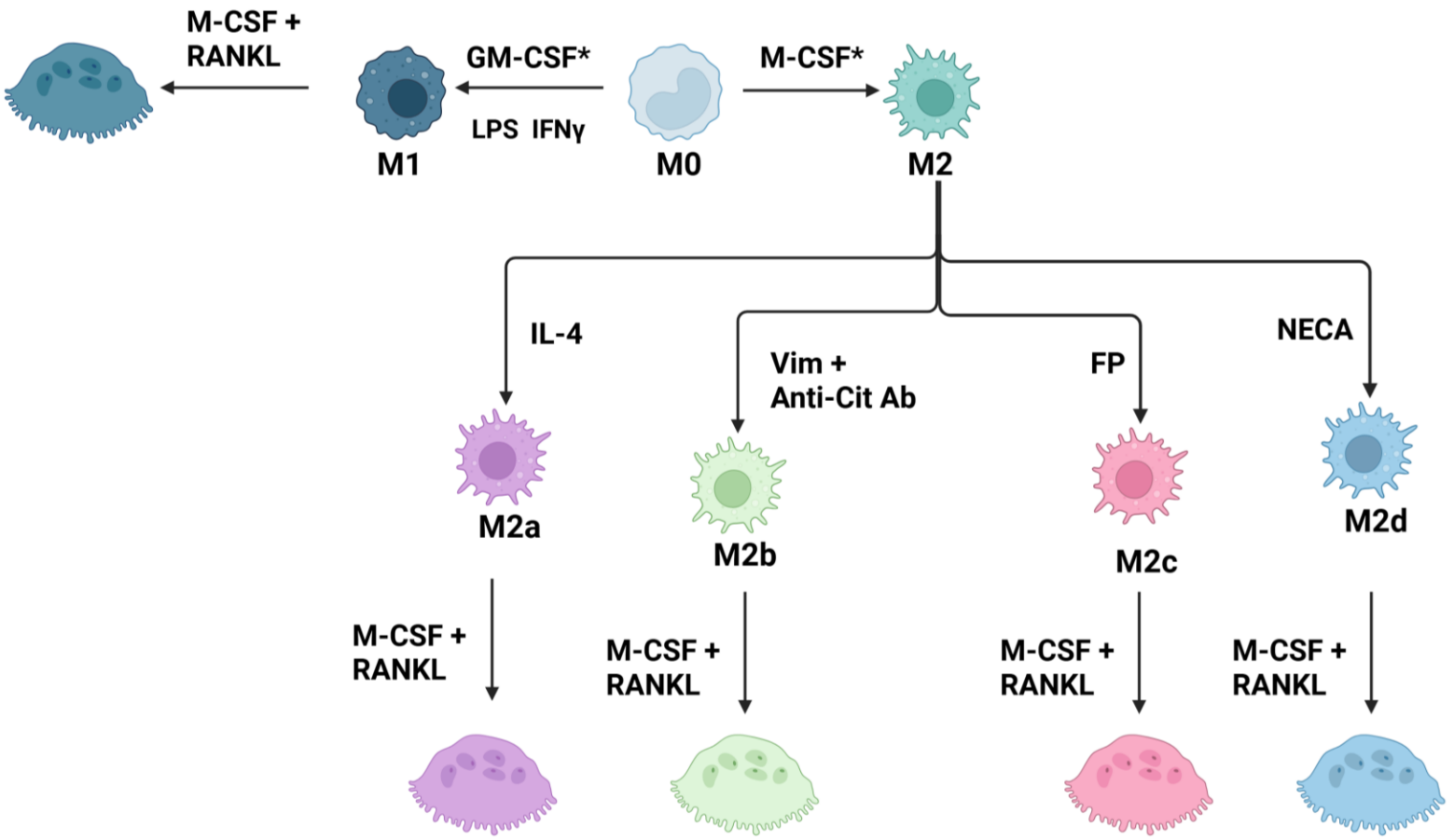
Increased CD86 and IRF4 in response to hypoxia by patient with RA macrophages.



Osteoclasts in RA

- Osteoclasts are progenitors of macrophages
- RANKL promotes OC formation
- Involved in bone erosion and resorption

Osteoclast Differentiation



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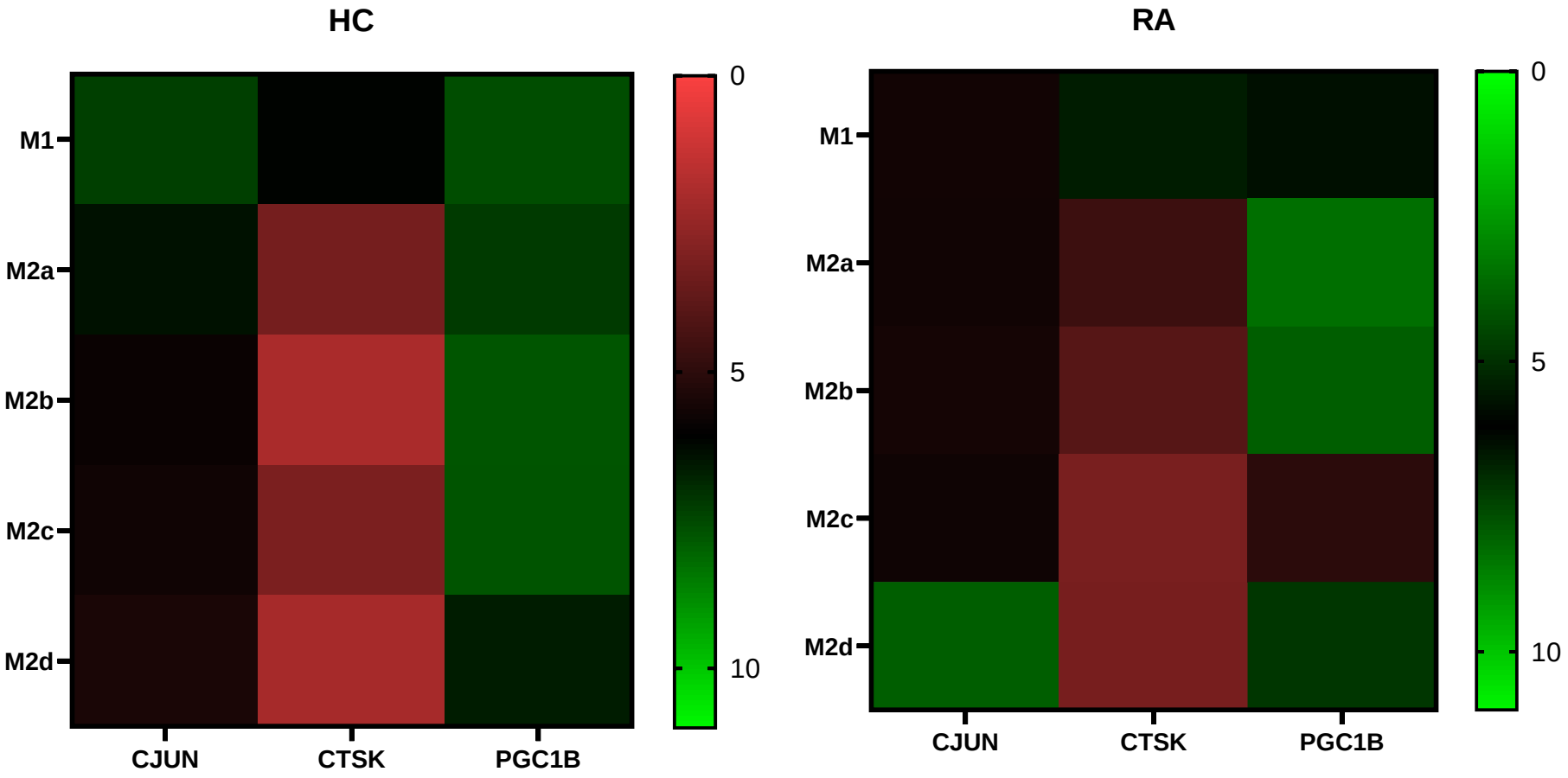
Different macrophage subsets give rise to distinct osteoclast populations

PhD Candidate Karina Kulakova



Gene	Function in osteoclasts
CTSK	Bone resorption, enhance OC activity
C-JUN	Differentiation and joint destruction
PGC1β	Regulates energy metabolism

Acknowledgements

Translational Immunology group

- PI Achilleas Floudas
- Dr. Konstantinos Panagiotidis
- Daphni Doulaptsi-Teeuwen
- Karina Kulakova
- Roozbeh Sanaei

- Patients who participated in the study.

