

Delineation of Fibroblast-Like Synoviocyte-mediated immune responses in the pathogenesis of rheumatoid arthritis

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PhD Candidate

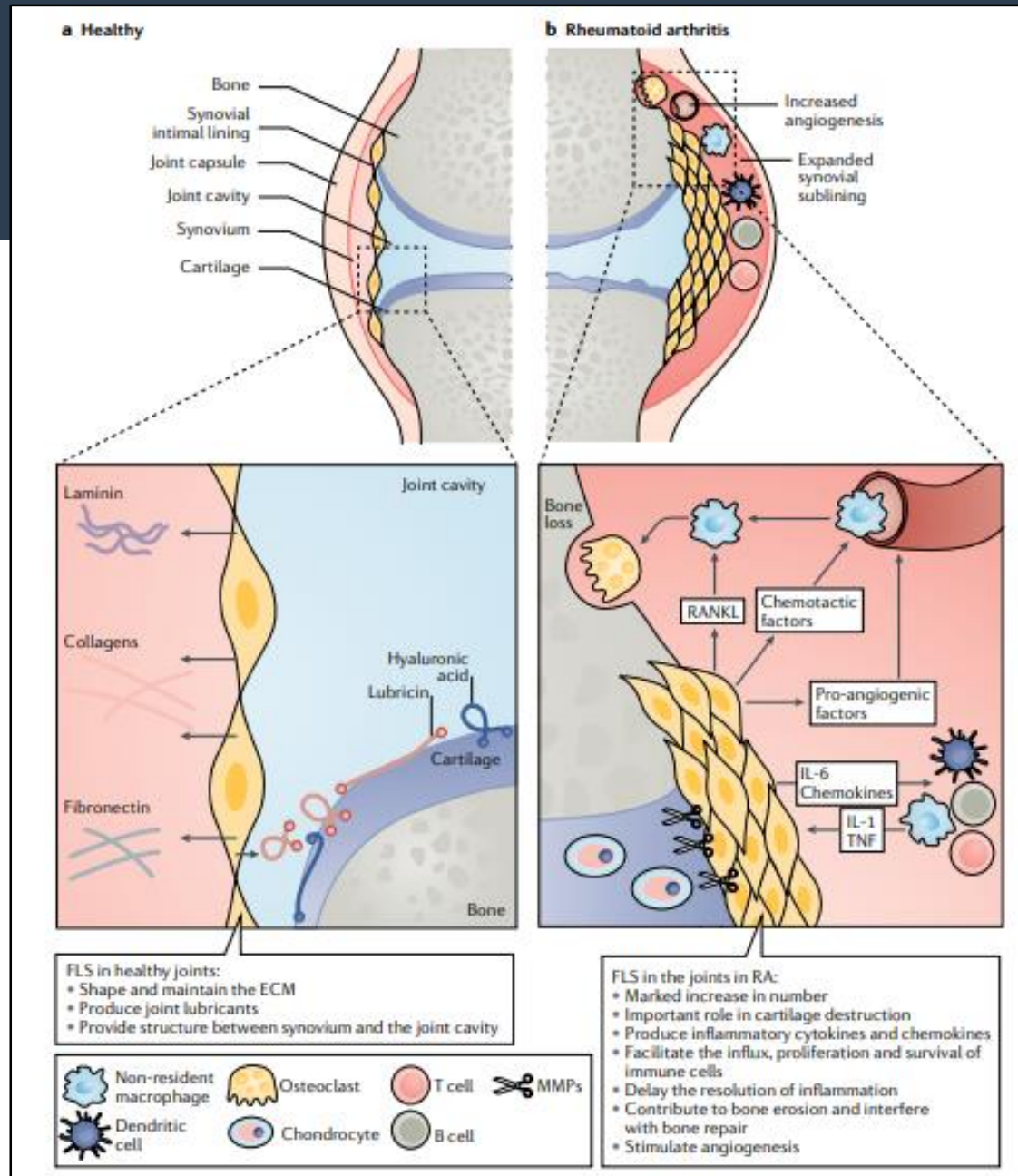
Laboratory of Rheumatology, Inflammation & Autoimmunity

School of Medicine, University of Crete

Introduction

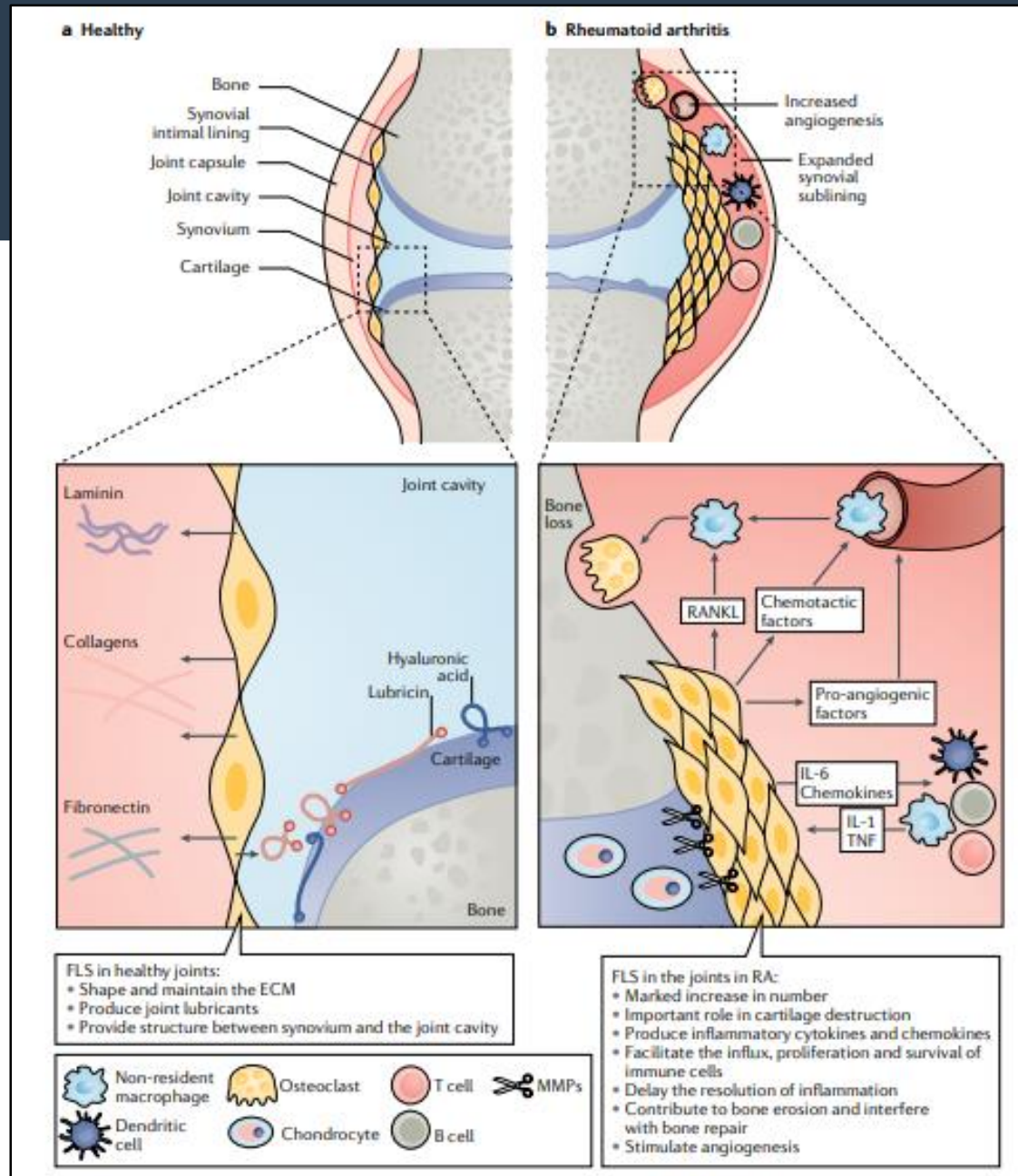
Rheumatoid Arthritis (RA):

- complex pathogenesis
- severe inflammation of the synovial membrane
- tissue destruction
- subchondral bone erosions



Introduction

FLS: Fibroblast-Like Synoviocytes
Main stromal cells of the joint



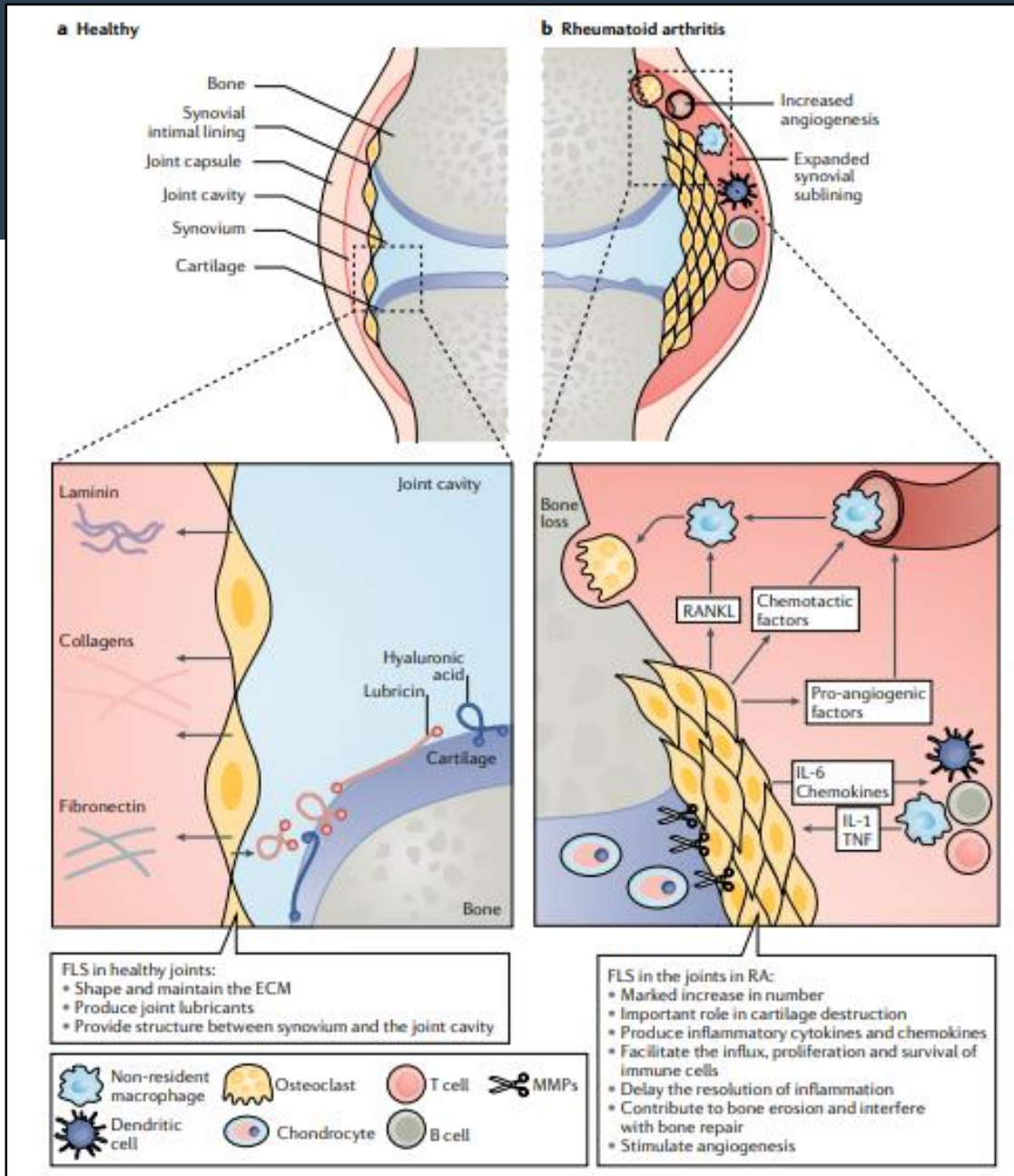
Introduction

FLS: Fibroblast-Like Synoviocytes

Main stromal cells of the joint

In the normal synovium:

