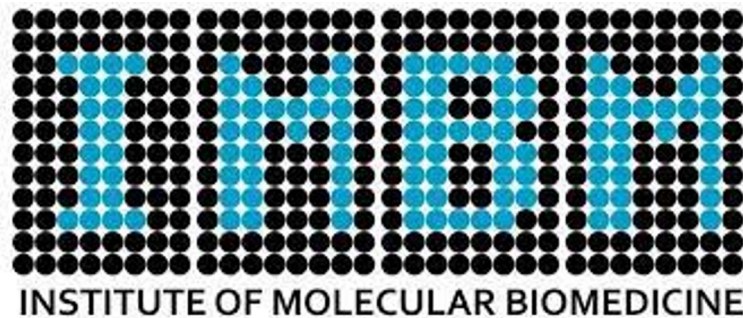


6th Central - Eastern European congress  
on cell free DNA and medical practice

# The role of extracellular DNA in pathogenesis of rheumatoid arthritis



**Kristína Macáková**

**Veronika Borbélyová, Bernard Maximilian Schuh, Peter Celec , Barbora Vlková**

**kristina.macakova@imbm.sk**

Review

## Cell-Free DNA in Rheumatoid Arthritis

Tepei Hashimoto <sup>1,\*</sup>, Kohsuke Yoshida <sup>2</sup>, Akira Hashiramoto <sup>2</sup> and Kiyoshi Matsui <sup>1</sup>

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  - <sup>2</sup> Department of Biophysics, Kobe University Graduate School of Health Sciences, Kobe 6540142, Japan; koh.yoshida1117@gmail.com (K.Y.); hash@med.kobe-u.ac.jp (A.H.)
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frontiers  
in Immunology

ORIGINAL RESEARCH  
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doi: 10.3389/fimmu.2020.00662



## Identification of Specific Joint-Inflammatogenic Cell-Free DNA Molecules From Synovial Fluids of Patients With Rheumatoid Arthritis

Cong Dong <sup>1</sup>, Yu Liu <sup>2</sup>, Chengxin Sun <sup>1</sup>, Huiyi Liang <sup>1</sup>, Lie Dai <sup>2\*</sup>, Jun Shen <sup>2\*</sup>, Song Wei <sup>1</sup>, Shixin Guo <sup>2</sup>, Kam W. Leong <sup>3</sup>, Yongming Chen <sup>1</sup>, Lai Wei <sup>2\*</sup> and Lixin Liu <sup>1\*</sup>

<sup>1</sup> Key Laboratory for Polymeric Composite and Functional Materials of Ministry of Education, Center for Functional Biomaterials, School of Materials Science and Engineering, Sun Yat-sen University, Guangzhou, China, <sup>2</sup> State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China, <sup>3</sup> Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China, <sup>4</sup> Department of Rheumatology, General Hospital of Guangzhou Military Command of PLA, Guangzhou, China, <sup>5</sup> Department of Biomedical Engineering, Columbia University, New York, NY, United States

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Edited by:  
Erminia Mariani,  
University of Bologna, Italy

Nucleus

ncDNA



RESEARCH ARTICLE

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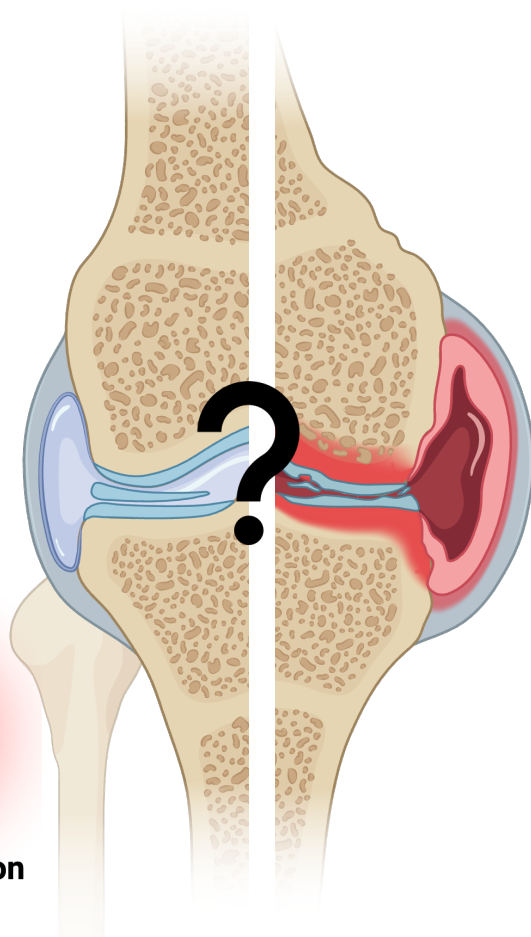


## Circulating DNA in rheumatoid arthritis: pathological changes and association with clinically used serological markers

Elena Rykova <sup>1,2</sup>, Aleksey Sizikov <sup>3</sup>, Dirk Roggenbuck <sup>4</sup>, Oksana Antonenko <sup>5\*</sup>, Leonid Bryzgalov <sup>6</sup>, Evgeniy Morozkin <sup>1,7</sup>, Kseniya Skvortsova <sup>1</sup>, Valentin Vlassov <sup>1</sup>, Pavel Laktionov <sup>1,7</sup> and Vladimir Kozlov <sup>3</sup>

Inflammation

## Treatment



## THE LANCET

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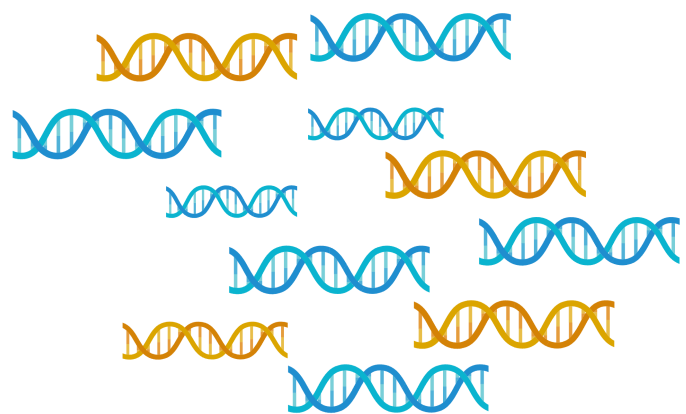
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Abatacept inhibits inflammation and onset of rheumatoid arthritis in individuals at high risk (ARIAA): a randomised, international, multicentre, double-blind, placebo-controlled trial

Juergen Rech, MD <sup>†</sup> • Koray Tascilar, MD <sup>†</sup> • Melanie Hagen, MD • Arnd Kleyer, MD • Prof Bernhard Manger, MD • Verena Schoenau, MD • et al. Show all authors • Show footnotes



Periodontitis  
Anti-citrullinated protein antibodies



**ecDNA**



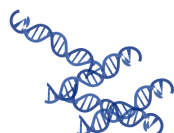
**Nucleus**



**ncDNA**



**Mitochondria**



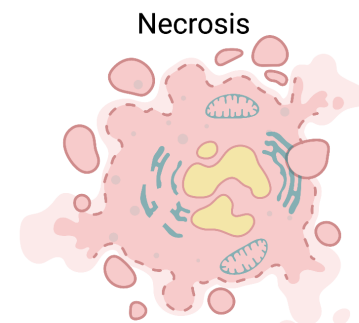
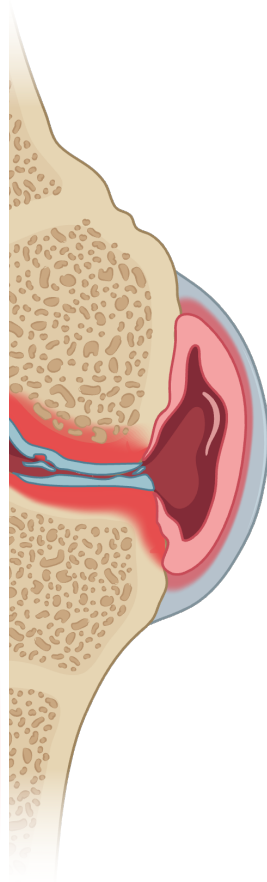
**mtDNA**