Collaborators: Michael Freely, Paul Leonard, Tanya Levingstone.

Eoghan McCarthy
Laura Durcan

Colm Kirby
Catherine Hughes

Beaumont Hospital
Ospidéal Beaumont

NGH
Naas General Hospital

Funders

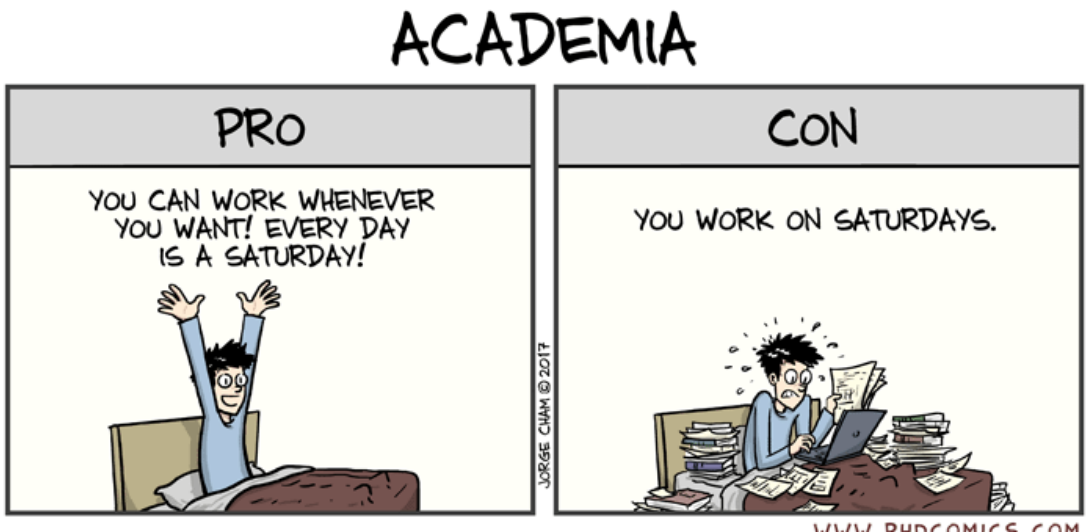
Health Research Board

HRB
An Bord Taighde Sláinte

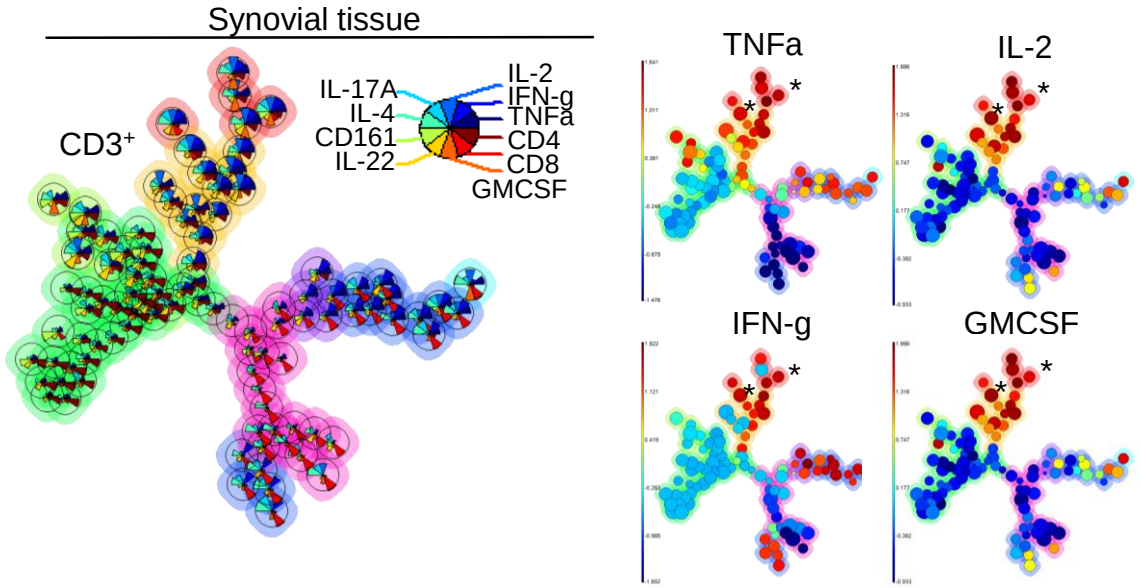
Dublin City University

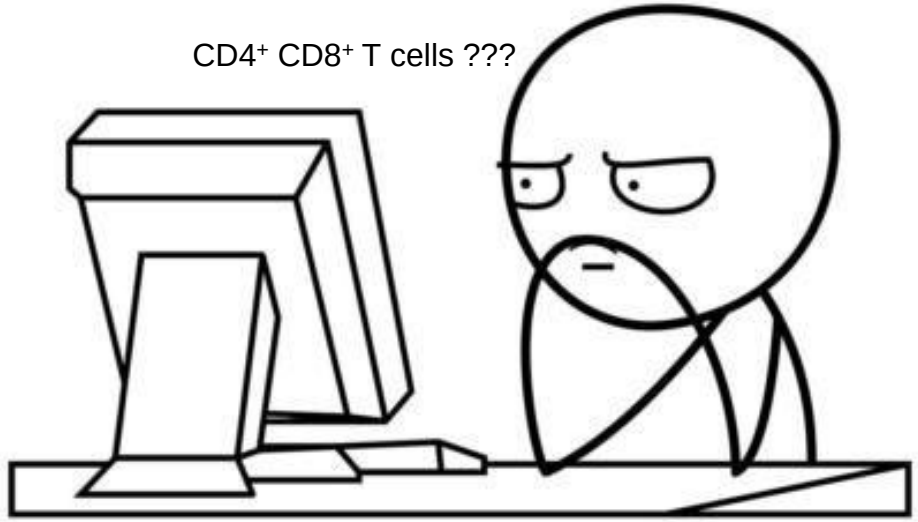
DCU
Ollscoil Chathair Bhaile Átha Cliath
Dublin City University

Looking for motivated students to join the lab!



Not all polyfunctional T cells are the same.