- Sub-lining and lining layers
- Secrete ECM components
- Synovial fluid components
- Ensure cartilage integrity, joint lubrication
- Express innate immune receptors



Introduction

FLS: Fibroblast-Like Synoviocytes

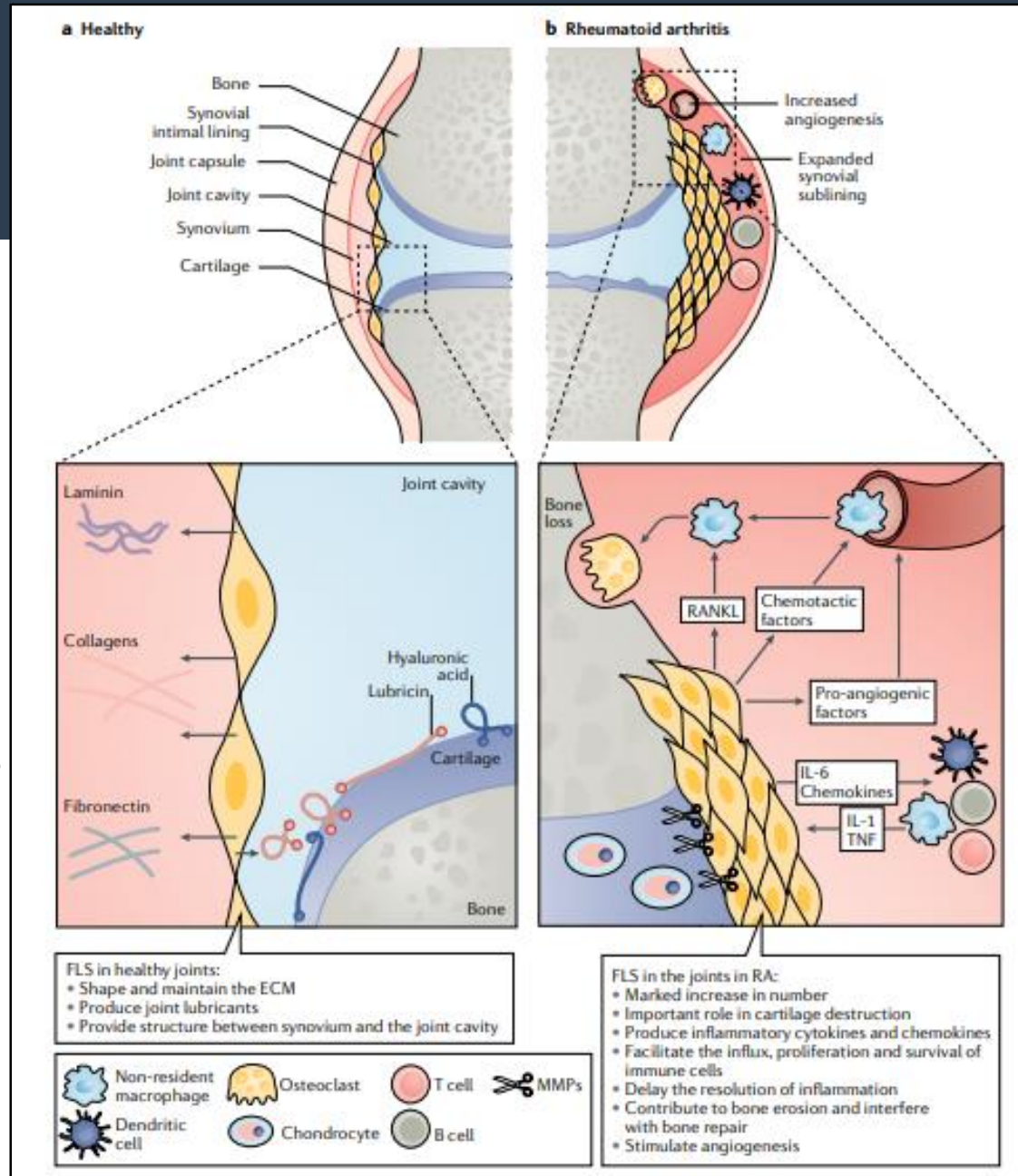
Main stromal cells of the joint

In the normal synovium:

- Sub-lining and lining layers
- Secrete ECM components
- Synovial fluid components
- Ensure cartilage integrity, joint lubrication
- Express innate immune receptors

In the RA Synovium:

- Proliferation
- Infiltration
- Cartilage attachment & damage
- Osteoclastogenesis
- Angiogenesis
- Immune cell stimulation



Introduction

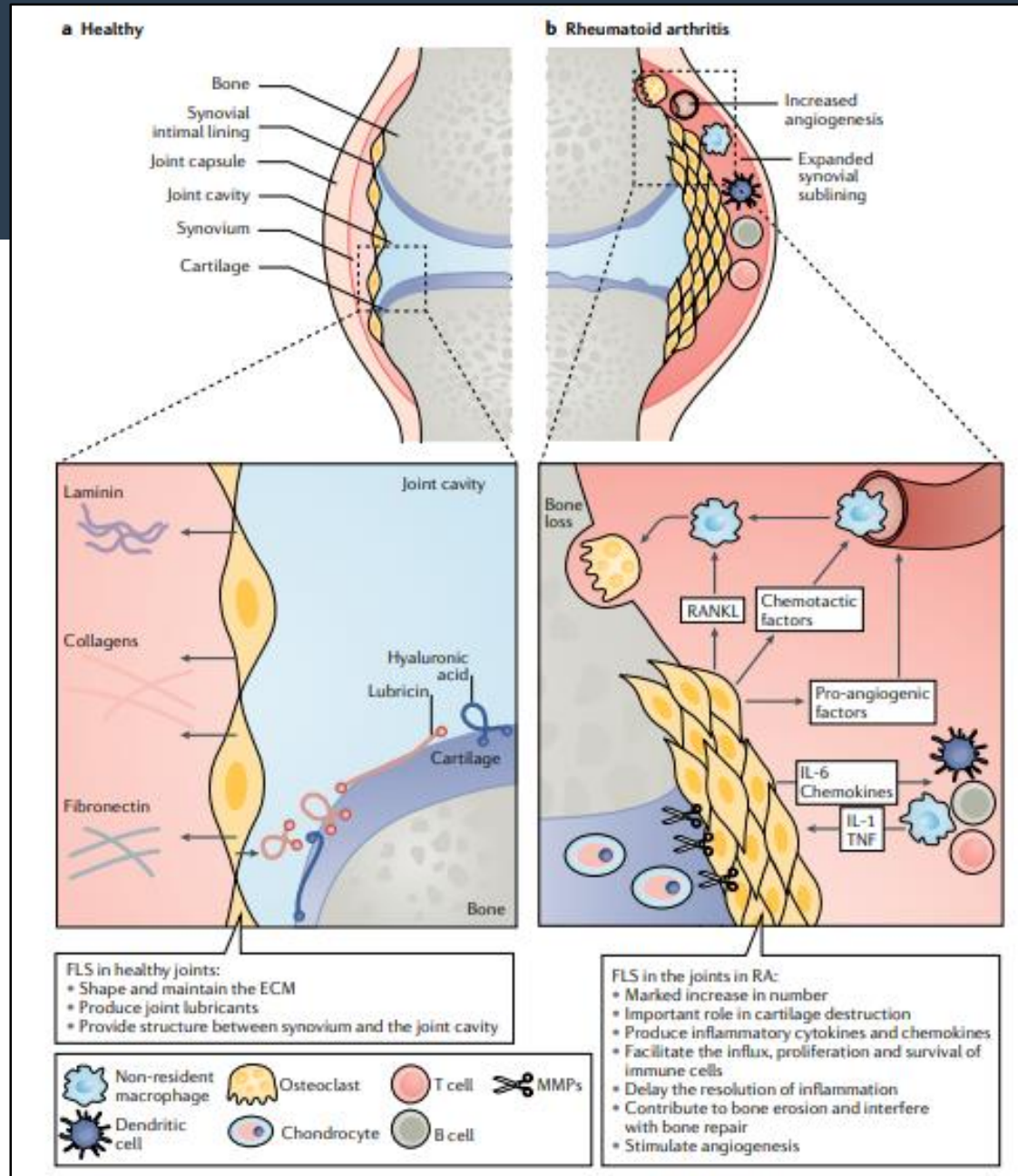
Treating RA

- Corticosteroids
- Anti-rheumatic Drugs
- Biological Agents

Unmet Needs:

- Low response rates (~30%)
- Side effects

Many approved expensive therapies may affect FLS function, directly or indirectly

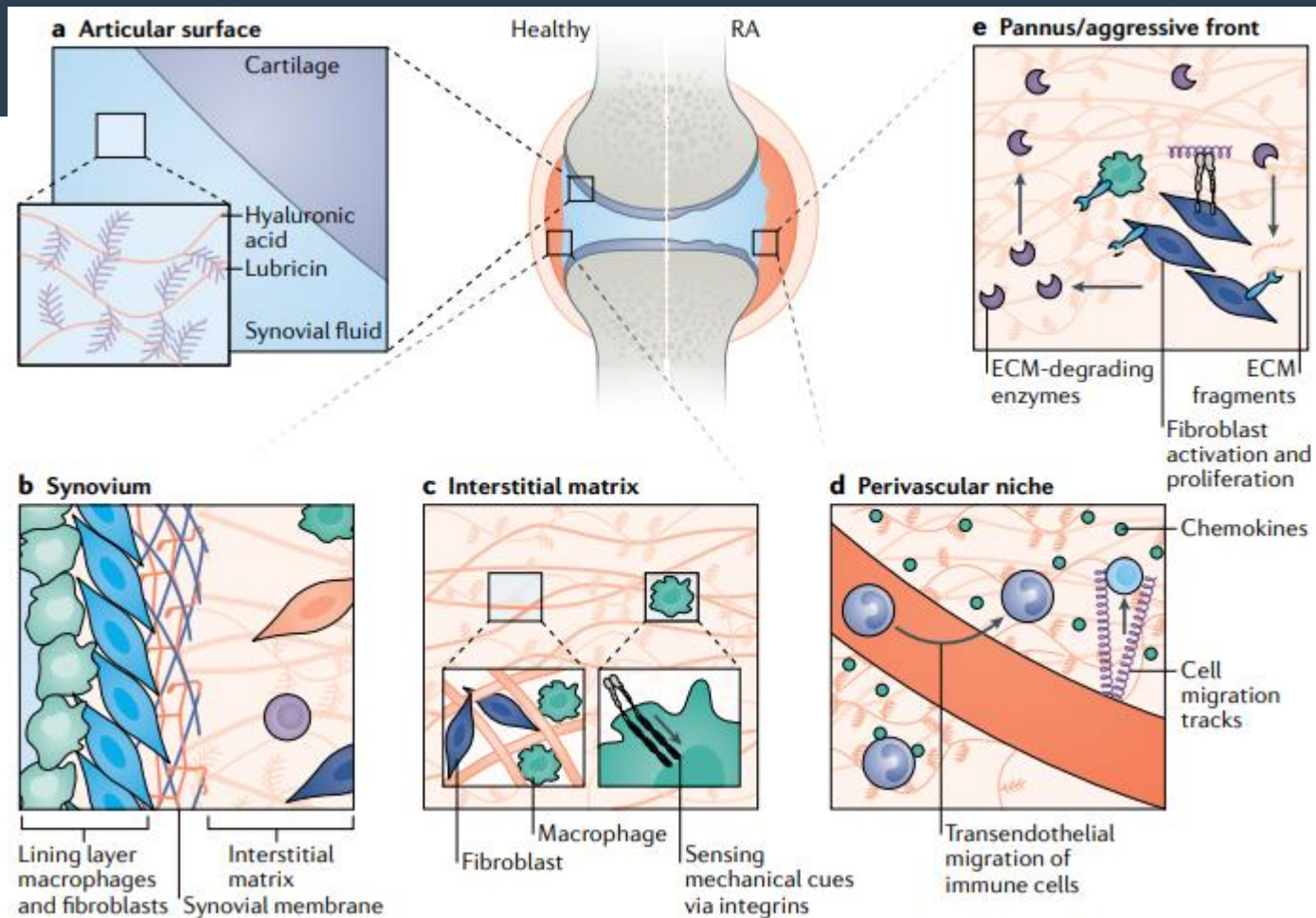


Objective

The focus in RA research has been on immune cells.