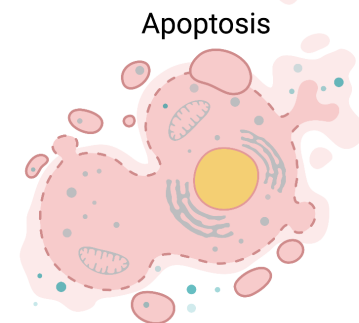
**TLR9**



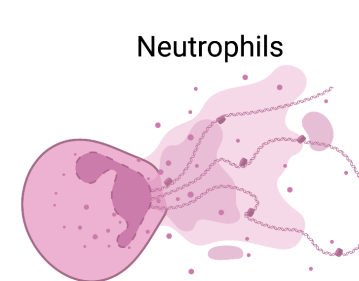
**Inflammation**



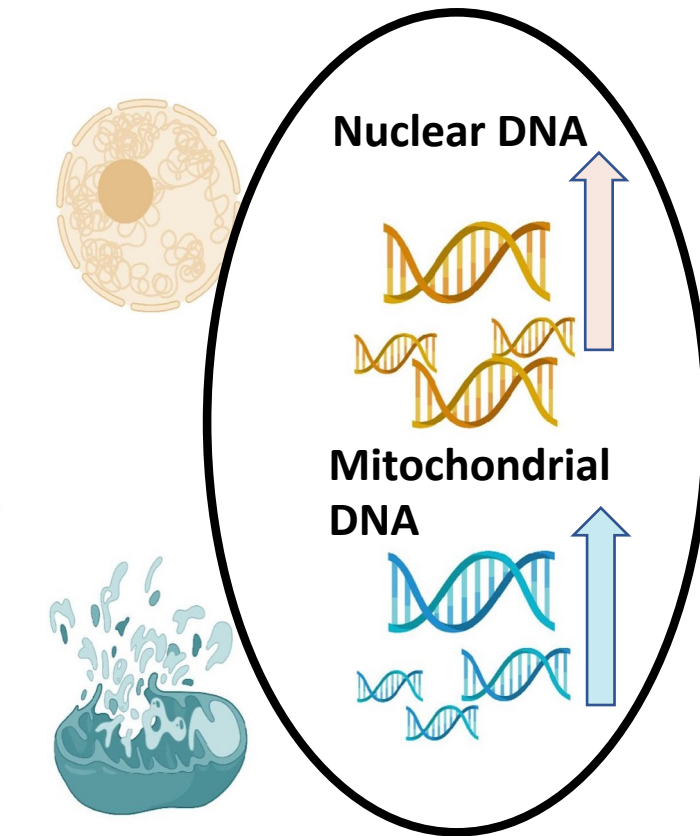
**Necrosis**



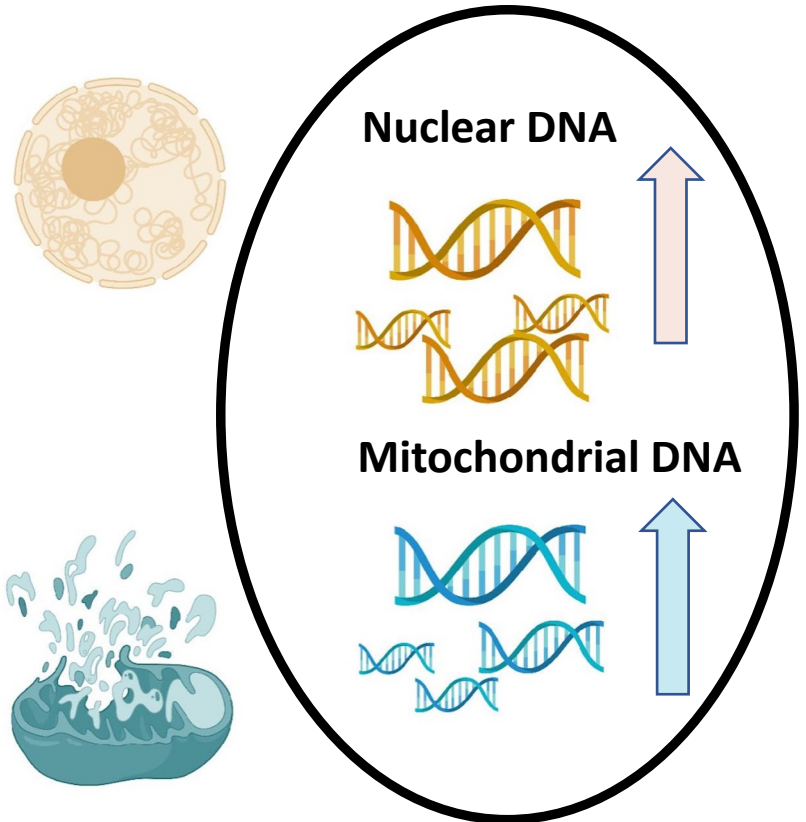
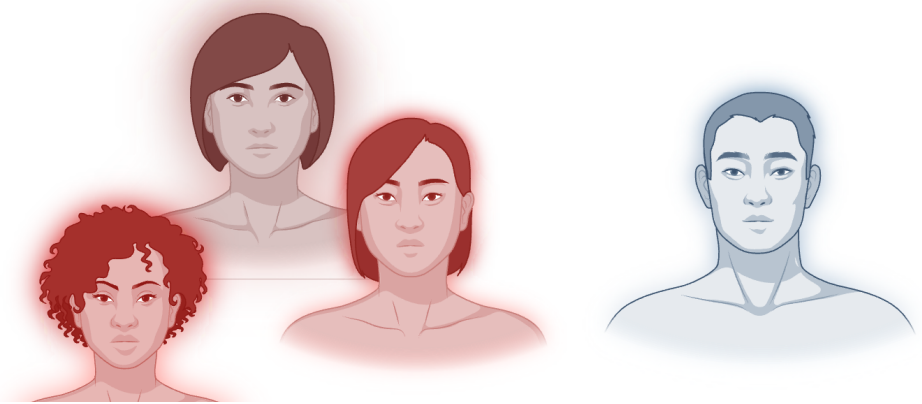
**Apoptosis**



**Neutrophils**



**Increased of total ecDNA concentration**



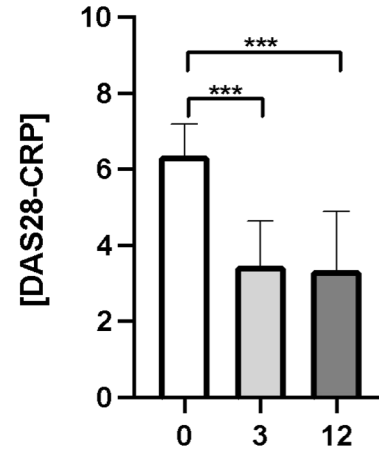
**Increased of total ecDNA concentration**

**Dynamics of ecDNA after bDMARDs ?**

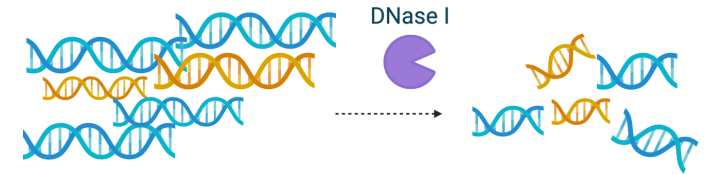
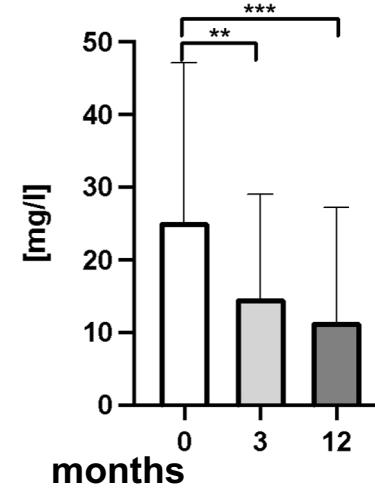