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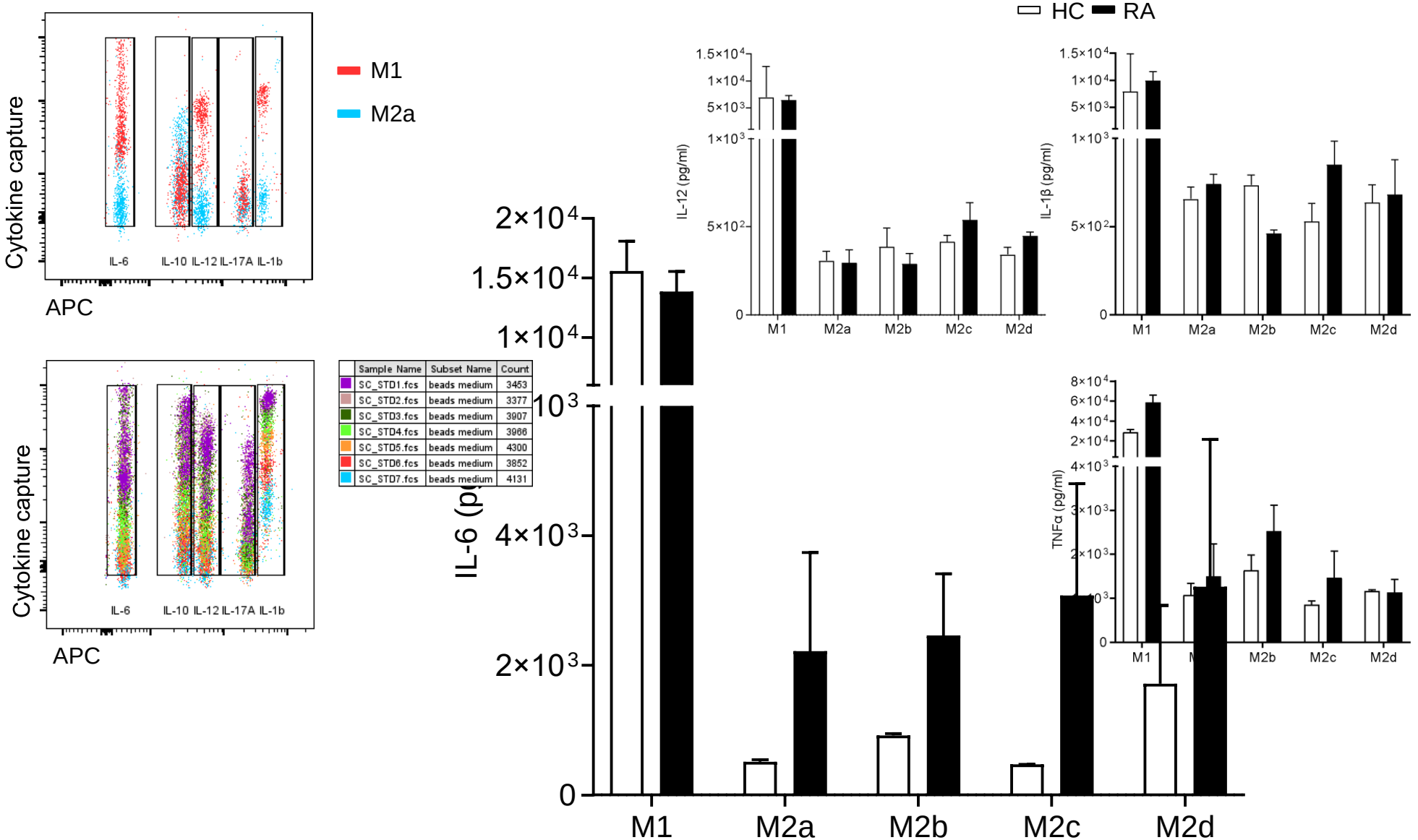
Next time……

PhD Candidate Karina Kulakova

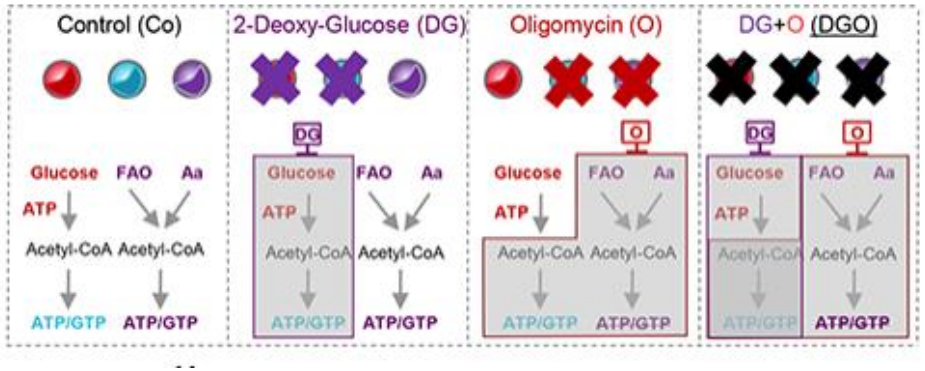
- Investigation of macrophage polarisation and plasticity under hypoxic conditions that simulate the inflamed joint.
- Metabolic comparison of whole blood and monocyte profiles.
- Bioinformatic characterisation of Macrophage – B cell interactions.

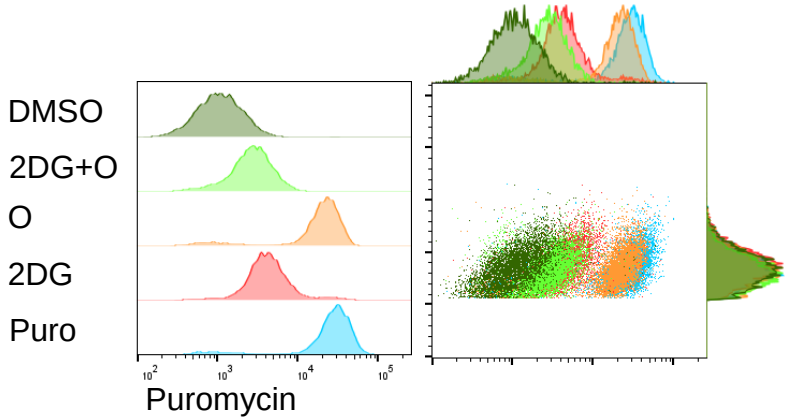
Elevated IL-6 and TNF-a by patient with RA M2 macrophage subsets.