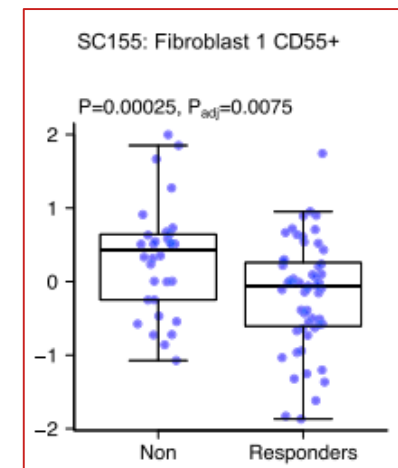
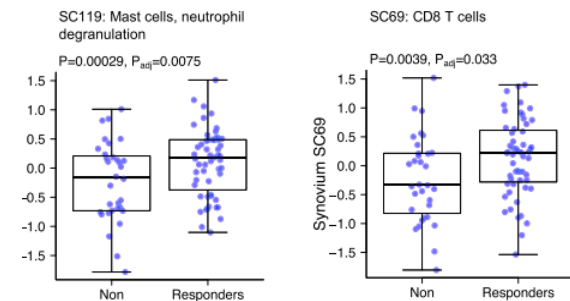
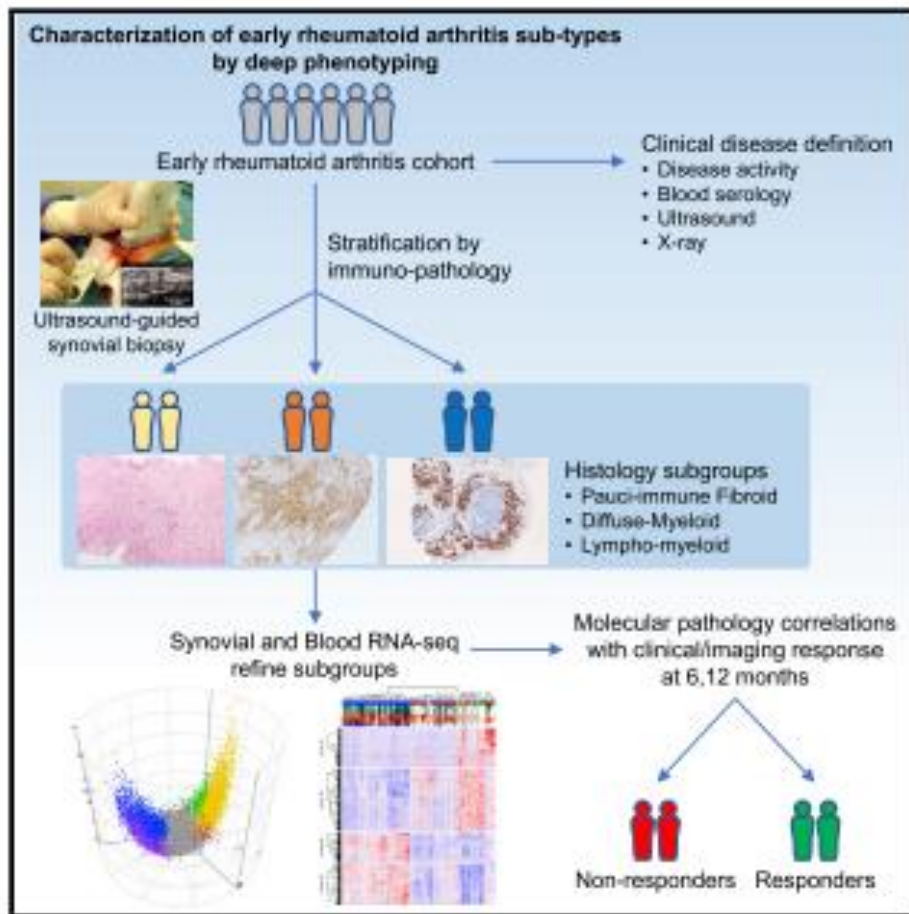
How do FLS contribute to the autoreactive immune response?

The tissue microenvironment affects inflammation in RA



Physical properties, mechanical cues
Patterning of soluble factors
Direct cell signalling

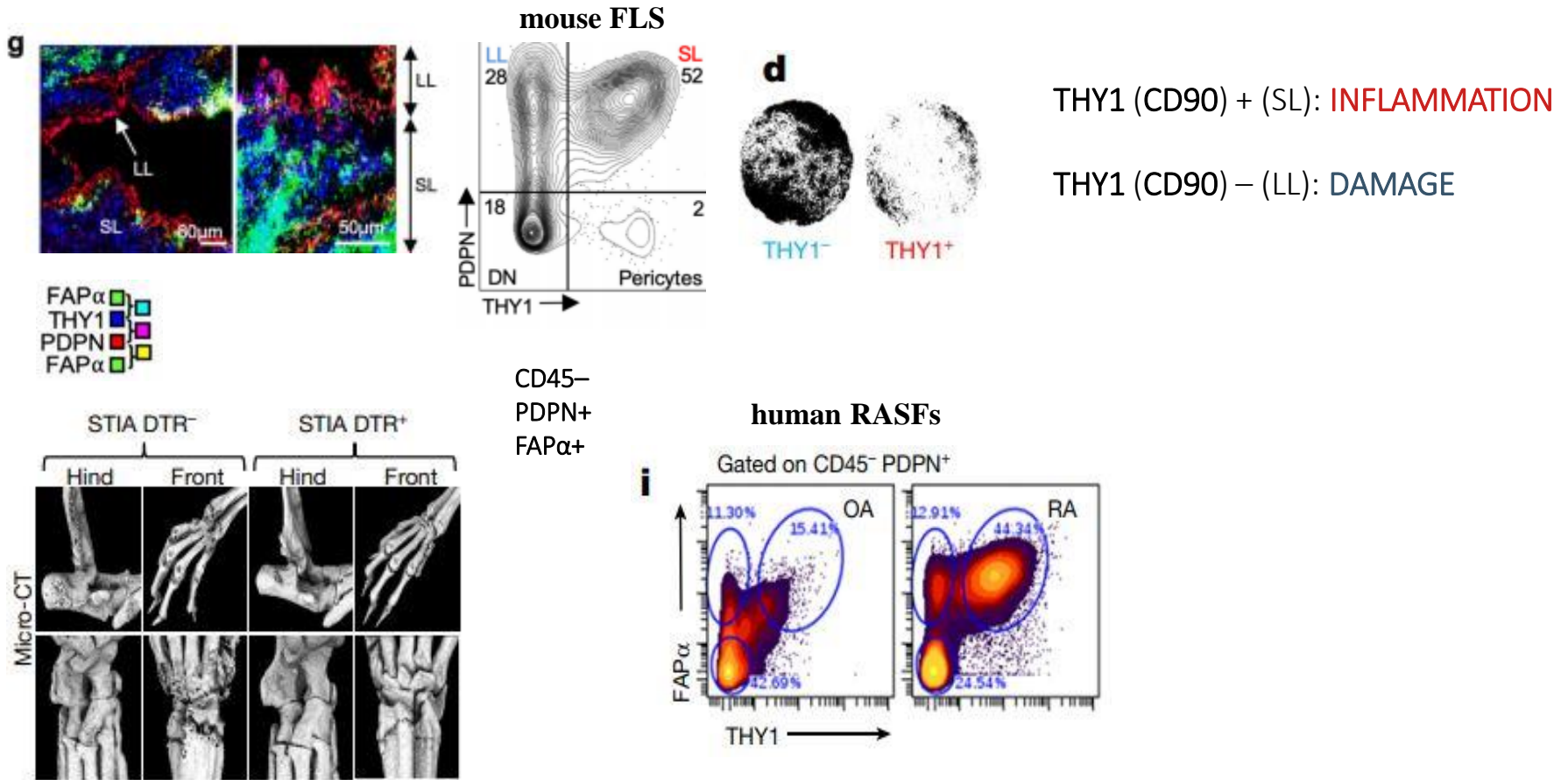
Molecular portraits of early RA identify clinical & treatment response phenotypes