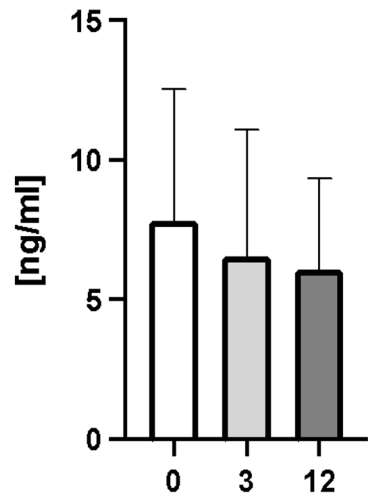
**Disease activity**



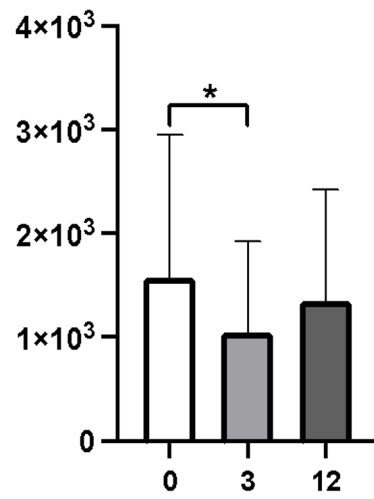
**CRP**



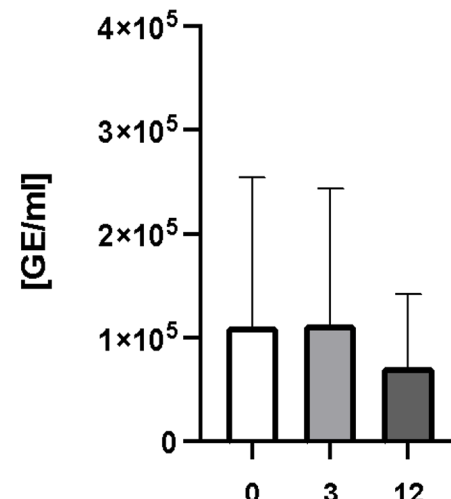
**ecDNA**



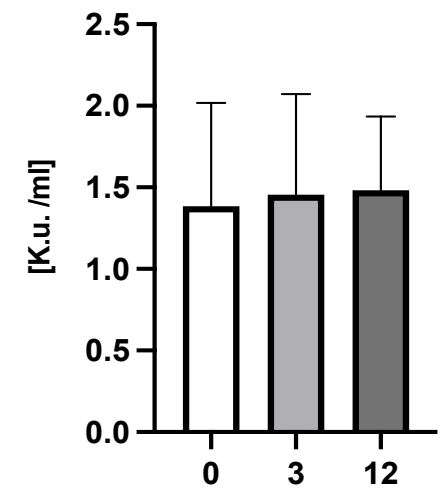
**ncDNA**



**mtDNA**



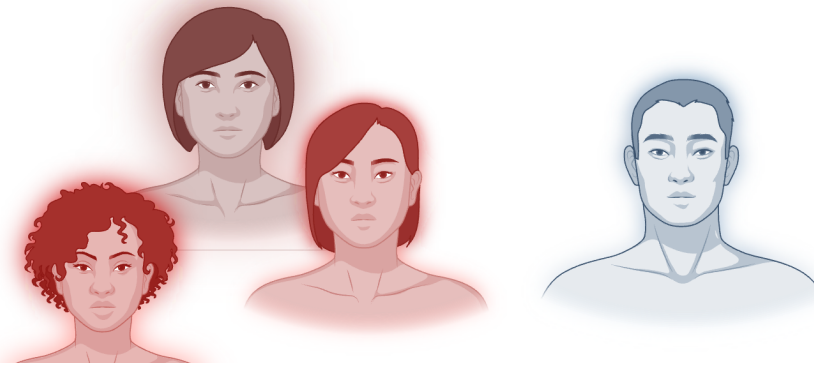
**DNase activity**



months

months

# RA patients



## EULAR CRITERIA



DAS28  
CRP



**Good responders**

n = 14



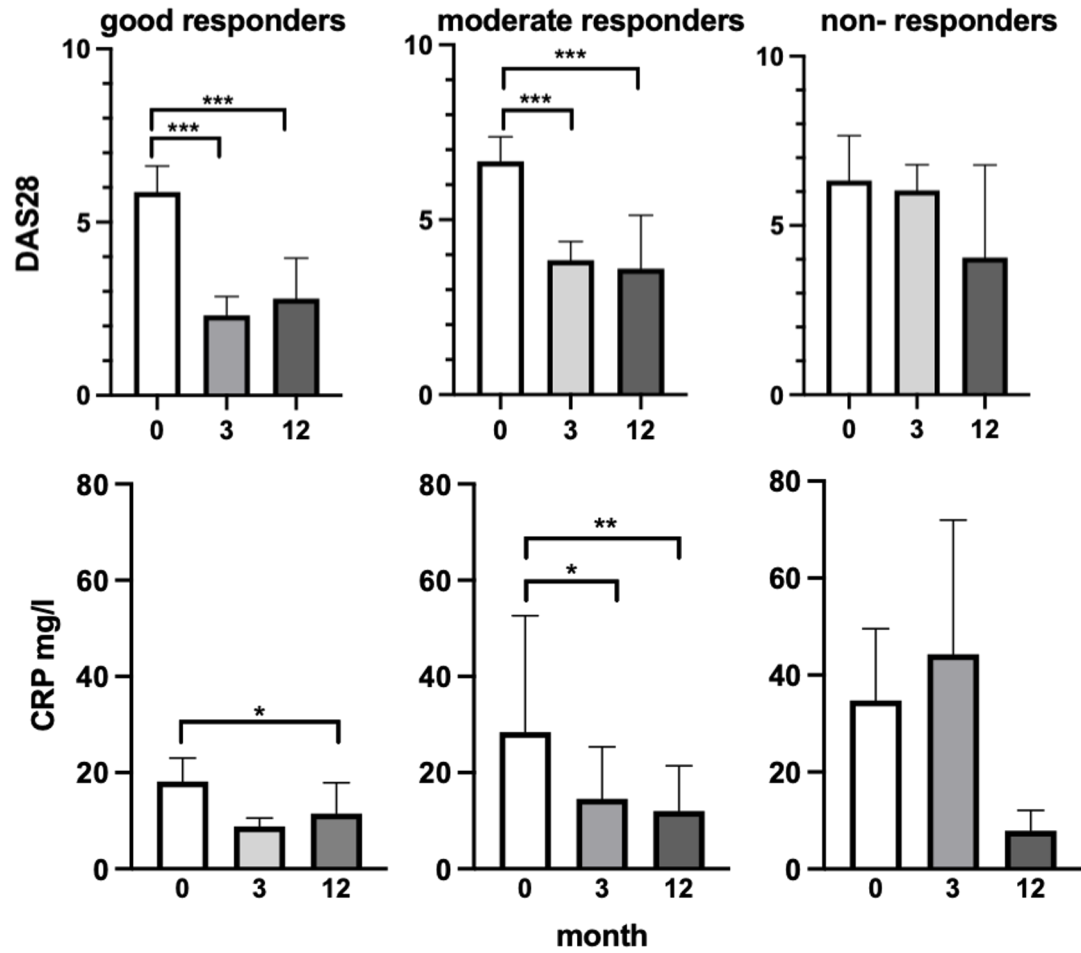
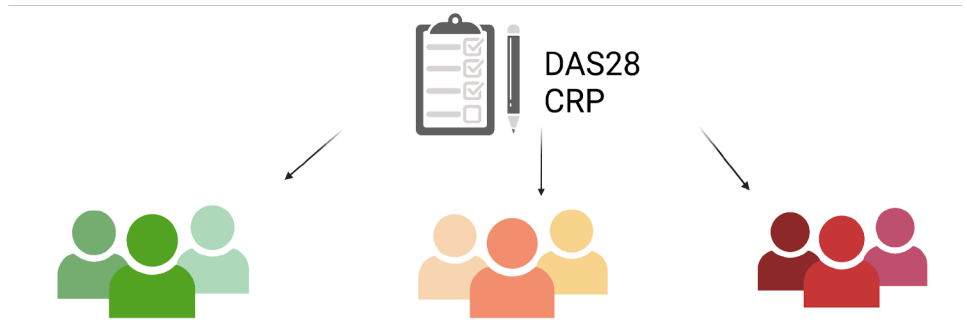
**Moderate responders**

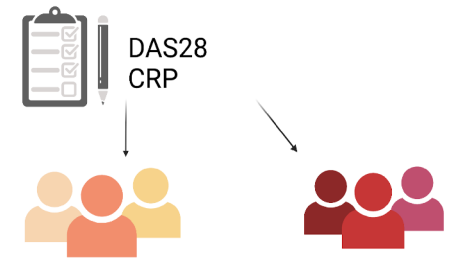
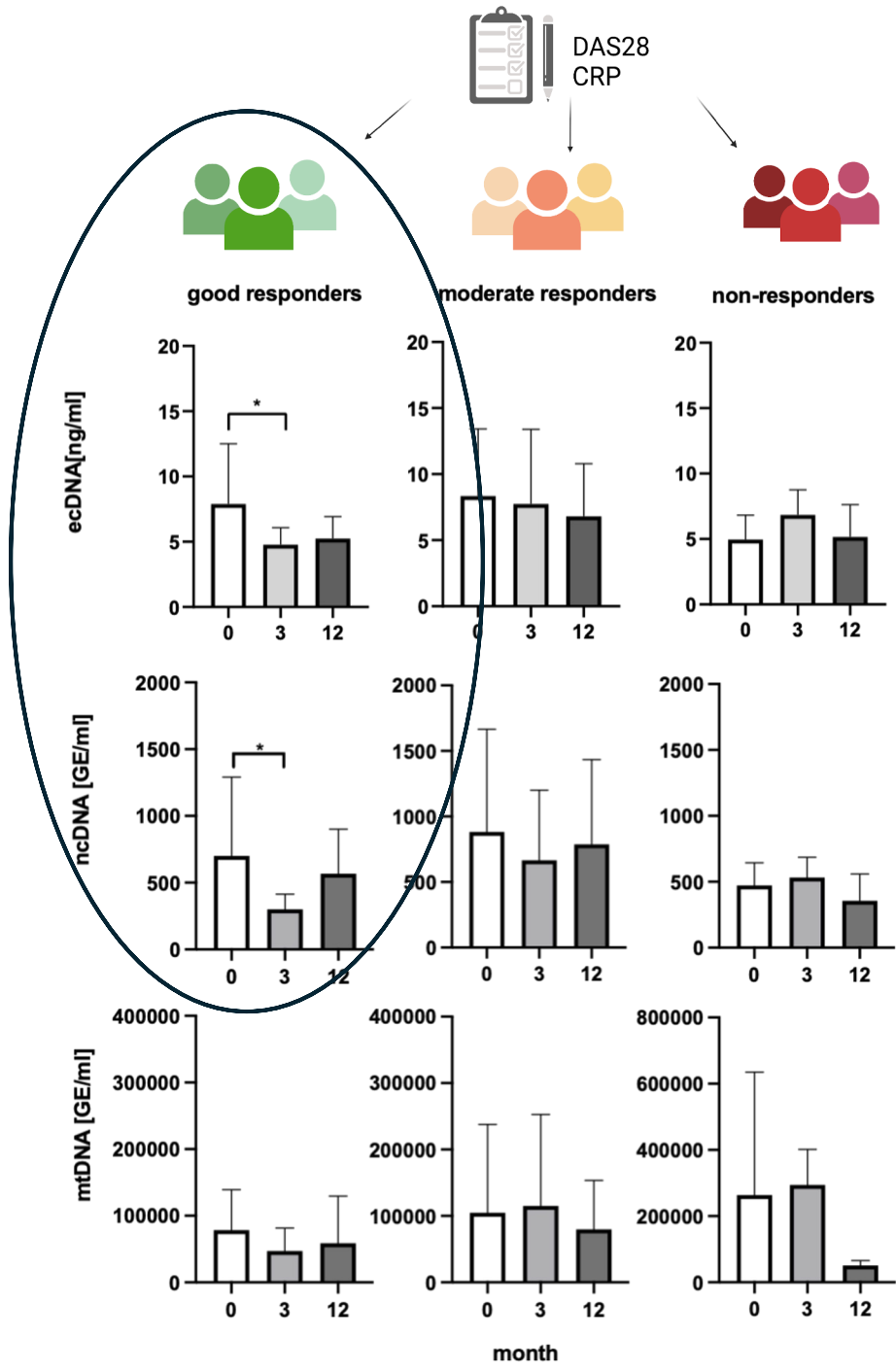
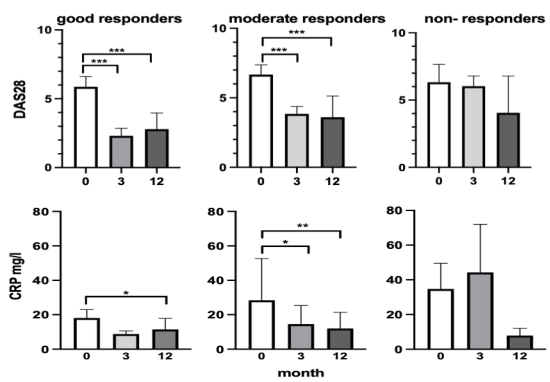
n = 23