PhD Candidate Karina Kulakova

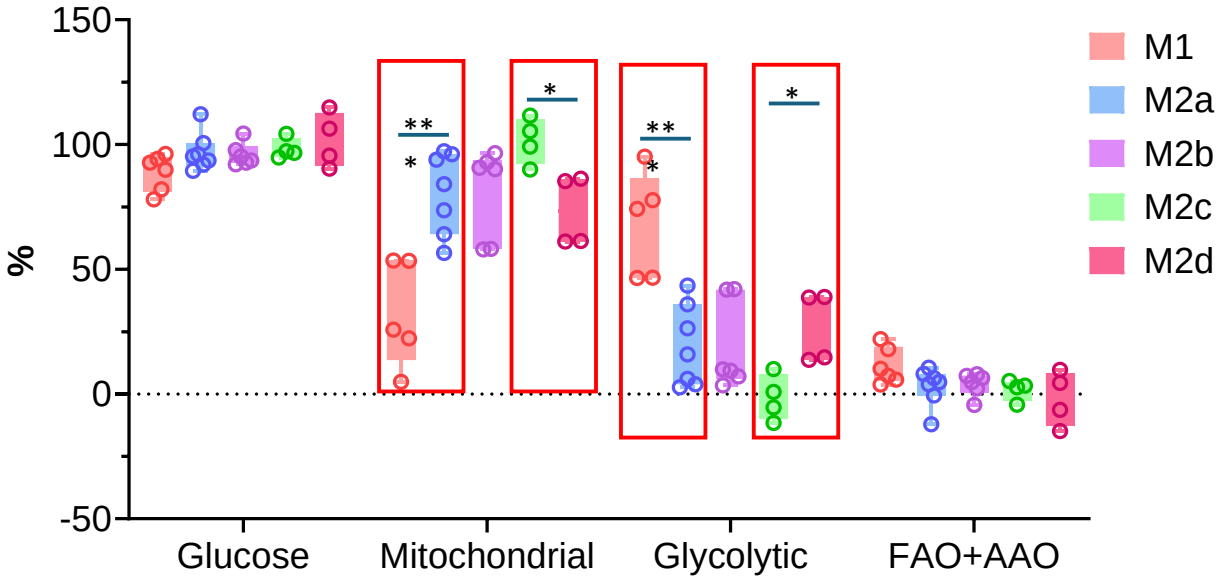


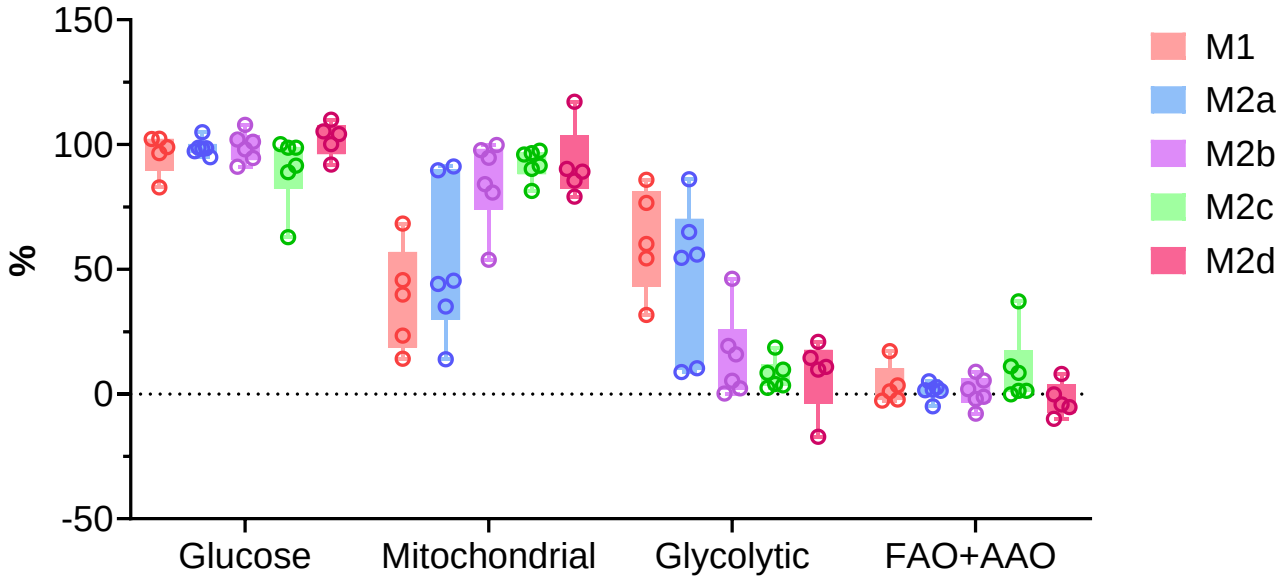
Differential metabolic profile of macrophage subsets





PhD Candidate Karina Kulakova





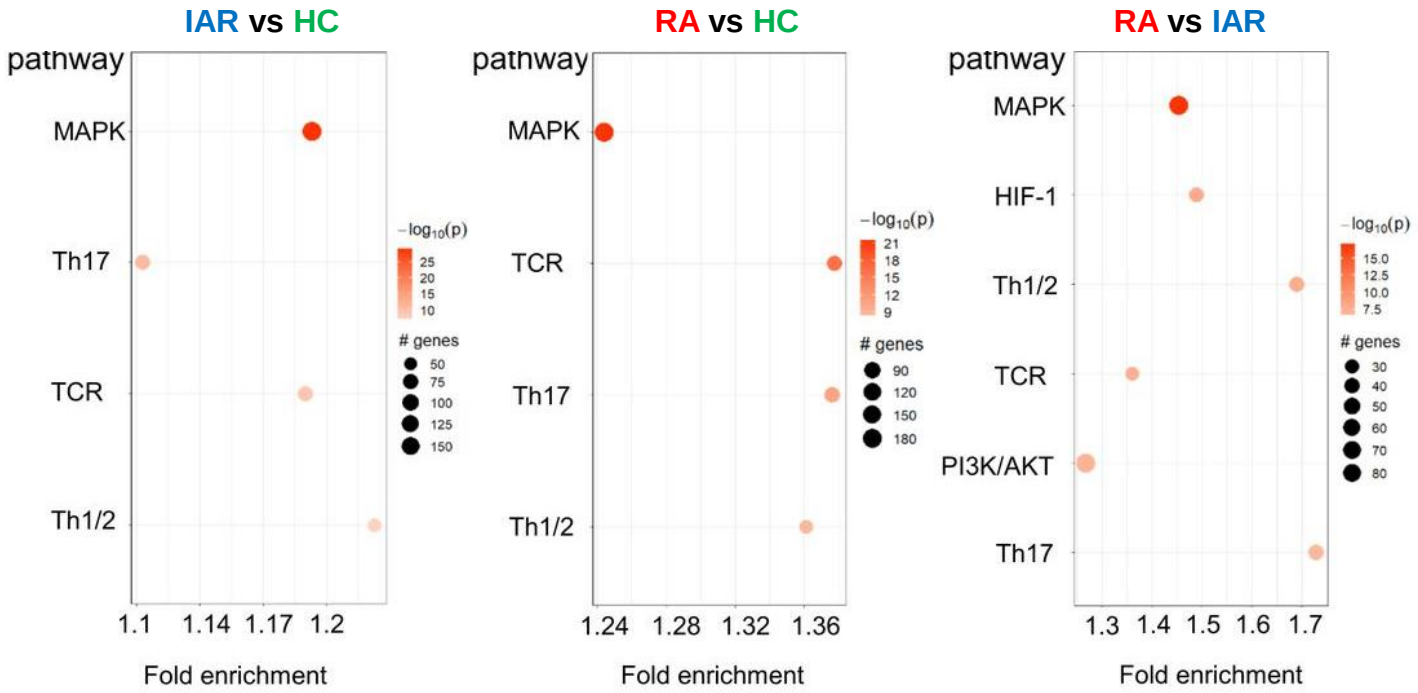
RNAseq analysis of HC, IAR and RA synovial tissue samples.