<https://peac.hpc.qmul.ac.uk/>

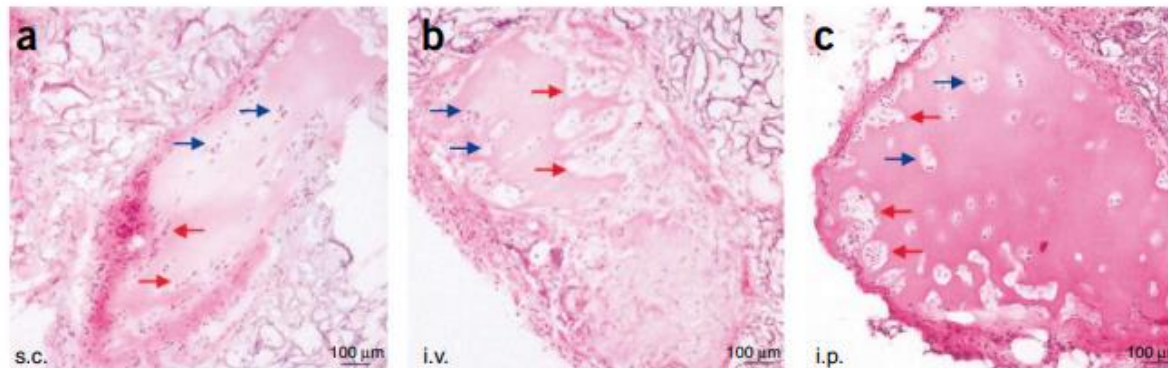
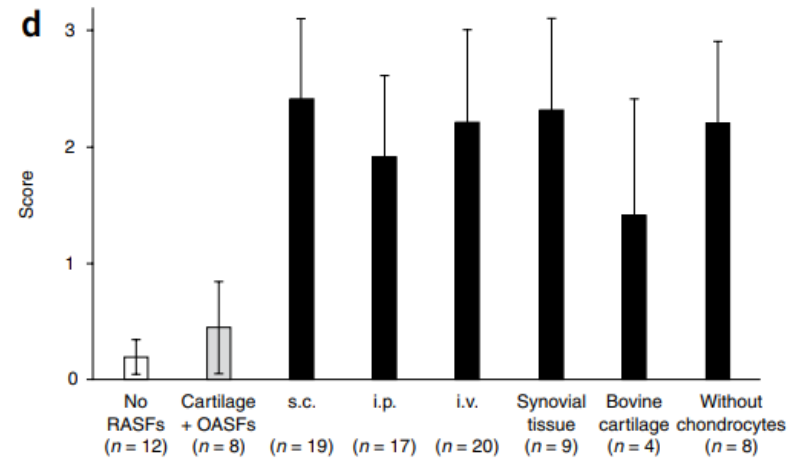
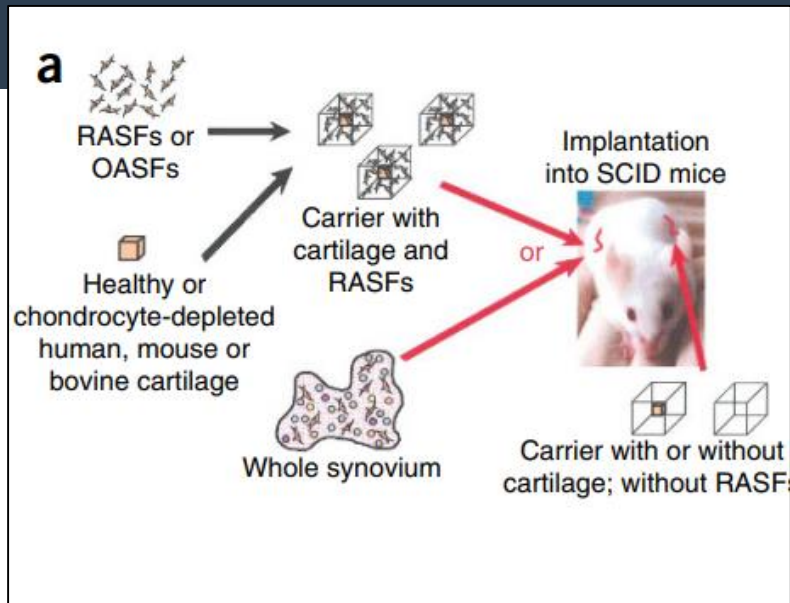
The fibroid phenotype was associated with reduced response to DMARD therapy

Distinct FLS subsets drive inflammation & damage in RA



FLS depletion ameliorates RA

FLS can spread RA to unaffected joints



The clinical progression of RA is mediated by RA FLS transmigration

FLS precursors can predict RA flares

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

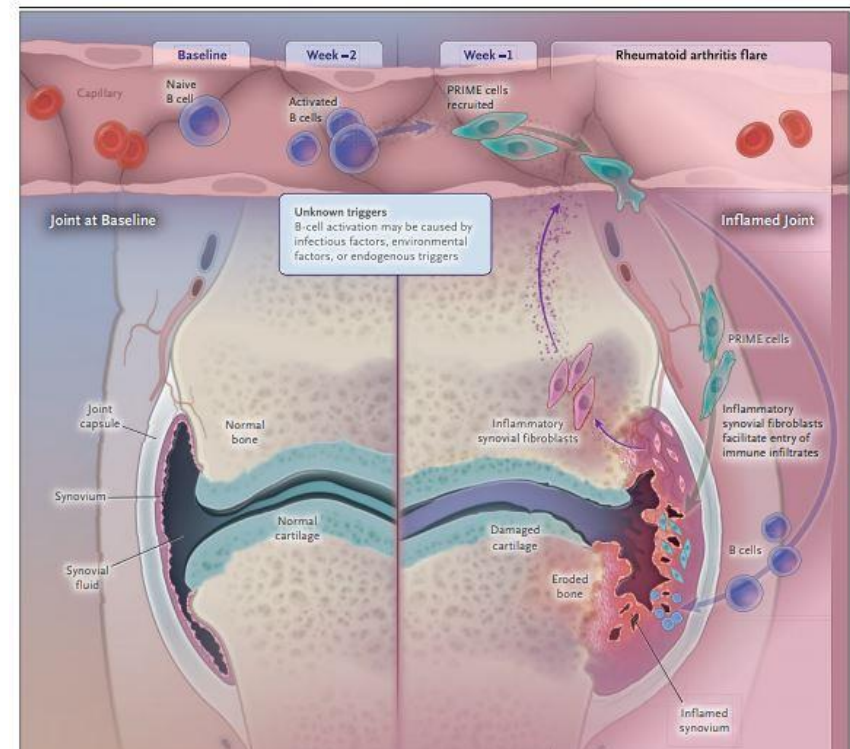
RNA Identification of PRIME Cells Predicting Rheumatoid Arthritis Flares

Dana E. Orange, M.D., Vicky Yao, Ph.D., Kirsty Sawicka, Ph.D., John Fak, M.S.,
Mayu O. Frank, N.P., Ph.D., Salina Parveen, M.A., Nathalie E. Blachere, Ph.D.,
Caryn Hale, Ph.D., Fan Zhang, Ph.D., Soumya Raychaudhuri, M.D., Ph.D.,
Olga G. Troyanskaya, Ph.D., and Robert B. Darnell, M.D., Ph.D.

ABSTRACT

BACKGROUND

Rheumatoid arthritis, like many inflammatory diseases, is characterized by episodes of quiescence and exacerbation (flares). The molecular events leading to flares are unknown.



CD45⁺ CD31⁻ PDPN⁺

FLS precursors are activated and migrate to the synovium via the circulation

Objective

The focus in RA research has been on immune cells.

How do FLS contribute to the autoreactive immune response?

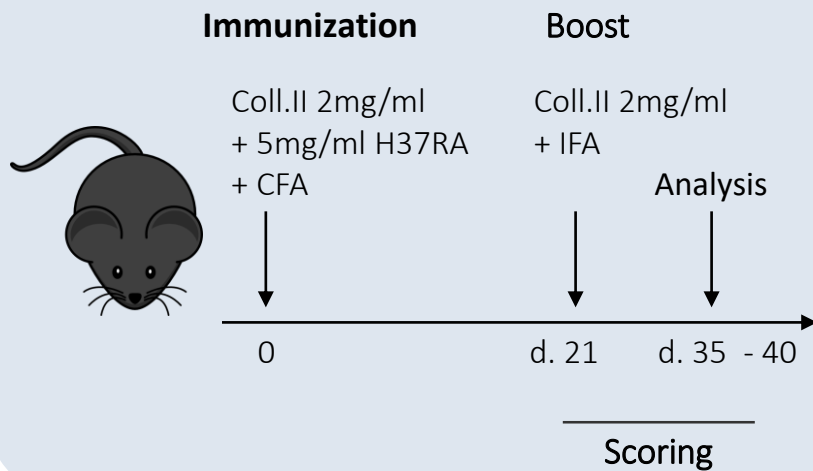
Aim 1: What is the immune profile of FLS and their interactions with the infiltrating immune populations in the mouse model of RA?

Aim 2: What metabolic pathways are implicated in FLS activity during arthritis?

Aim 3: How do current RA treatments alter the baseline status of FLS, their inflammatory capacity and metabolic reprogramming?

Tools:

Collagen-Induced Arthritis (CIA)



C57BL/6

aSMA-RFP



RFP-tagged FLS

aSMA-TK



Fibroblast-specific,
inducible depletion

Experimental Design

Aim 1: Immune profiling

- Confocal → spatial interactions
- FACS → immune infiltrate, FLS characteristics
- Sorting → *in vitro* experiments, rtPCR

Aim 2: Study pathways activated in FLS

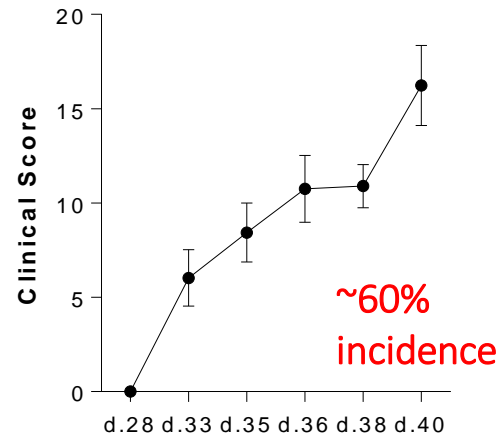
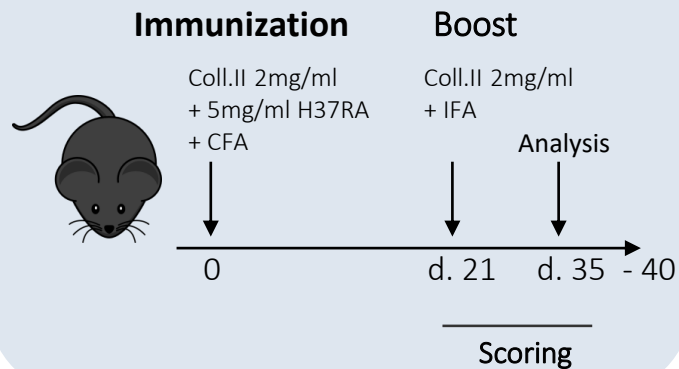
- Meta-analysis
- Seahorse → metabolic profiling

Aim 3: How do those profiles change upon therapy?

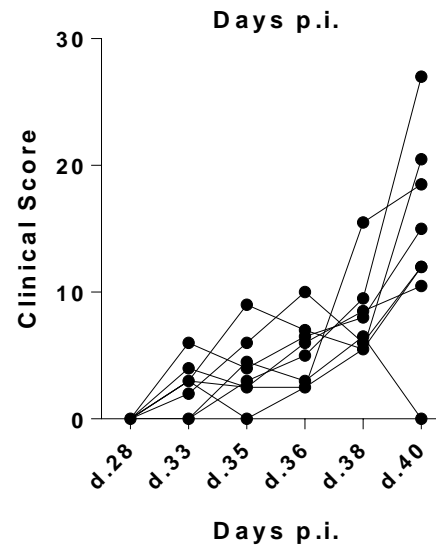
- Administration of JAK/STAT inhibitors or aTNF → assessment of FLS function
- FLS depletion → assessment of disease progression

CIA-induced synovial inflammation

Collagen-Induced Arthritis (CIA)



Normal limb (Score = 0)

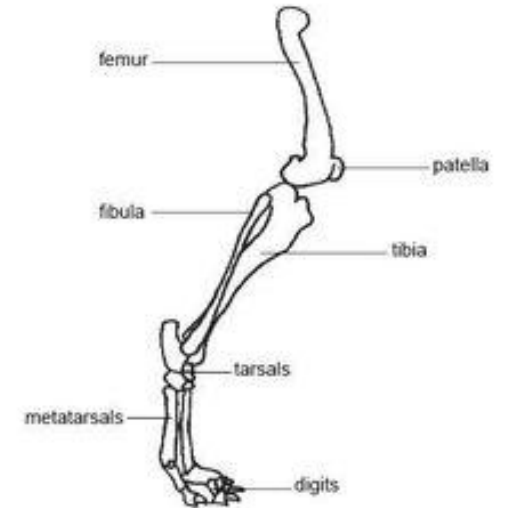
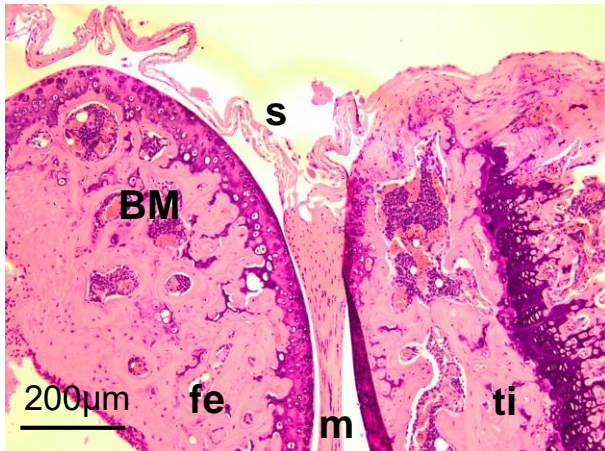