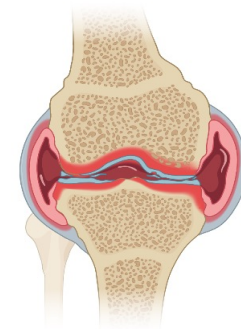
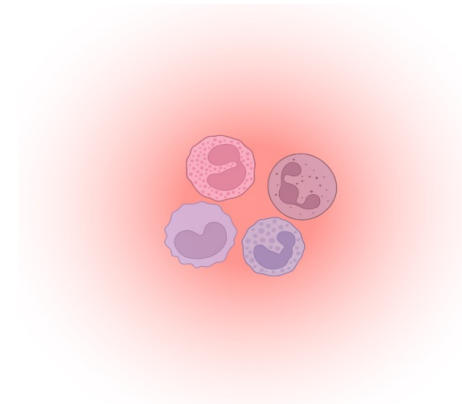
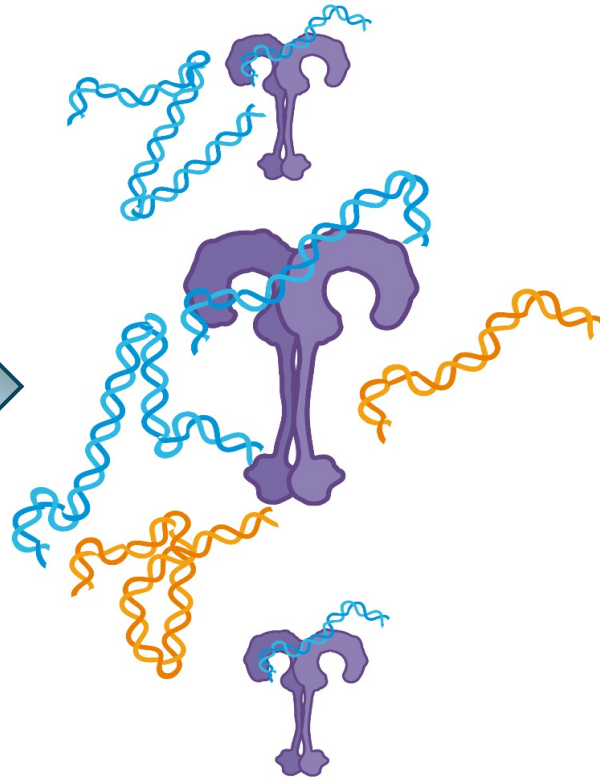
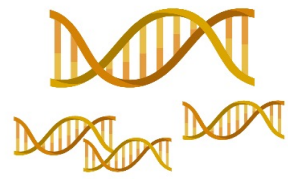
**Non responders**

n = 3





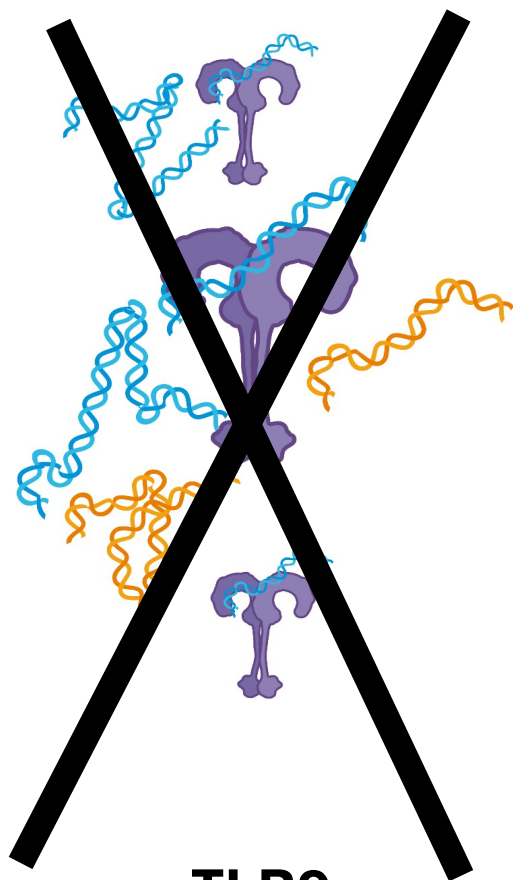
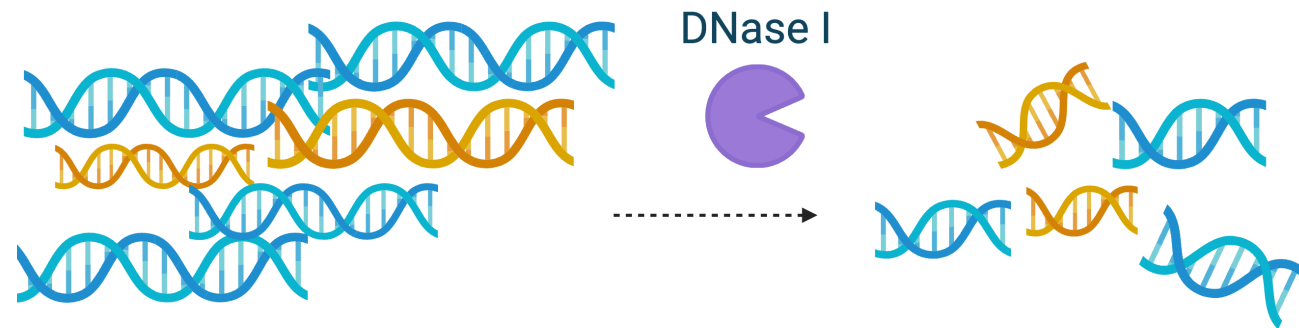




**Increased of ecDNA  
concentration**

**TLR9**

**Rheumatoid  
arthritis**



TLR9

Rheumatoid  
arthritis

# Animal model

## CAIA

(Collagen antibody induced arthritis)



18 DBA/IJ  
females  
mice

CTRL

n=6



0,9 % NaCl

CAIA

n=6



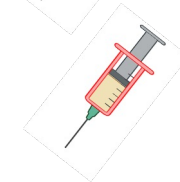
0,9 % NaCl



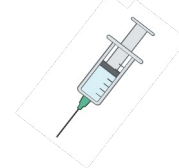
Ab

CAIA + **DNase I**

n=6



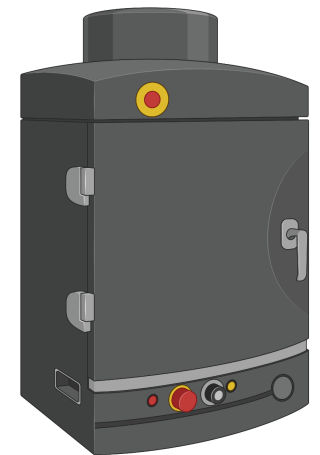
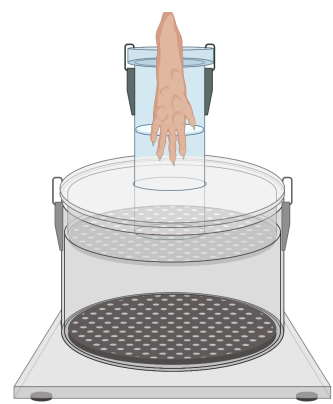
Ab