HC = healthy control synovial tissue

IAR = arthralgia synovial tissue

❑ no signs of clinical inflammation

RA = RA patient synovial tissue



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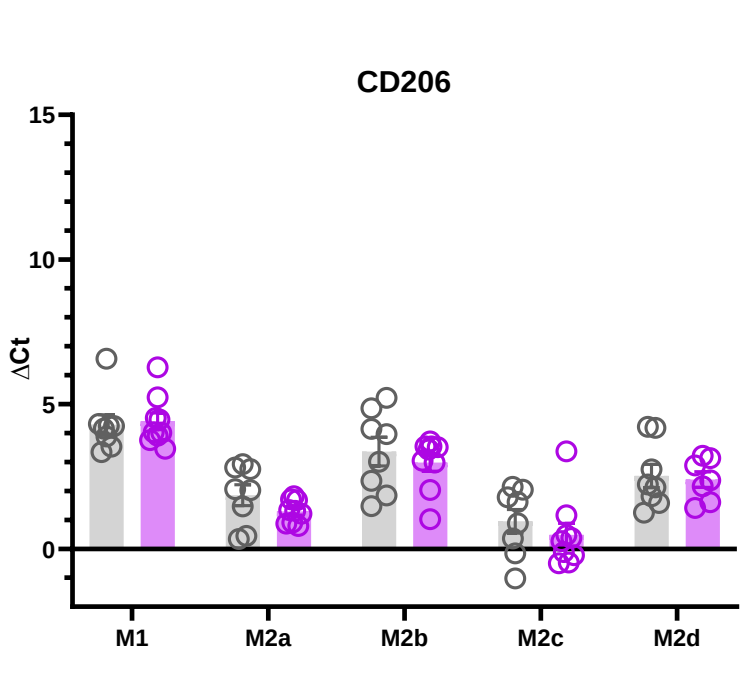
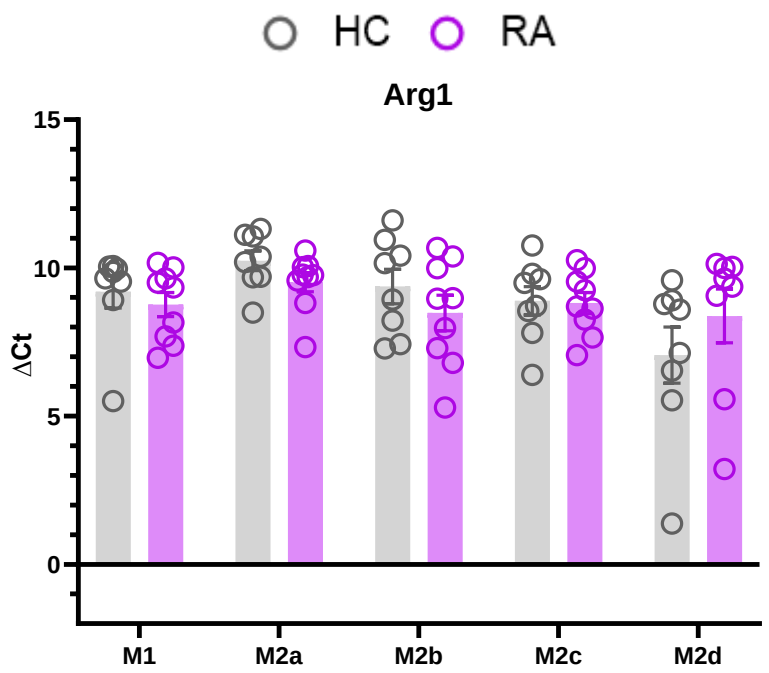
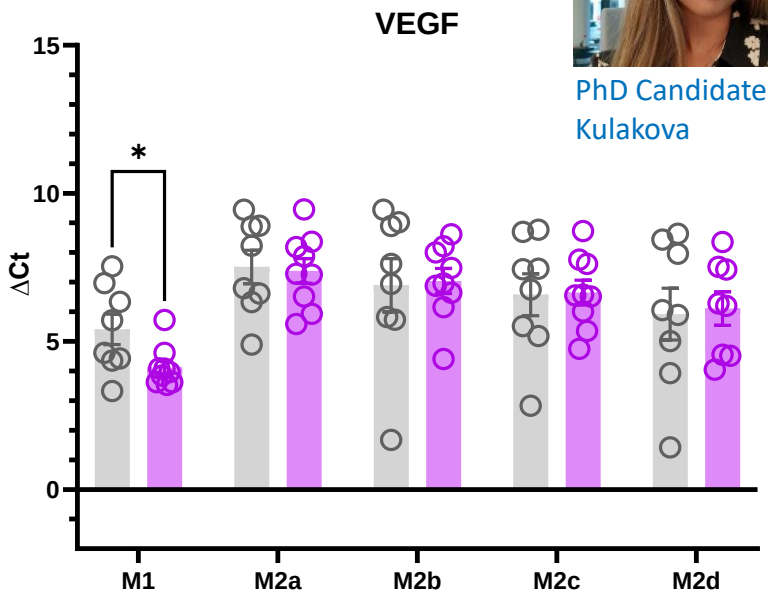
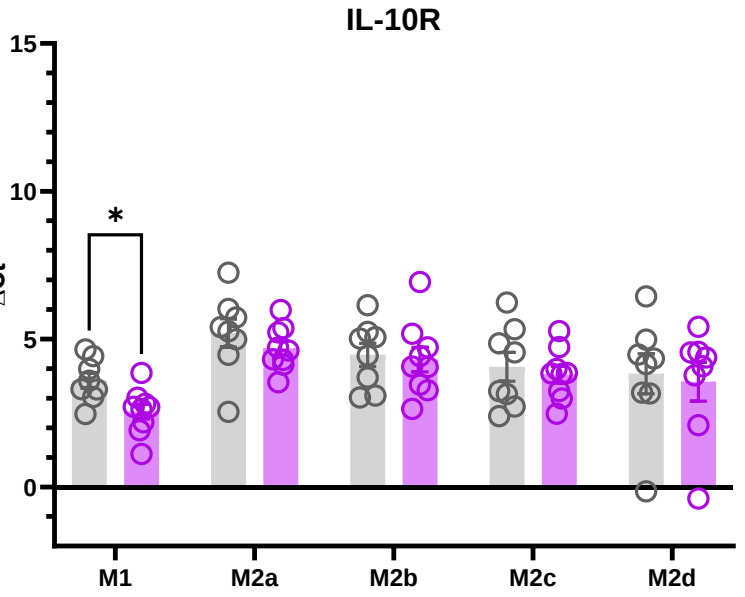
HC and patient with RA macrophage polarisation profile