Arthritic limb (Max score)

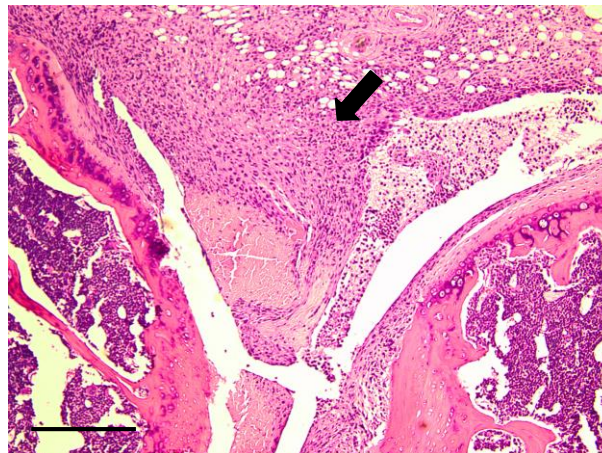
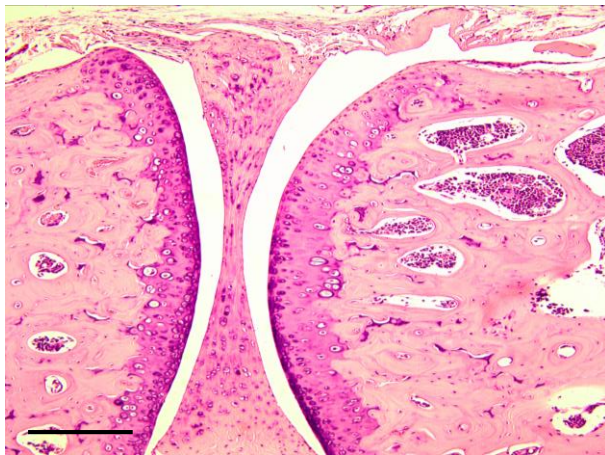


Histological assessment of inflammation

Normal limb (Score = 0)



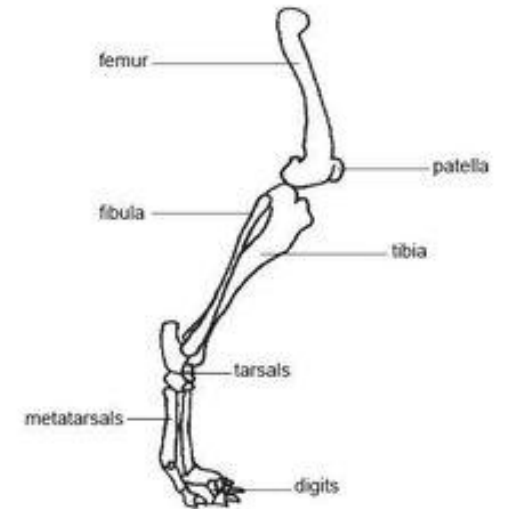
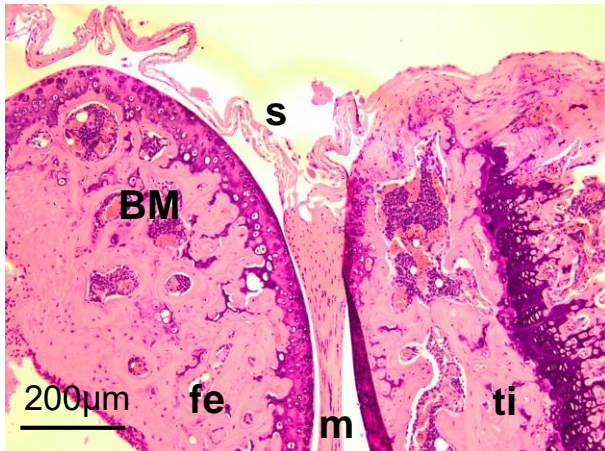
Arthritic limb (Score = 25)



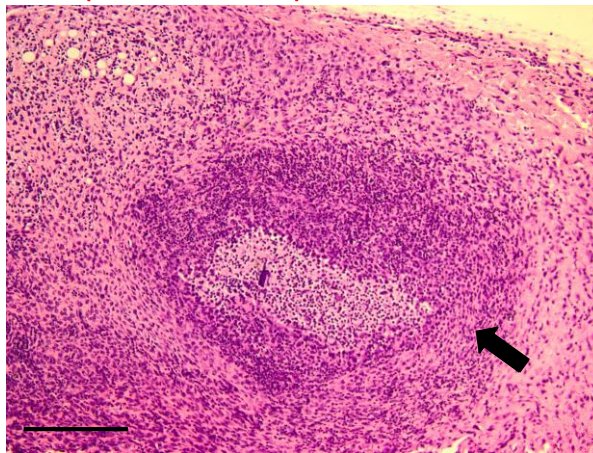
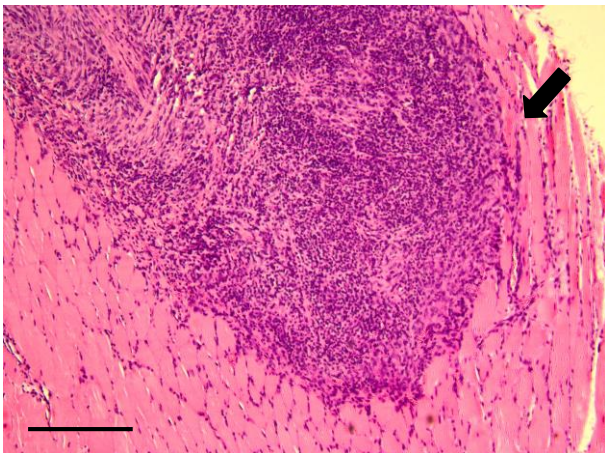
fe: femur
ti: tibia
BM: bone marrow
s: synovial membrane
m: meniscus

Histological assessment of inflammation

Normal limb (Score = 0)



Arthritic limb (Score = 29)

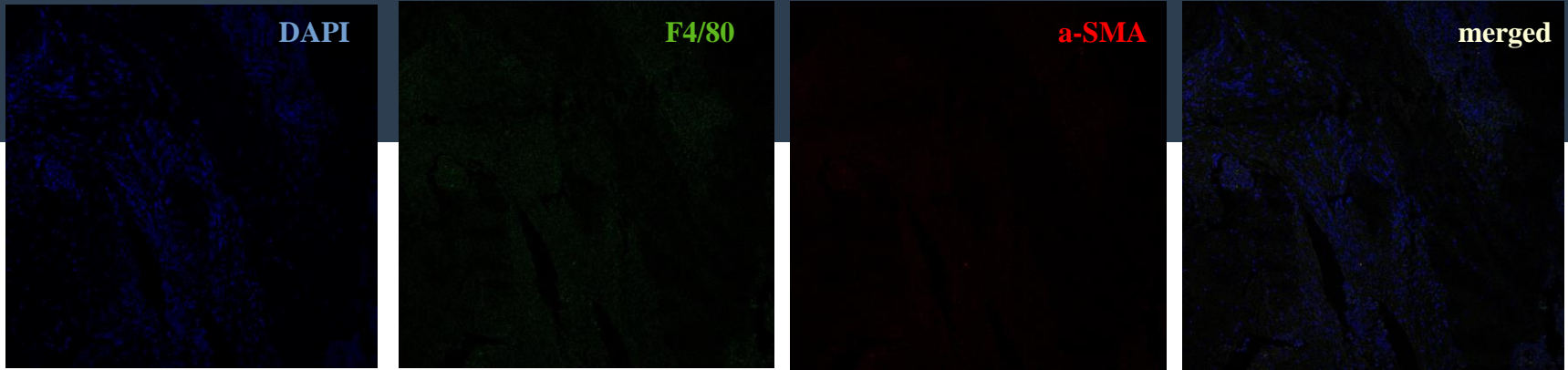


fe: femur
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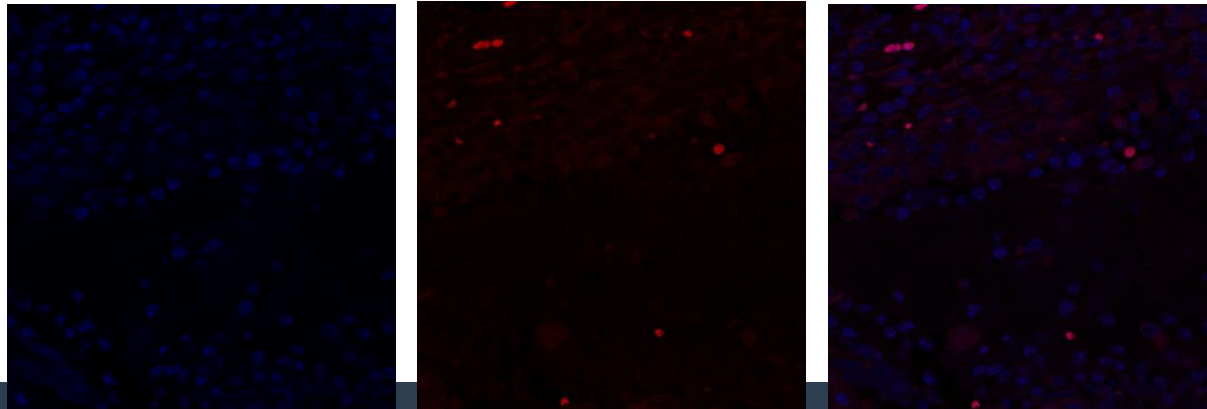
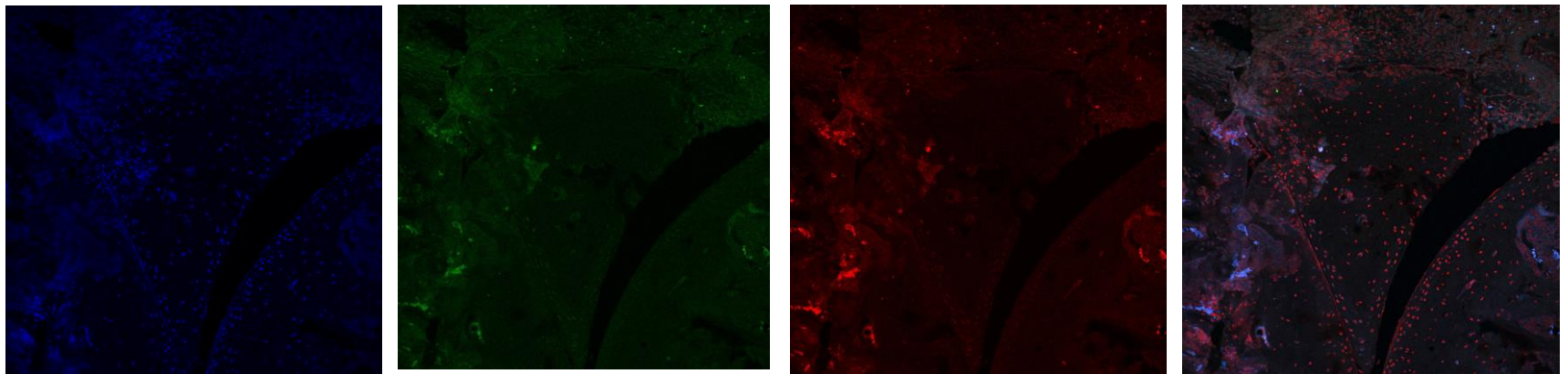
Immune infiltrate in muscle