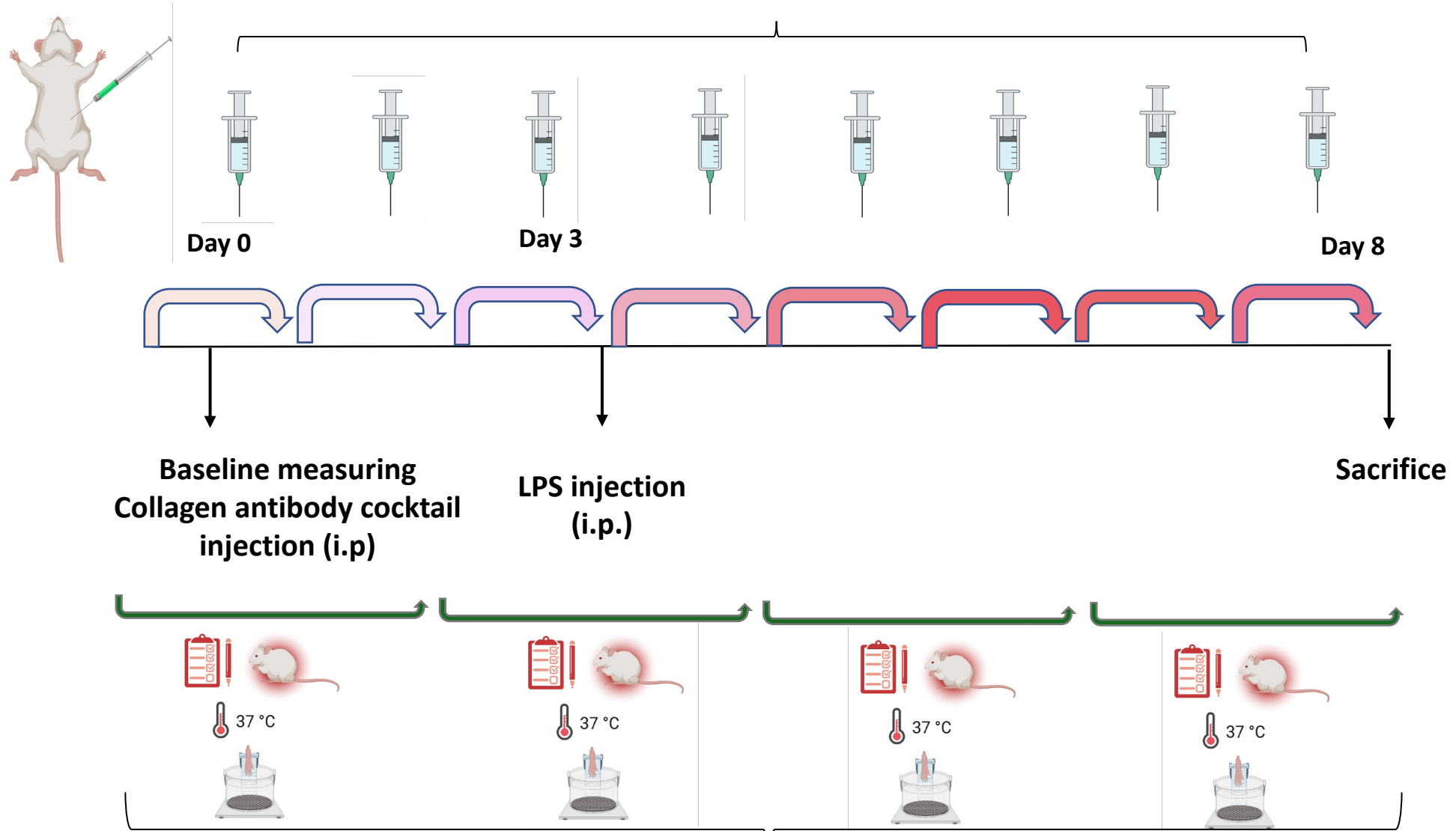
DNase I



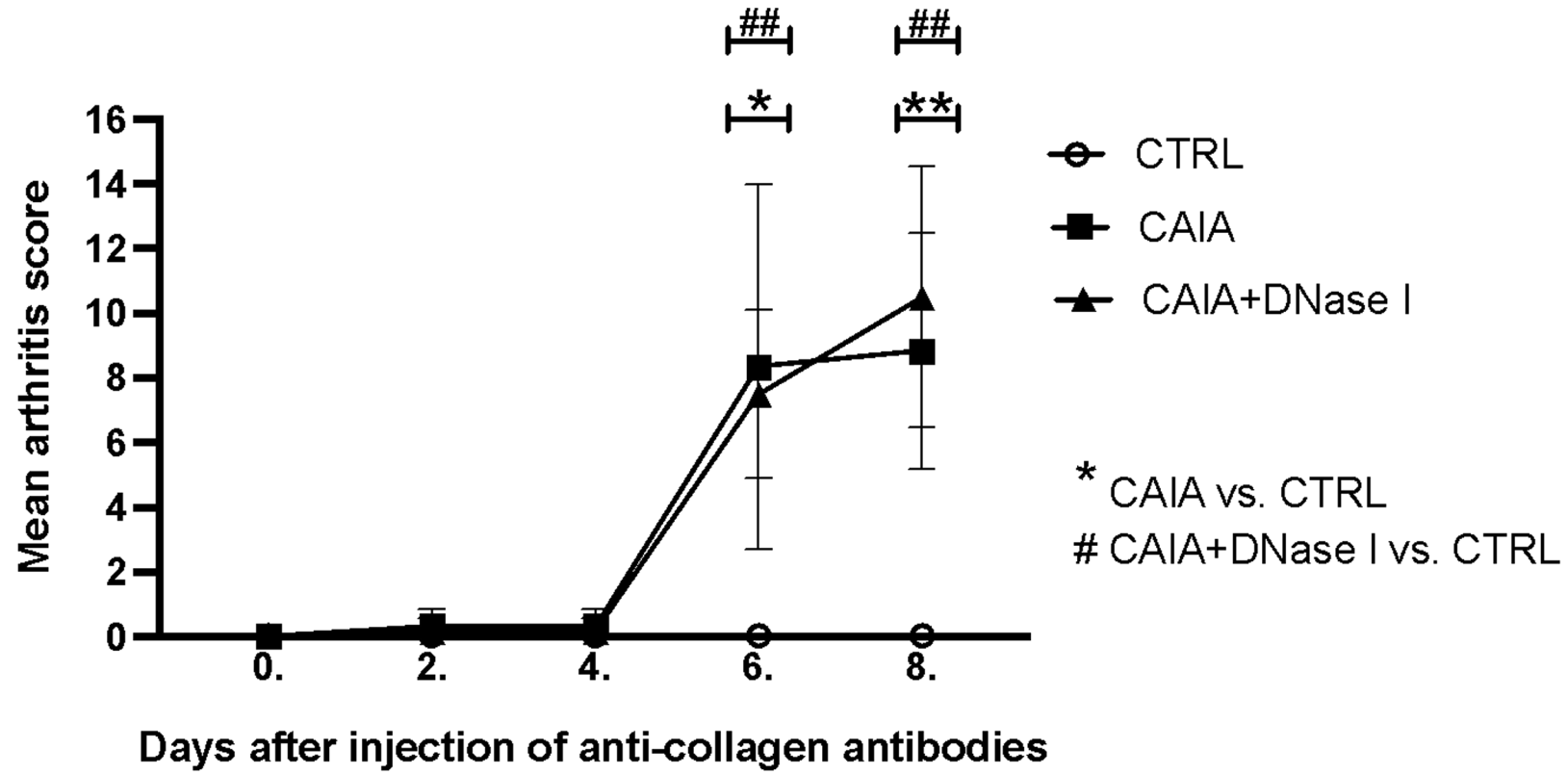
37 °C

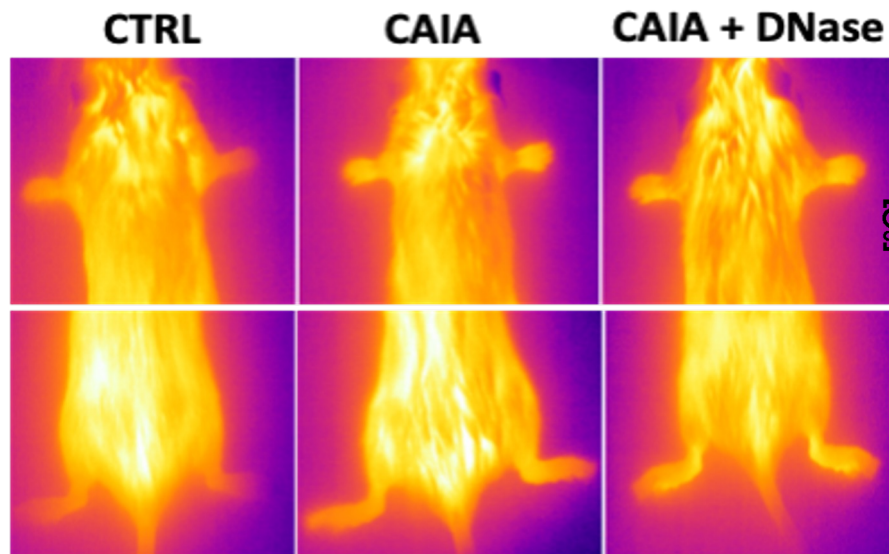


Application of DNase I (every 12 hours, 10 mg/kg)

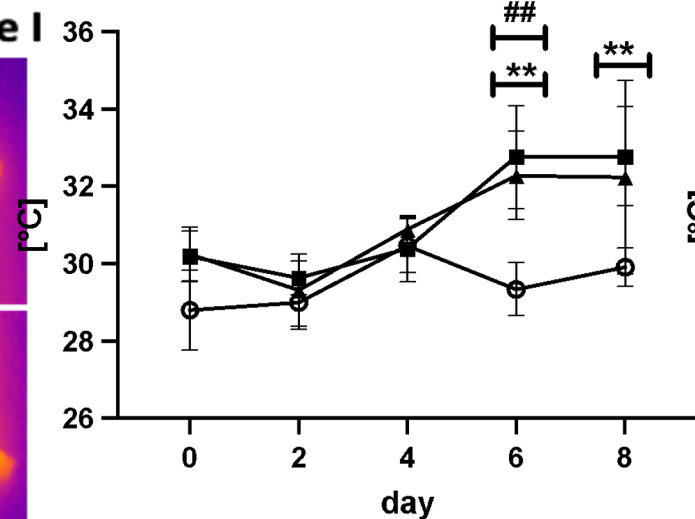


RA scoring, measuring of body temperature and temperature of paws. measuring of paws volume with plethysmometer (every 2 day)

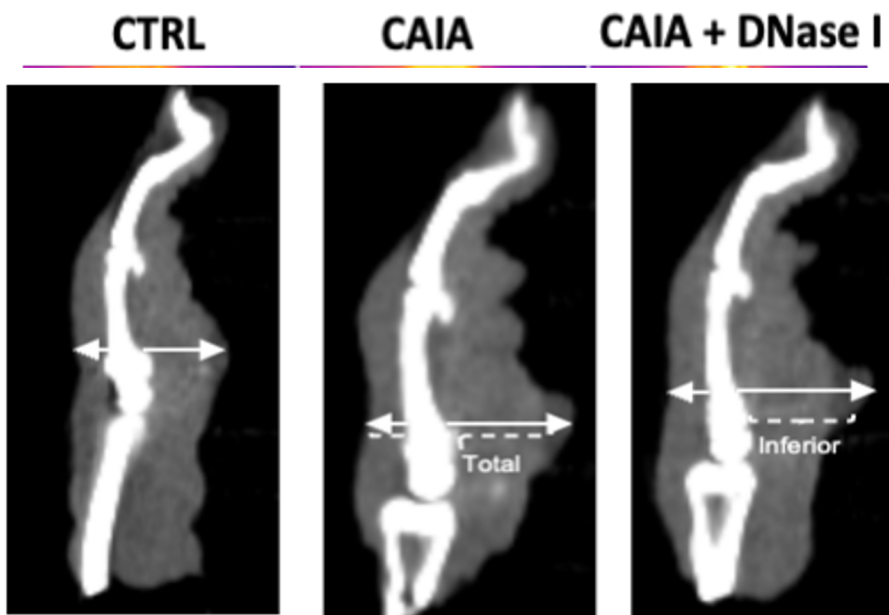
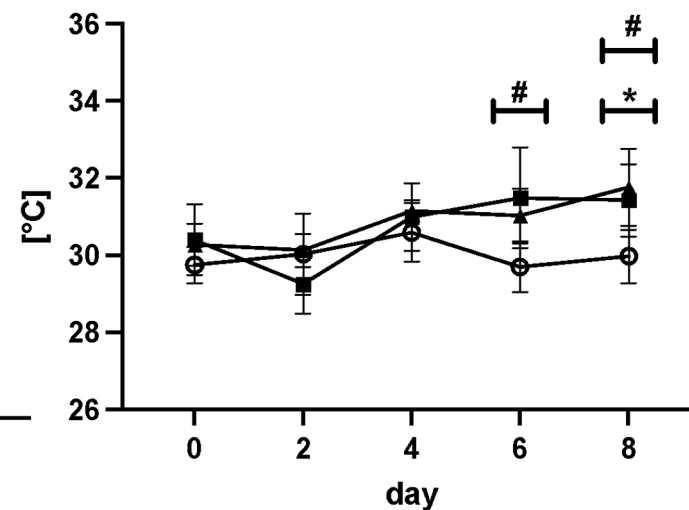