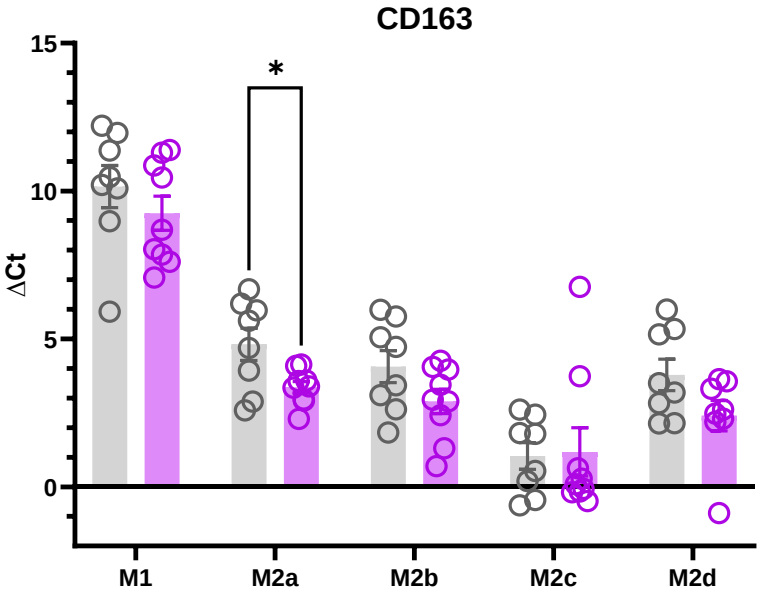
PhD Candidate Karina Kulakova









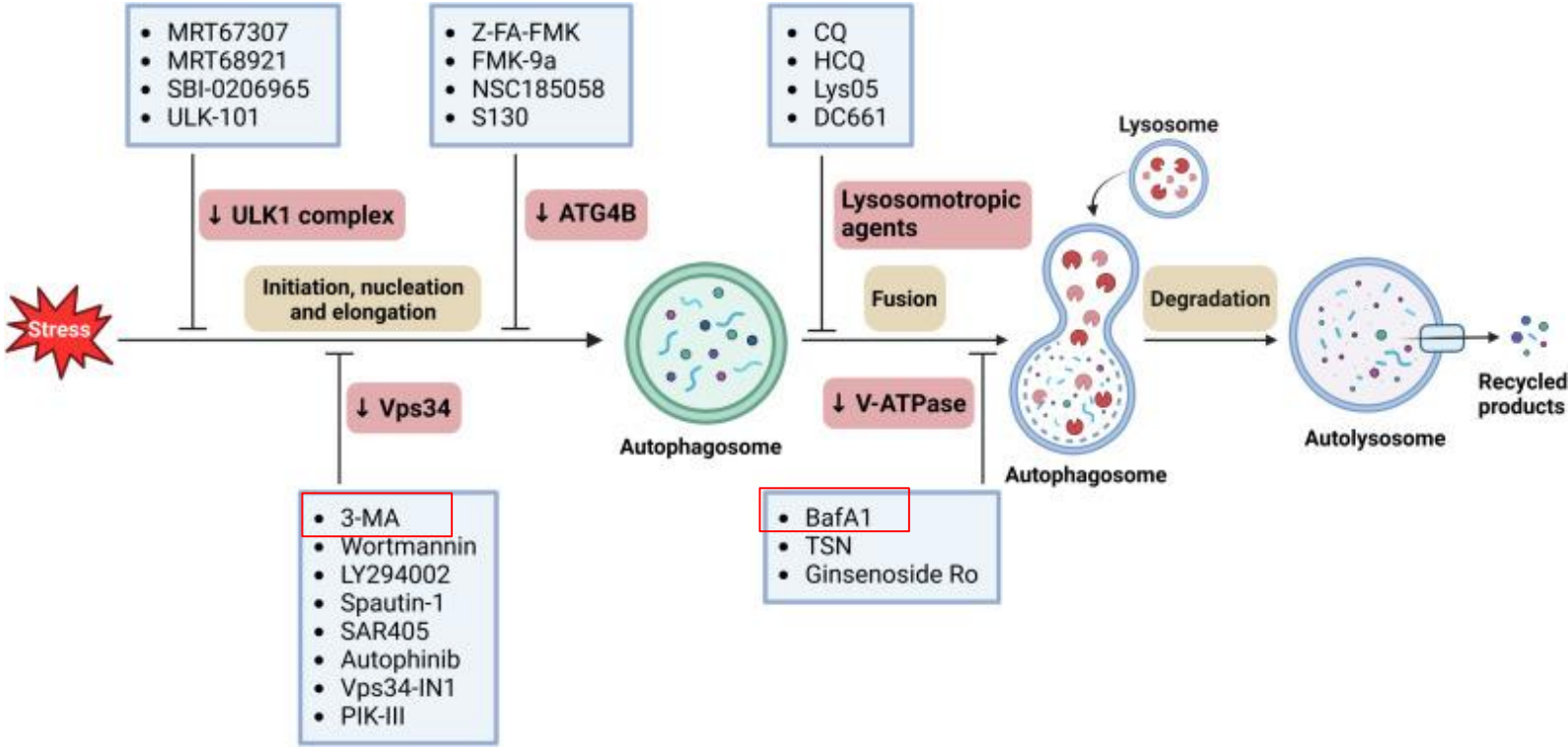


Gene	Function in macrophages
CD206	Expressed by M2a, tissue repair and wound healing
IL-10R	Released by M2b
Arg1	M2c marker, collagen synthesis, fibrosis and cell proliferation
CD163	M2c local anti-inflammatory response by reducing haemoglobin levels
VEGF	M2d proangiogenic properties

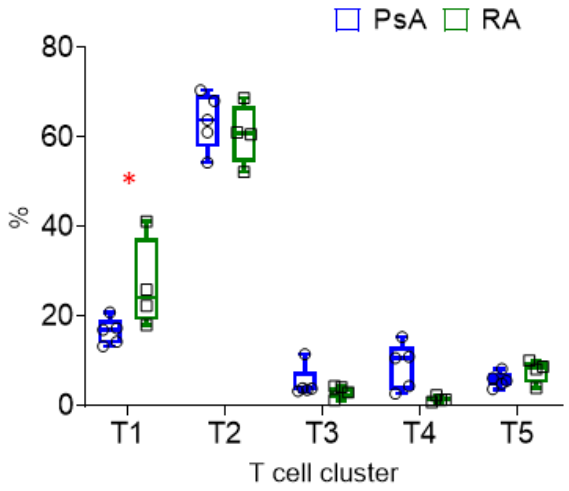
PhD Candidate Daphni Doulaptsi-Teeuwen

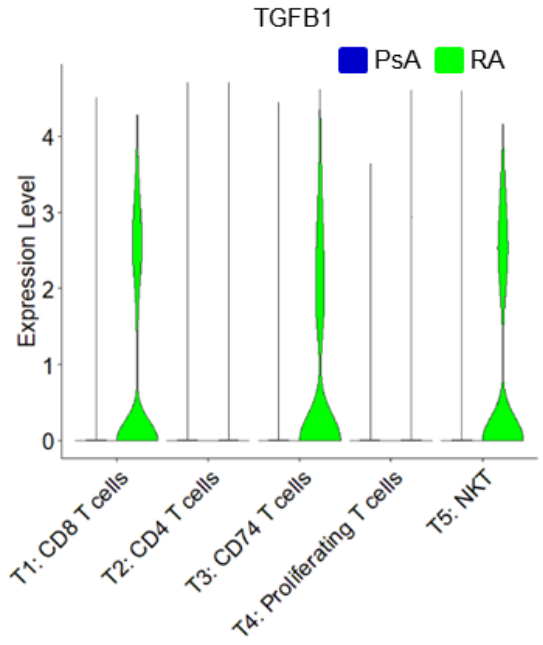
The interplay between CD4+ T cells autophagy, metabolism and fitness in Rheumatoid arthritis.