Investigating cell interactions within the CIA joint

B6 CIA

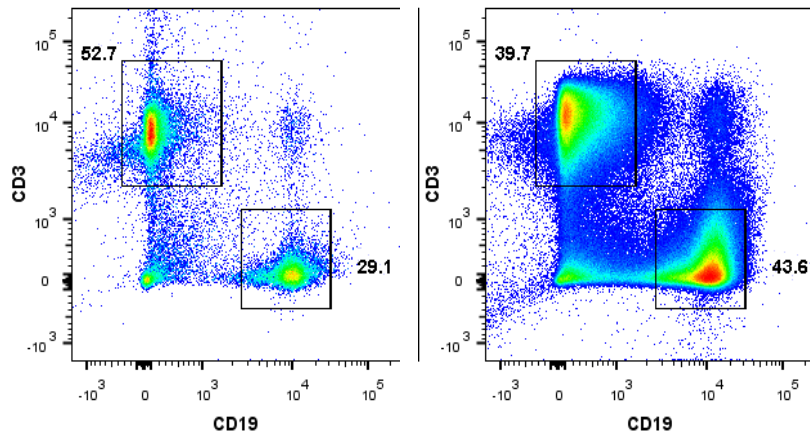


RFP+ CIA

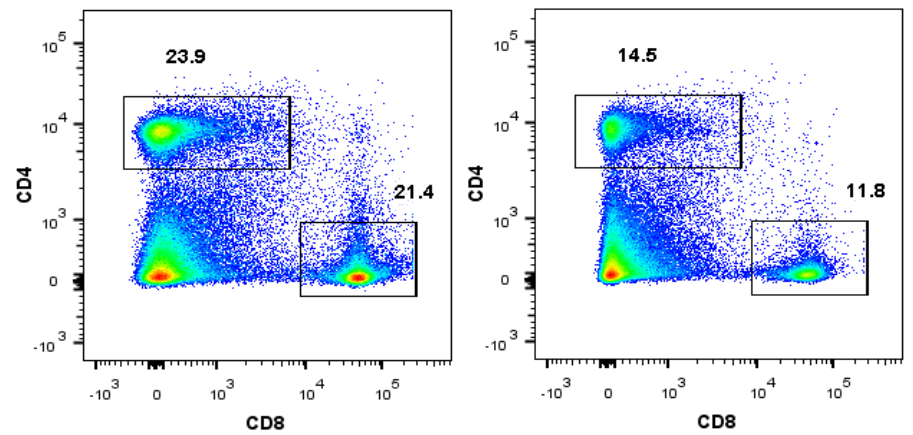


Involvement of draining inguinal lymph nodes

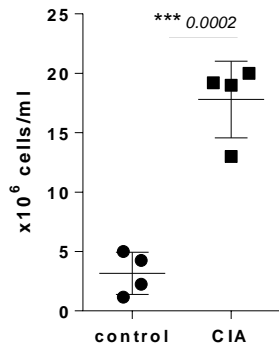
control



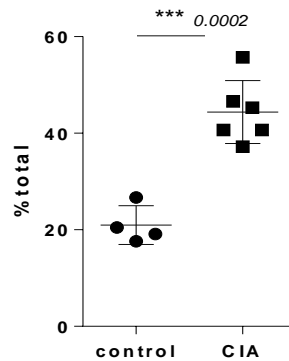
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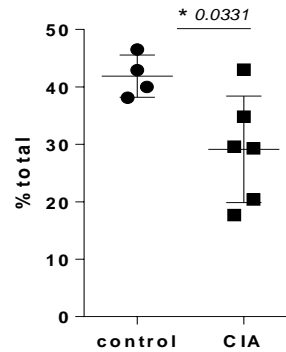
Cellularity
(per lymph node)



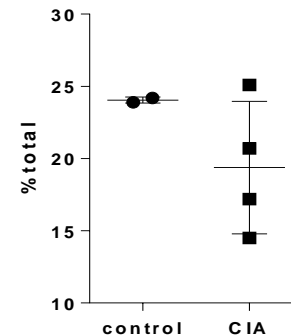
CD 19



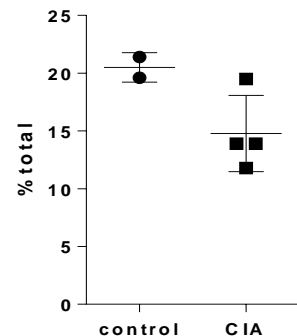
CD 3



CD 4

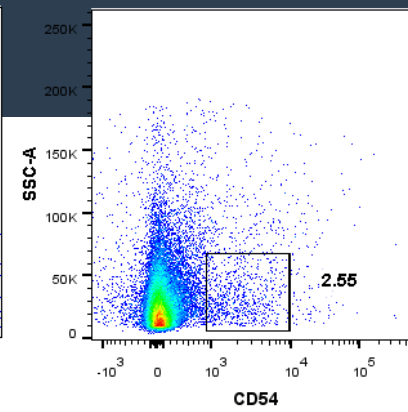
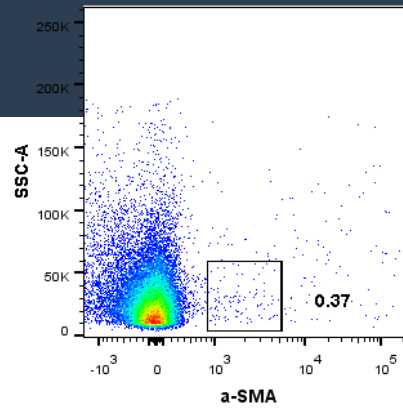
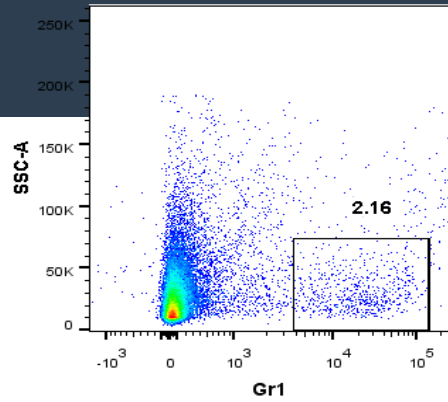


CD 8

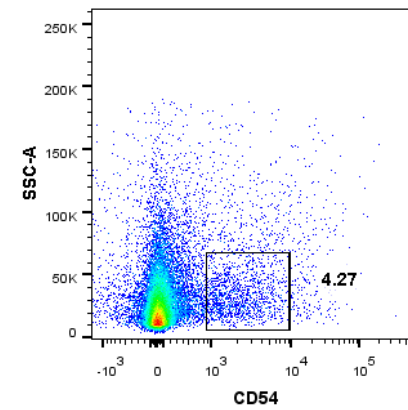
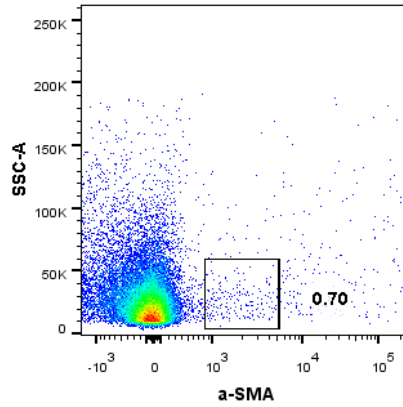
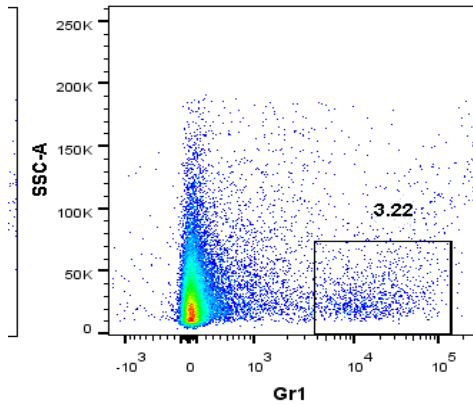


Cellular content of the inflamed joint

control



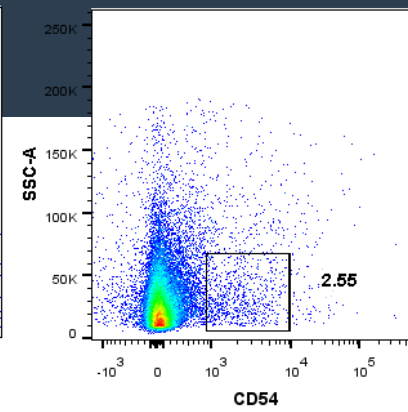
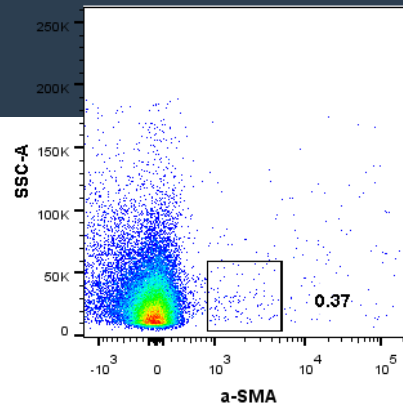
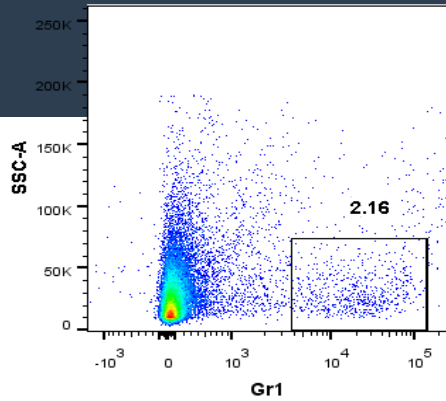
CIA



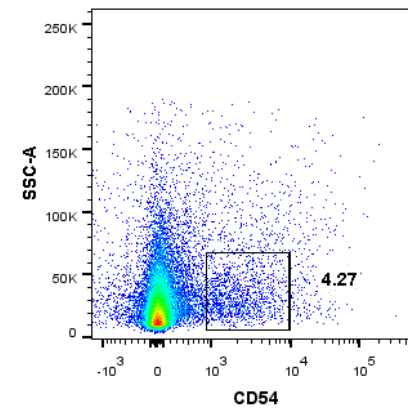
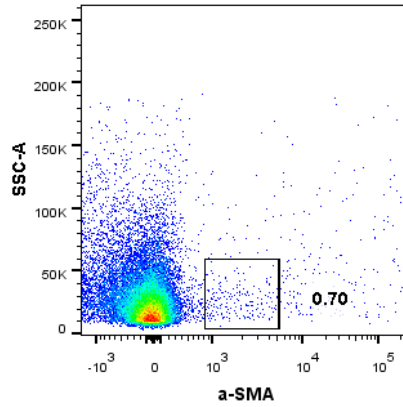
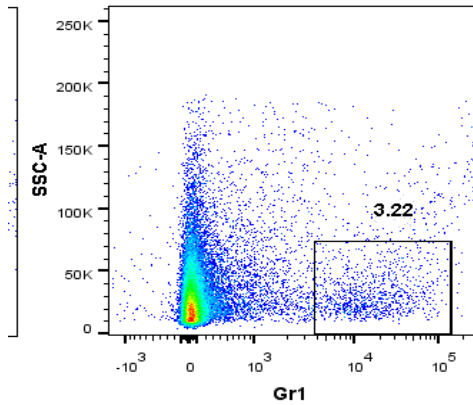
d.40