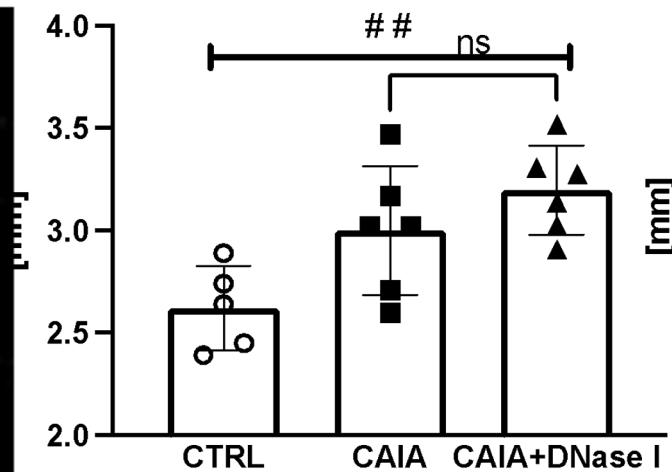
Temperature of front paws



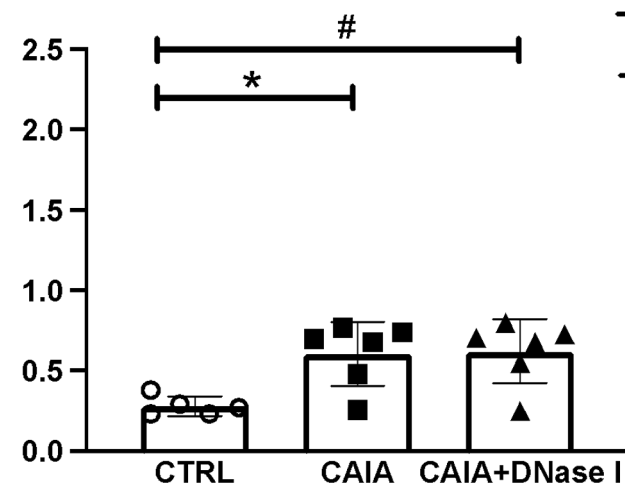
Temperature of back paws



Total paw thickness



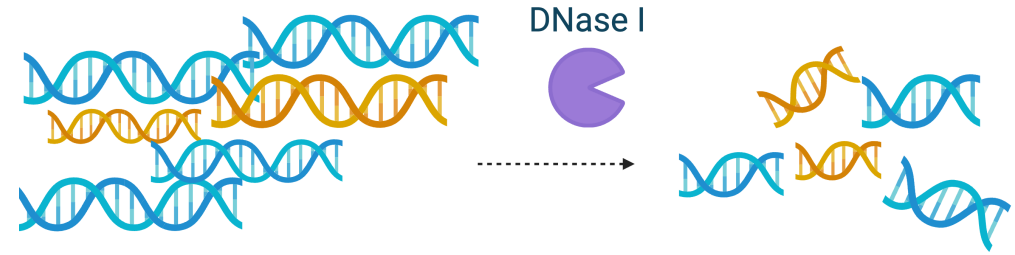
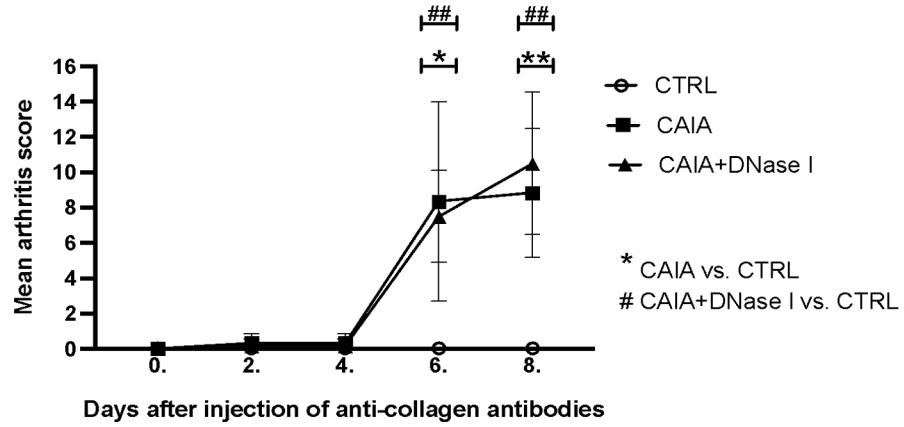
Inferior paw thickness



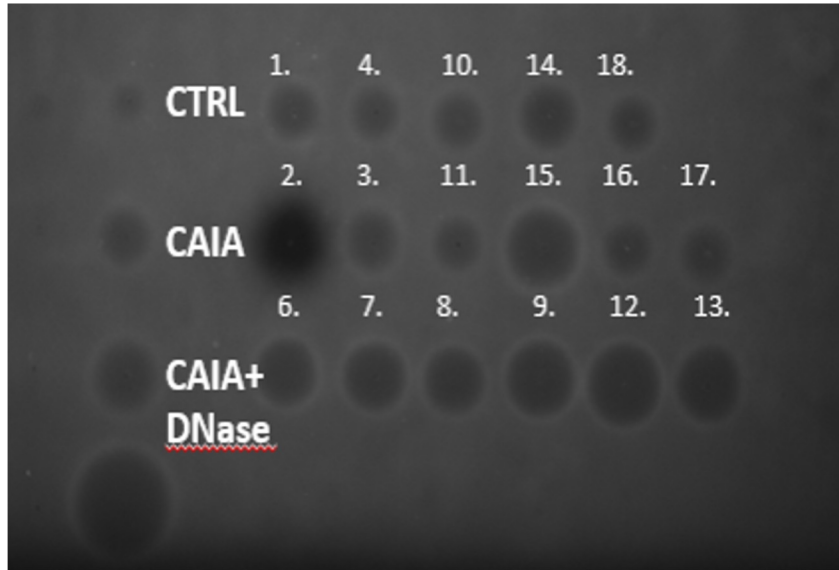
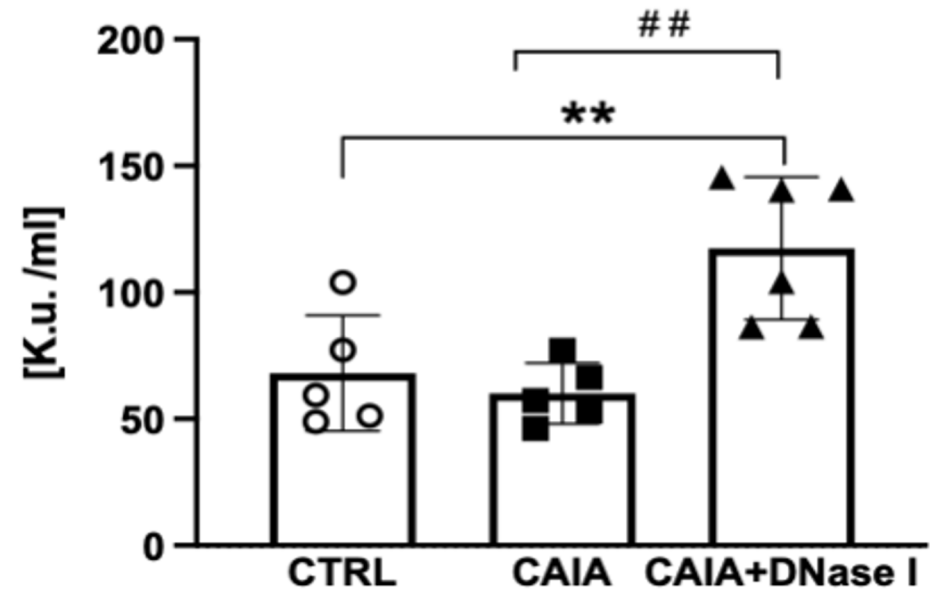
○ CTRL  
 ■ CAIA  
 ▲ CAIA+DNase I