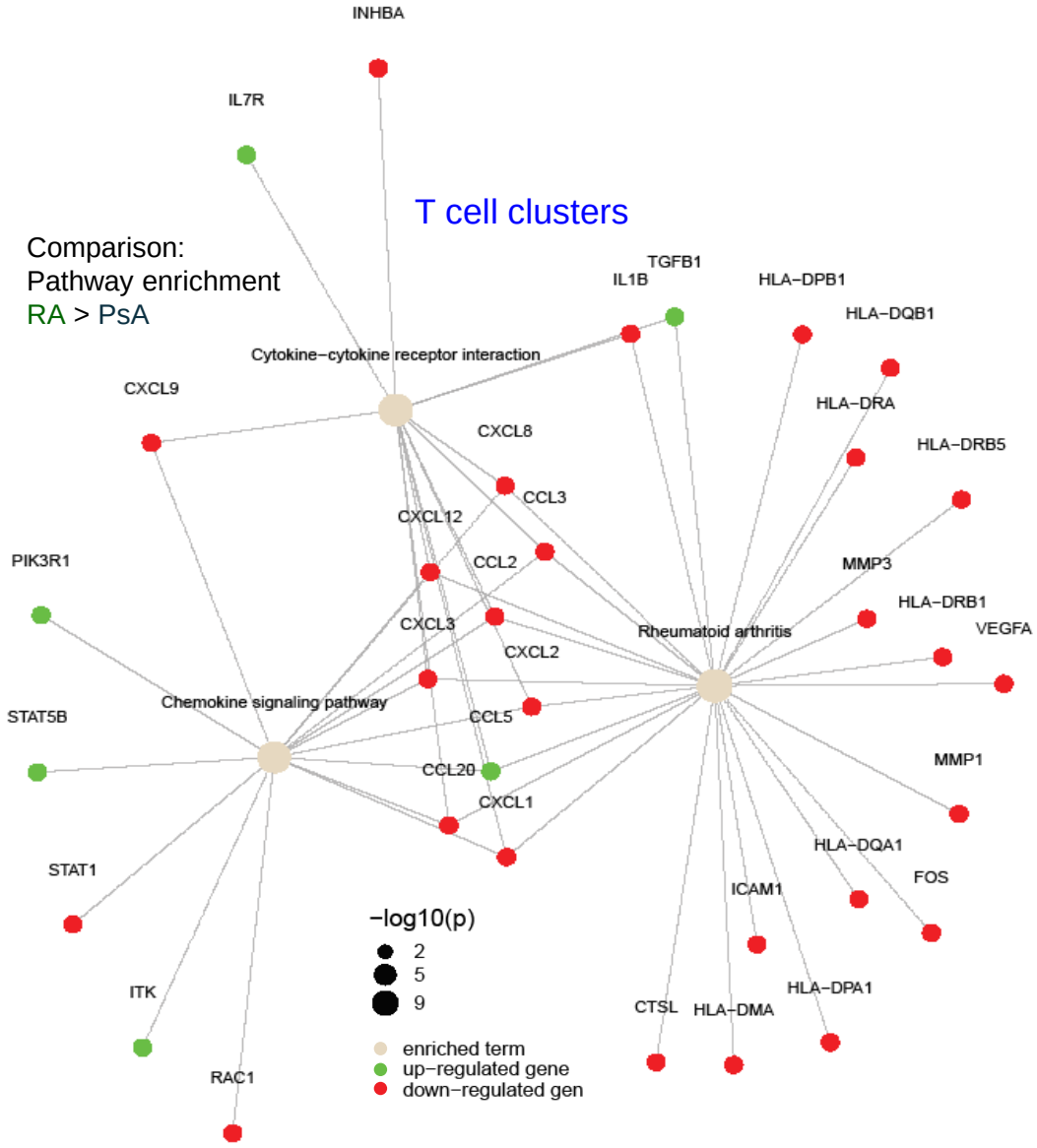
- **Hypothesis:** T cell autophagy is potentially promoting T cell survival and fitness in the inflamed joint.
- Mitigation of cellular stress conditions.
- Promotion of proliferation under Hypoxia.



RA but not PsA patient synovial T cells maintain high expression of TGFB1







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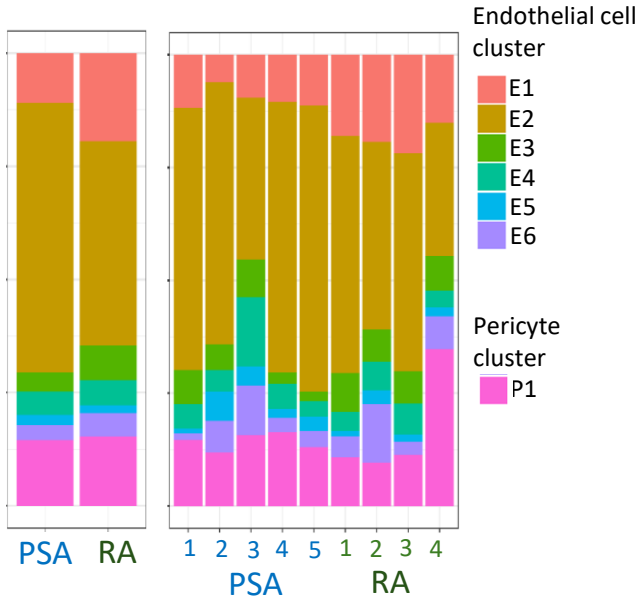
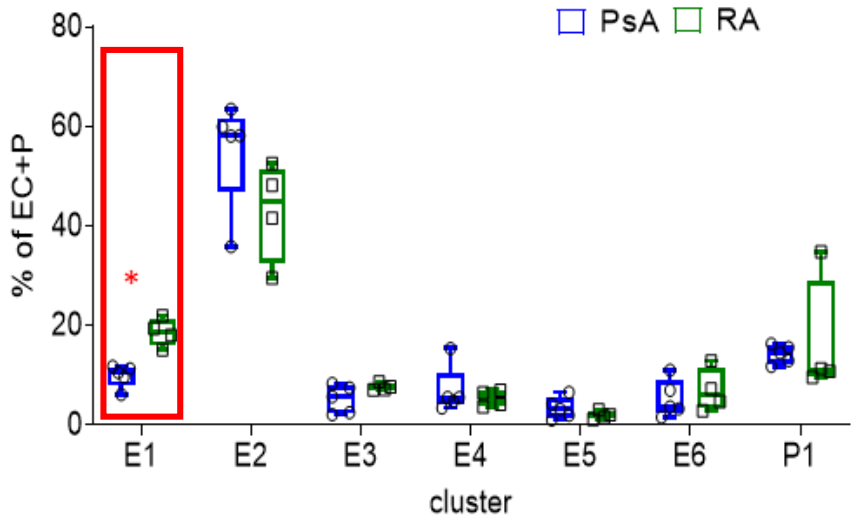
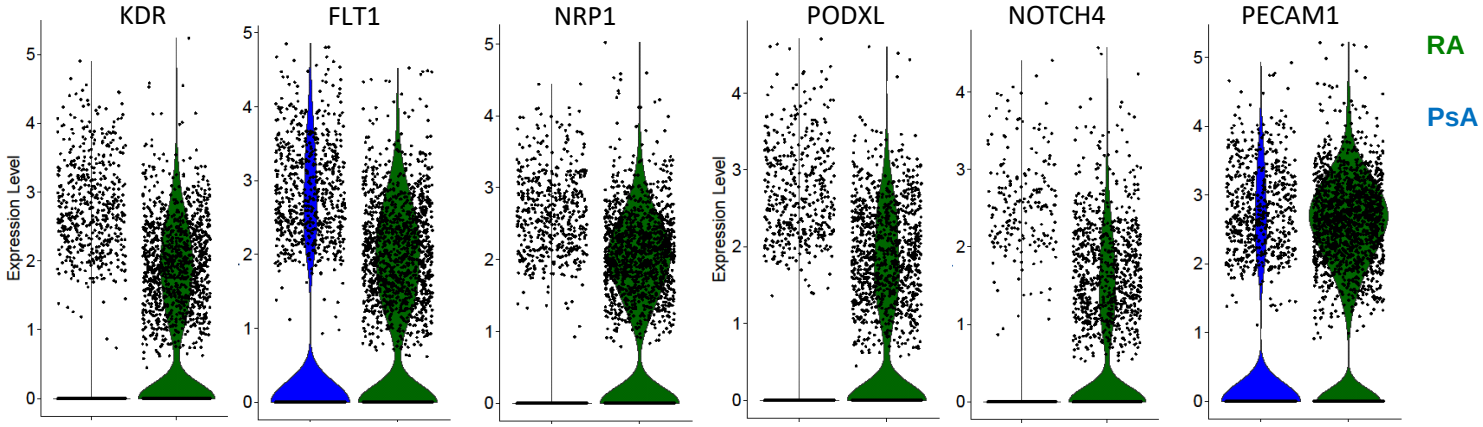
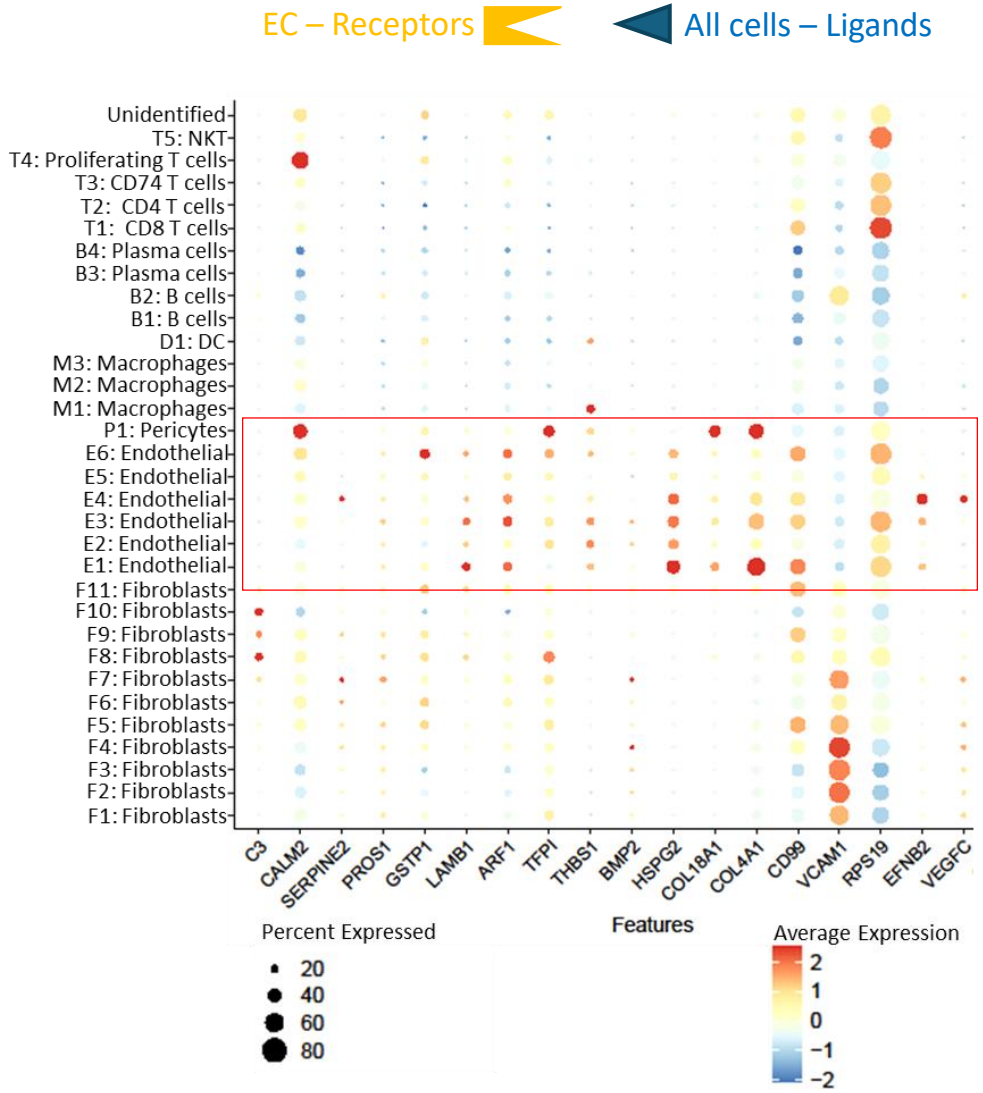
Differential EC cluster abundance and transcriptomic profile in RA and PsA