Cellular content of the inflamed joint

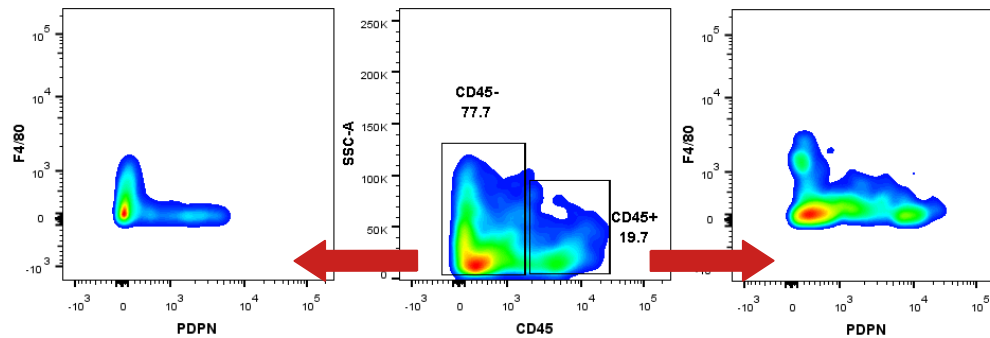
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CIA



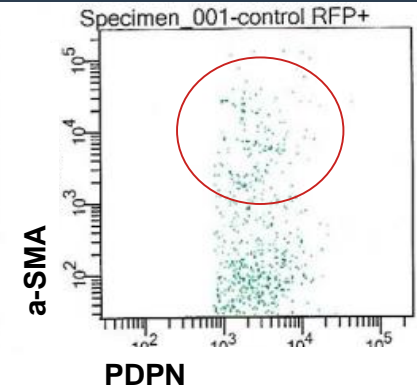
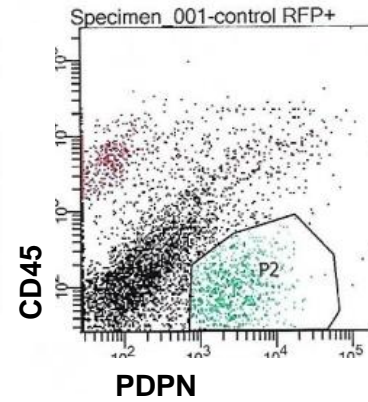
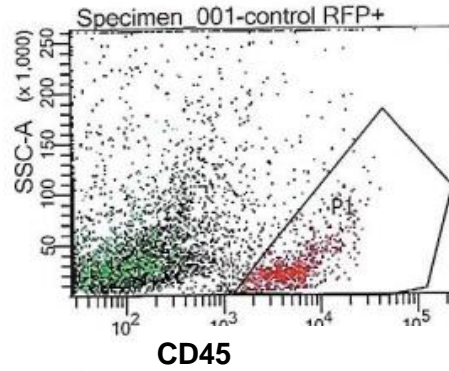
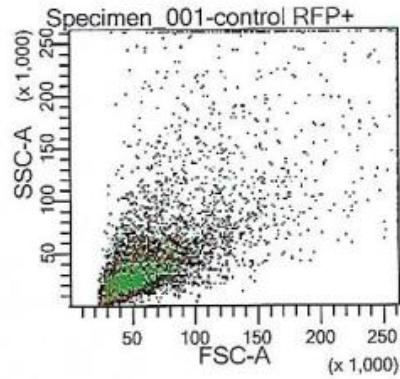
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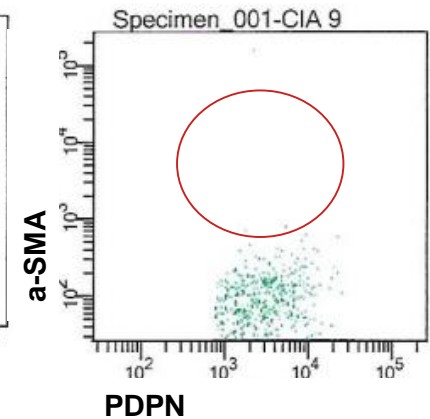
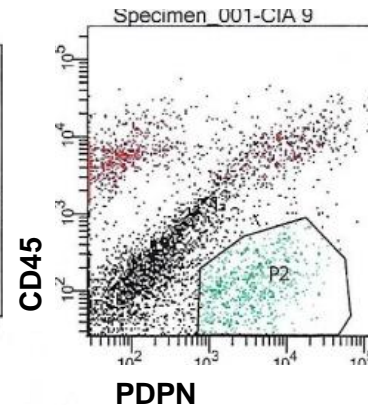
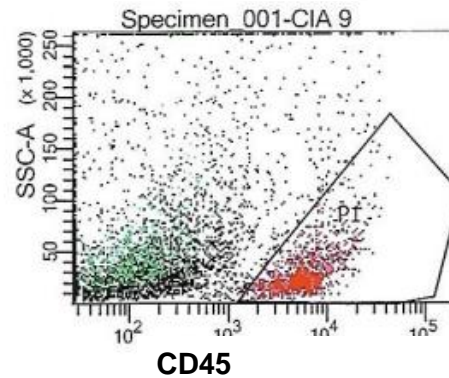
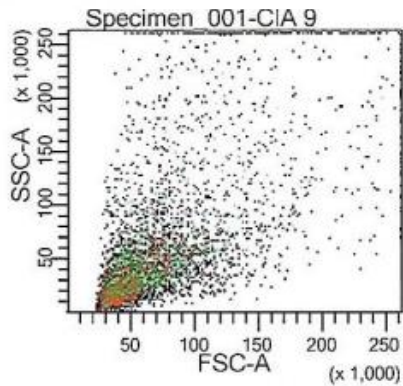
d.8 of culture

Sorting FLS...

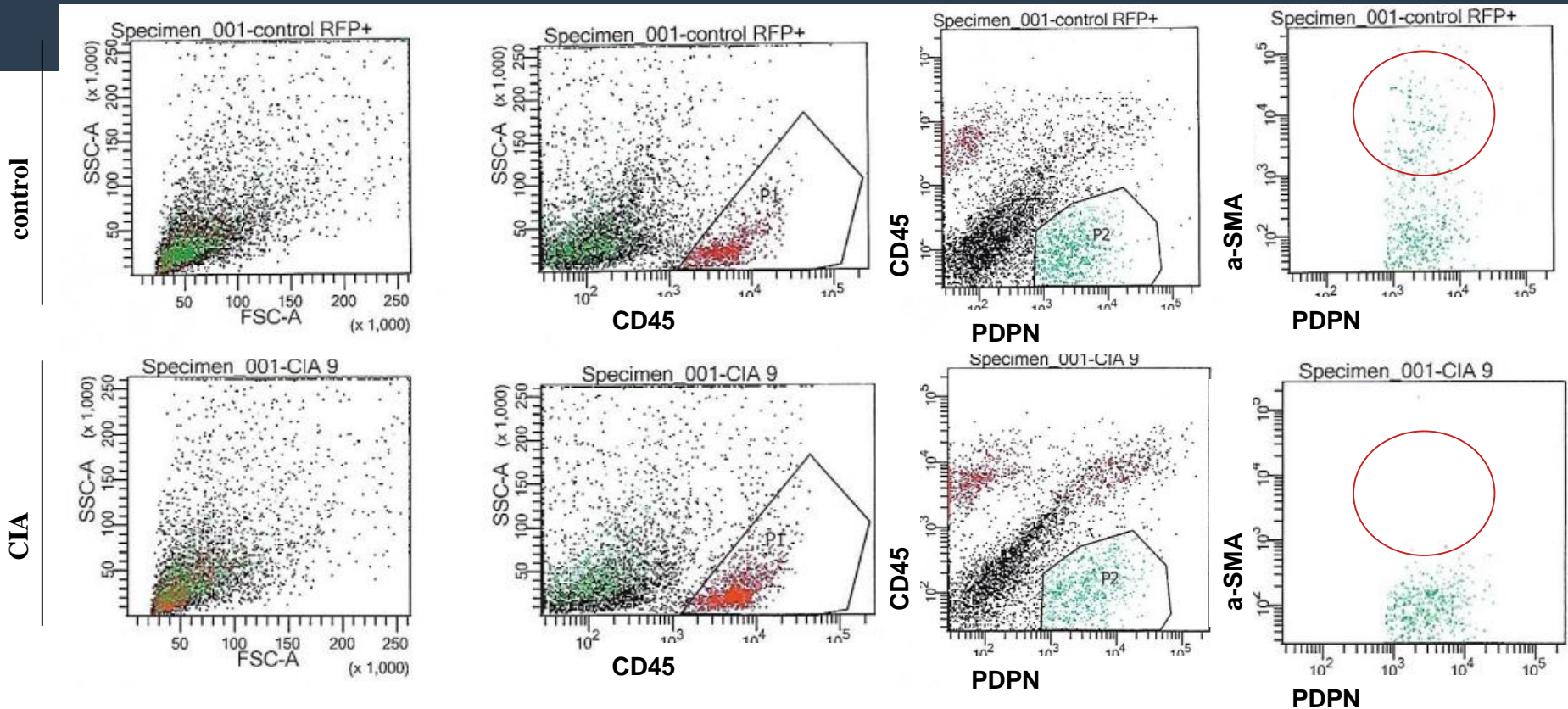
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CIA

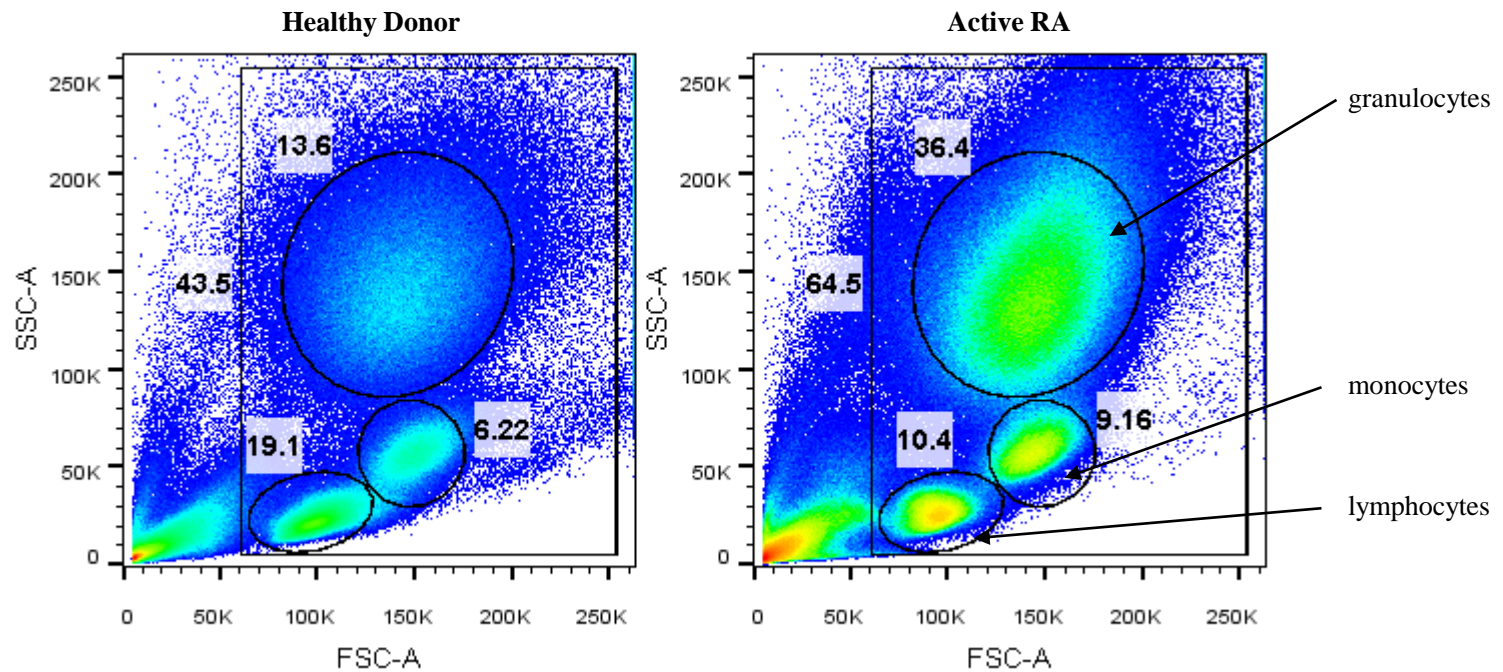


Sorting FLS...