\* CAIA vs. CTRL  
 # CAIA+DNase I vs. CTRL

# CAIA + DNase I



## DNase activity in serum



\* CAIA vs. CTRL  
 # CAIA+DNase I vs. CTRL



CAIA + DNase I

CTRL

0. day



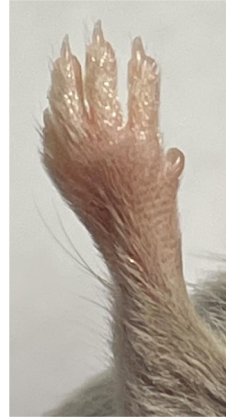
2. day



4. day



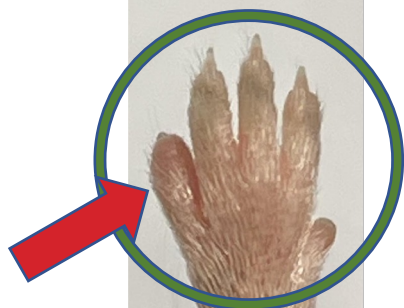
6. day



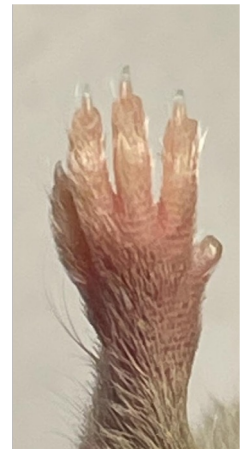
8. day



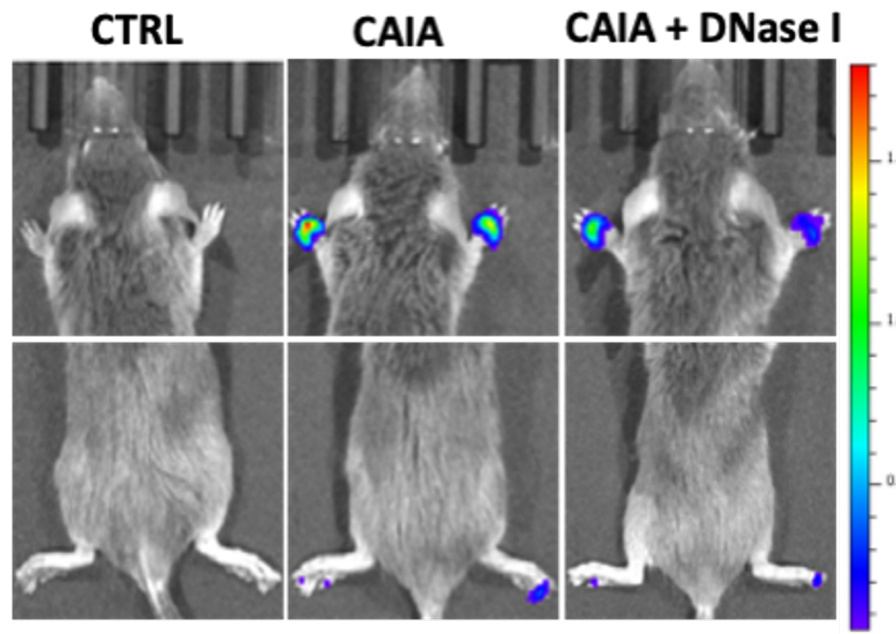
CAIA



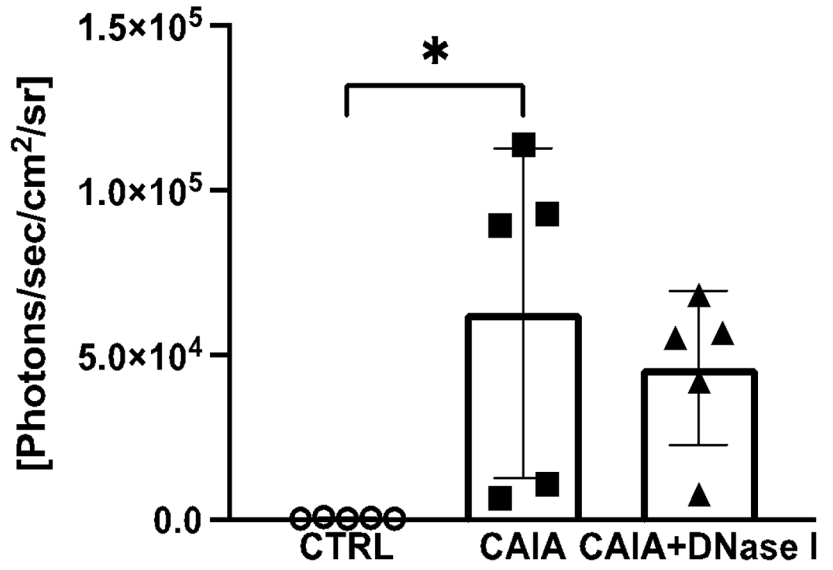
CAIA + Dnase I



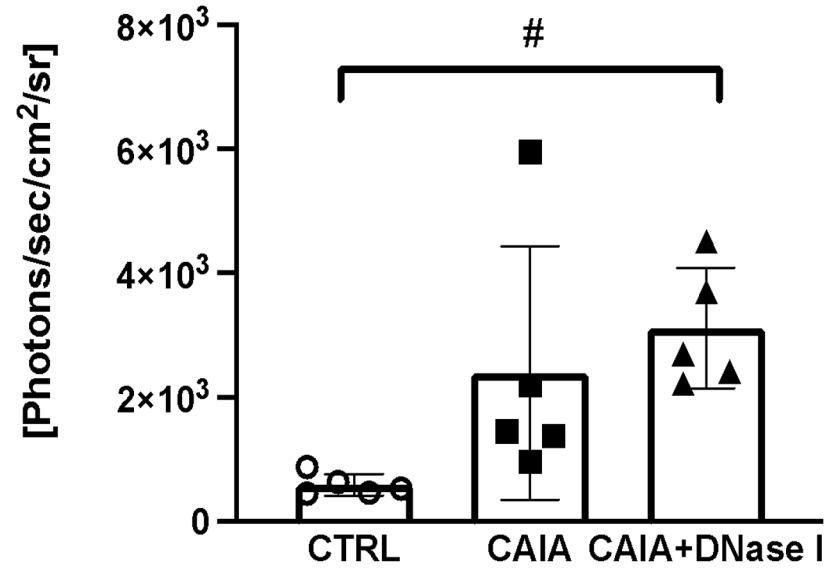




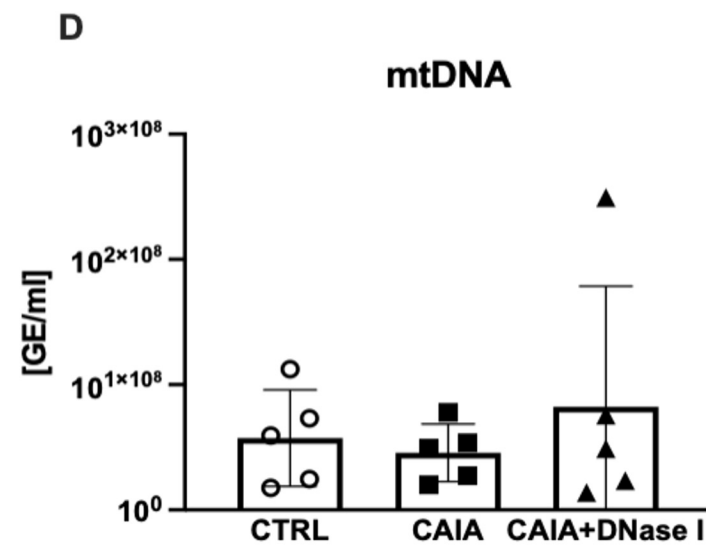
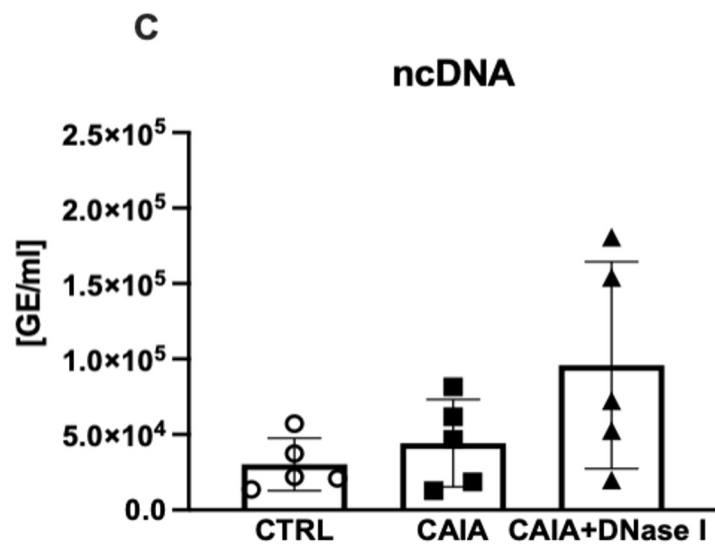
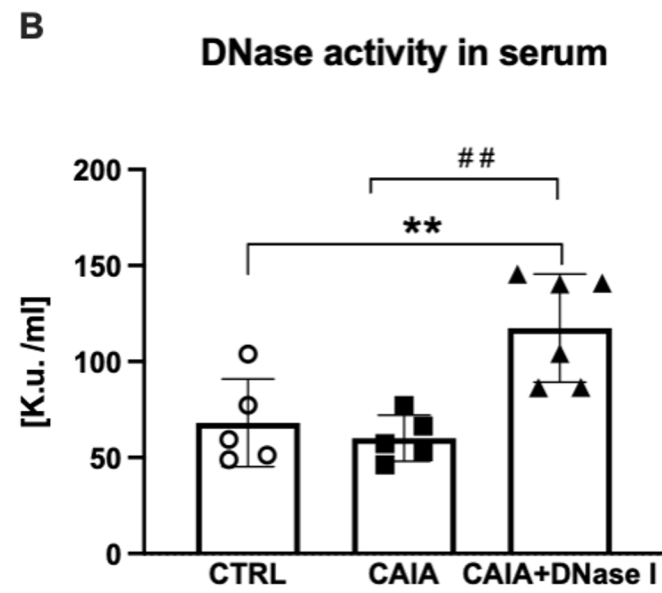
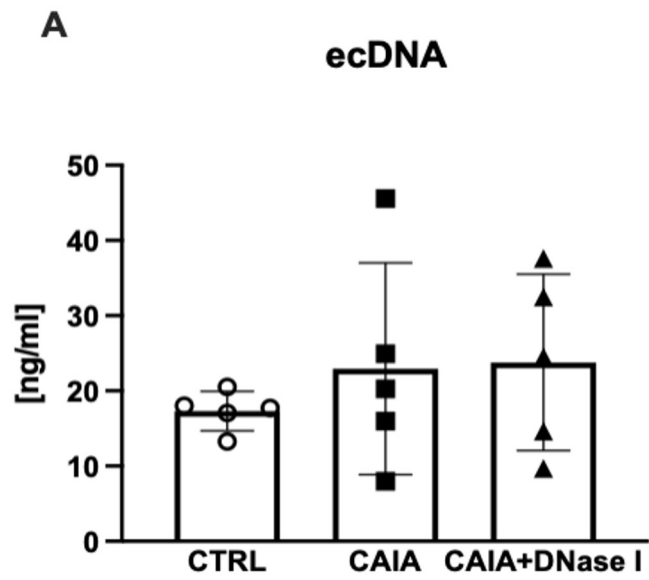
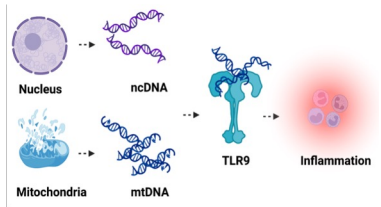
Bioluminescence front paws