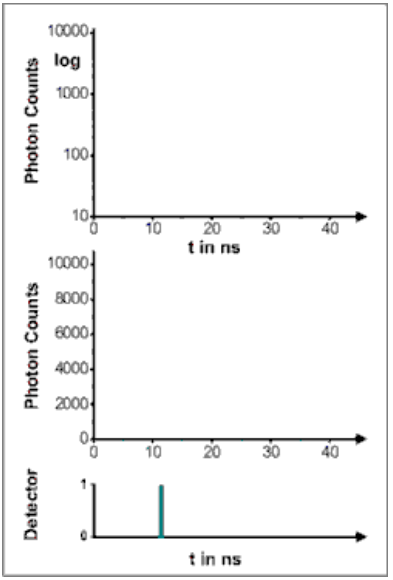


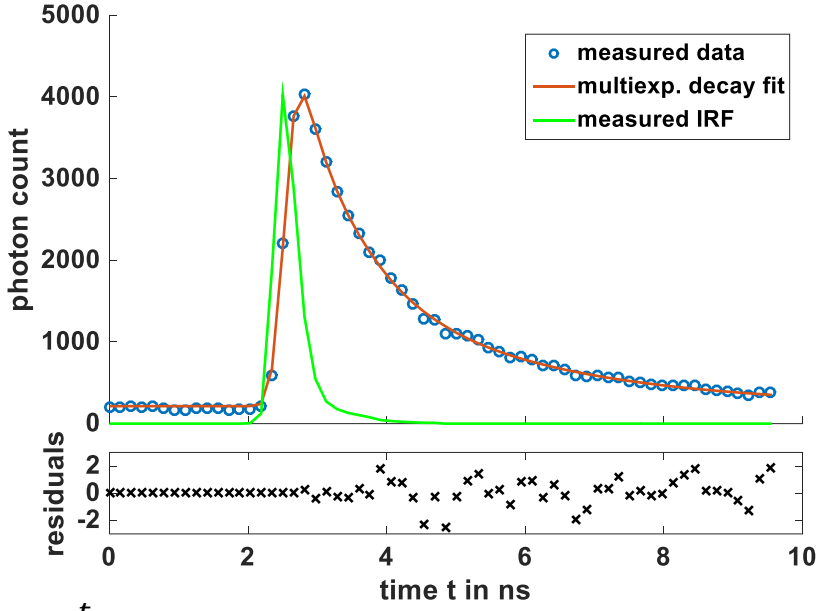




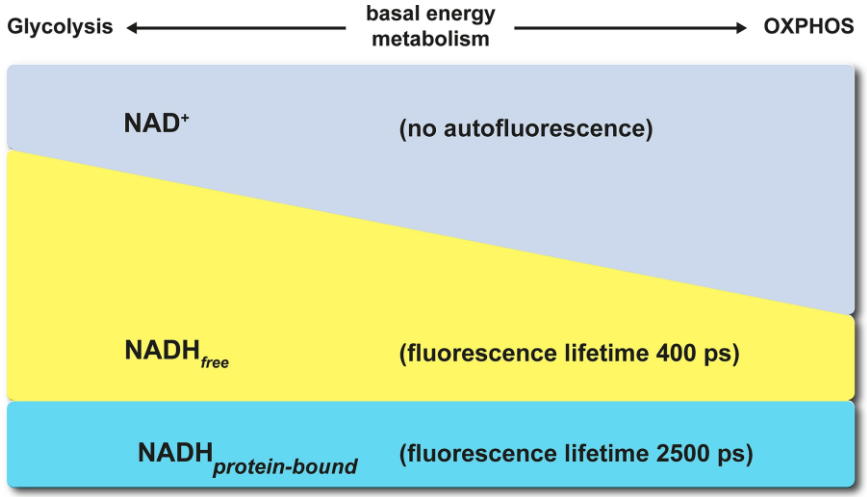
Floudas et al., ARD, 2022b

Multi-exponential decay fitting of Fluorescence lifetime imaging microscopy (FLIM)





$$I(t) = \alpha_1 e^{-\frac{t}{\tau_1}} + \alpha_2 e^{-\frac{t}{\tau_2}} + c$$



Lakner, P. H., **Monaghan, M.G**., Moller, Y., Olayioye, M.A., and Schenke-Layland, K. Scientific Reports, 7, 42730, 2017

Serra, V.V., Neto, **Neto, N.G.B**., Andrade S.M., and Costa, S.M.B. Langmuir, 33, 7680, 2017.

Nuno Neto, Monaghan lab

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