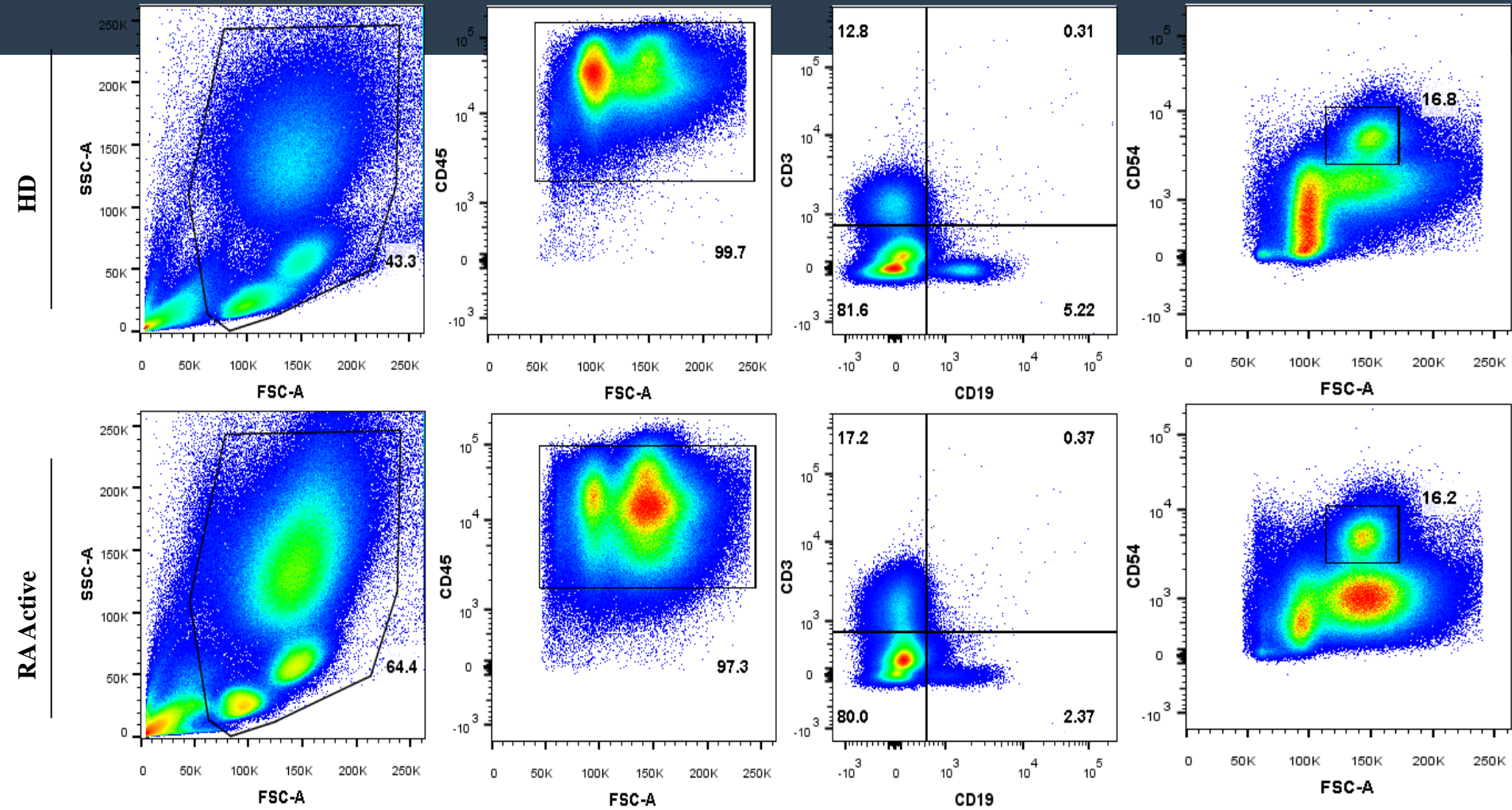


- FLS markers are not uniformly expressed – intrinsic heterogeneity
- Different isolation techniques
- Temporal diversity (disease stage) – timecourse of disease

Circulating FLS in human samples

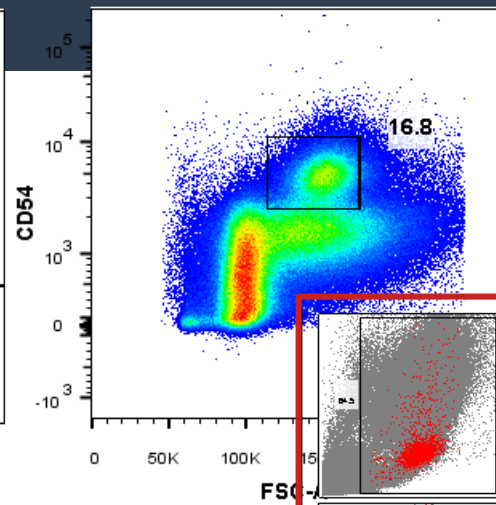
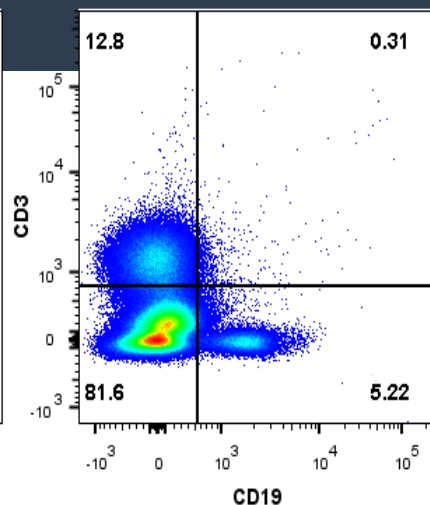
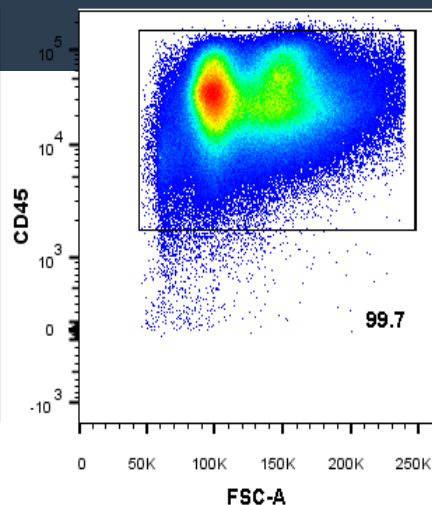
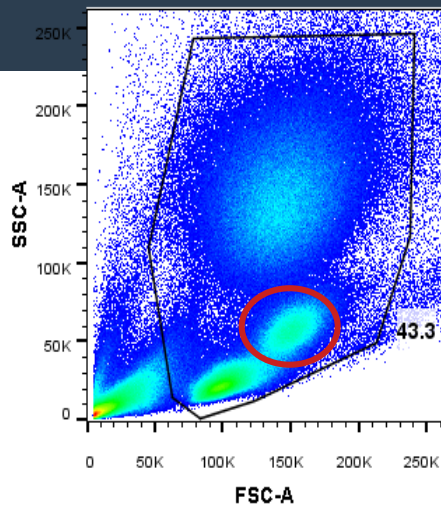


[FLS in human samples]

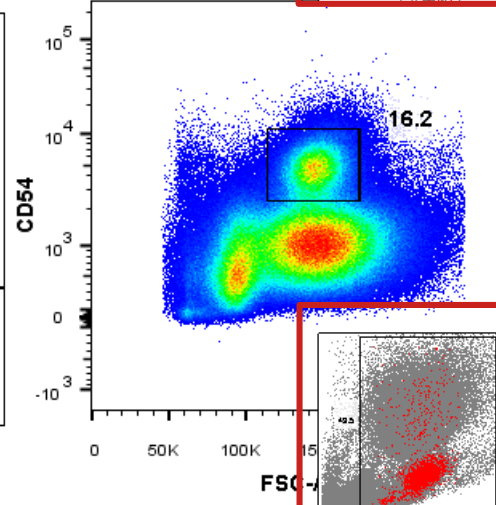
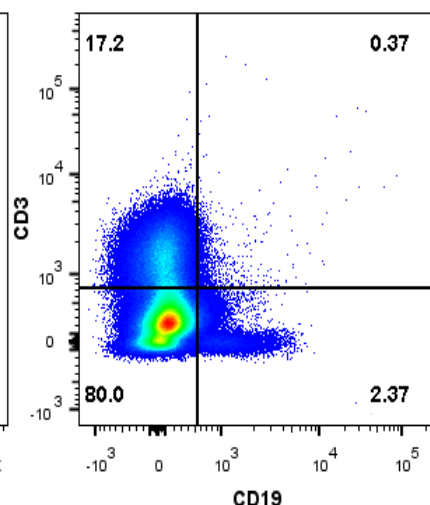
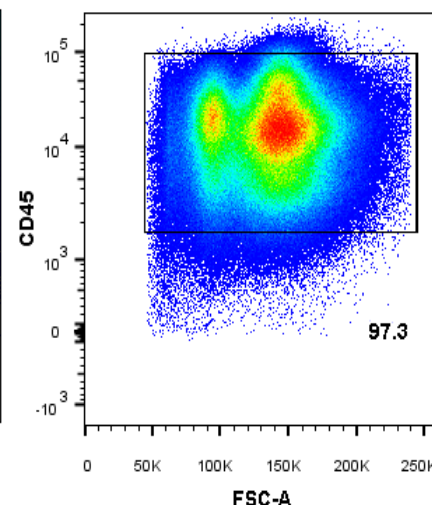
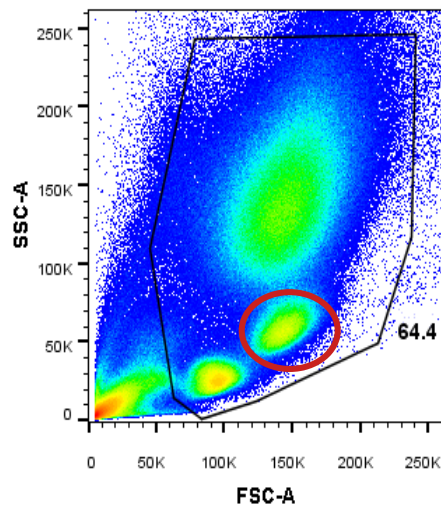


Circulating FLS in human samples

HD



RA Active





Delineation of Fibroblast-Like Synoviocyte-mediated immune responses in the pathogenesis of rheumatoid arthritis

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PhD Candidate

Laboratory of Rheumatology, Inflammation & Autoimmunity

School of Medicine, University of Crete