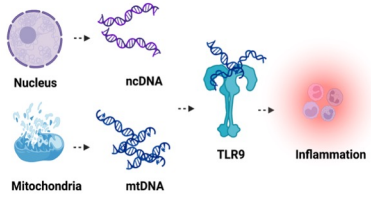
Bioluminescence back paws



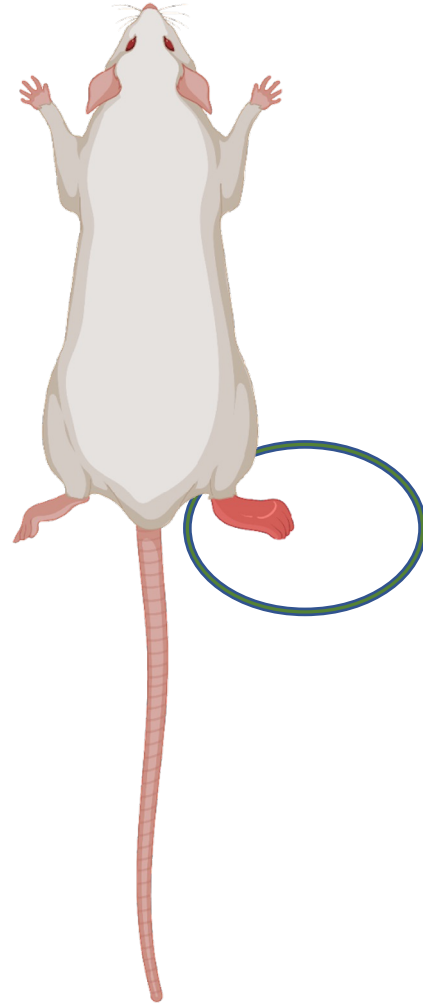
CAIA + DNase I  
X



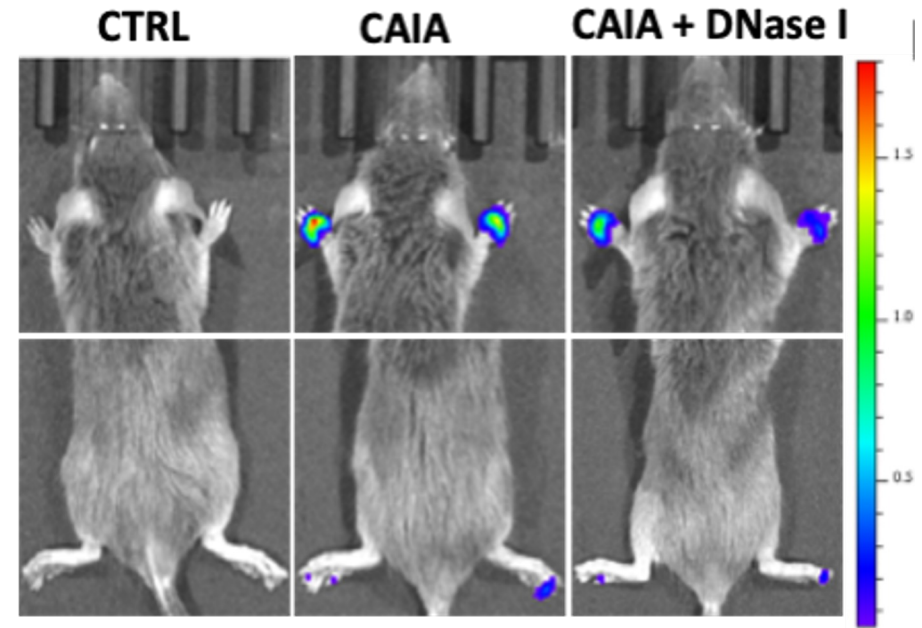
# CAIA + DNase I



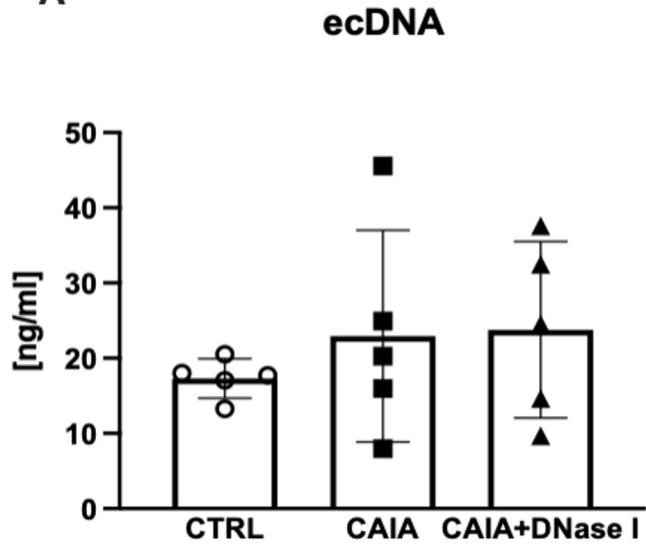
# CAIA



?

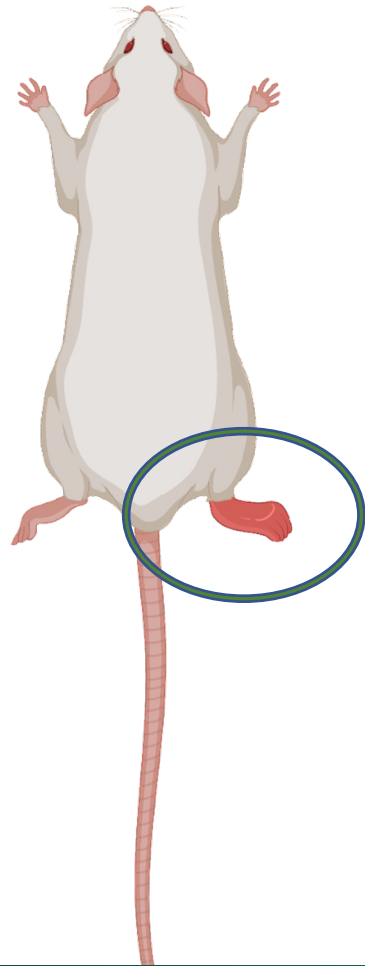


A

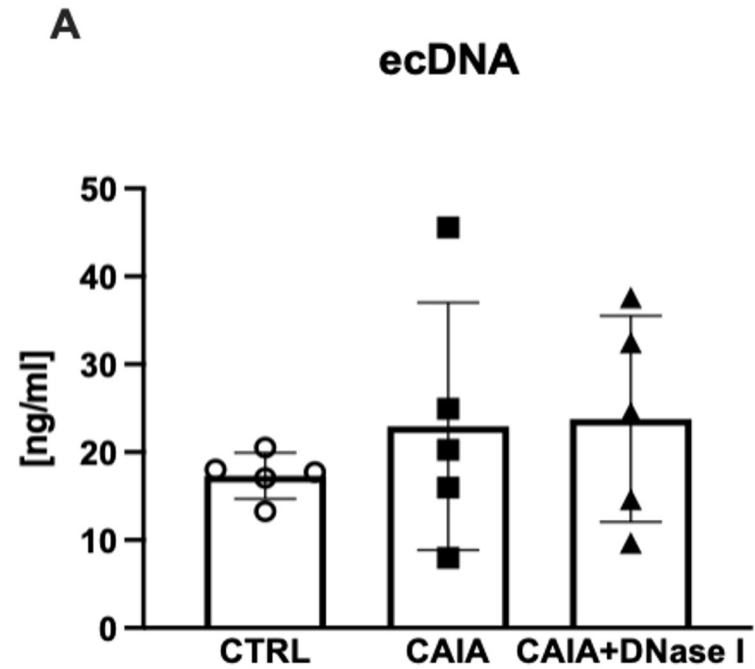
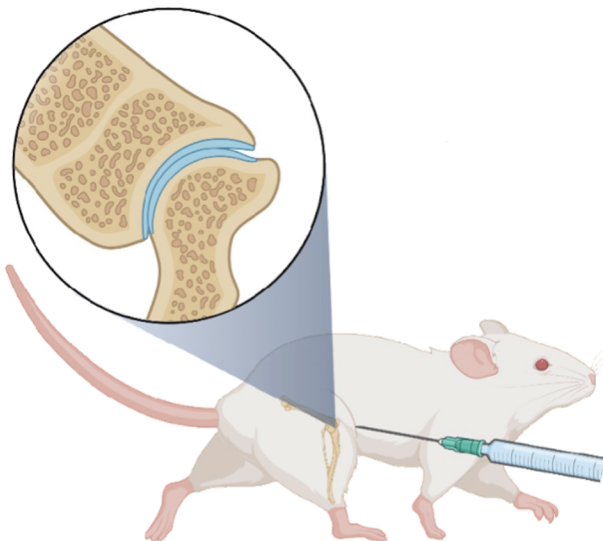




CAIA + DNase I



?

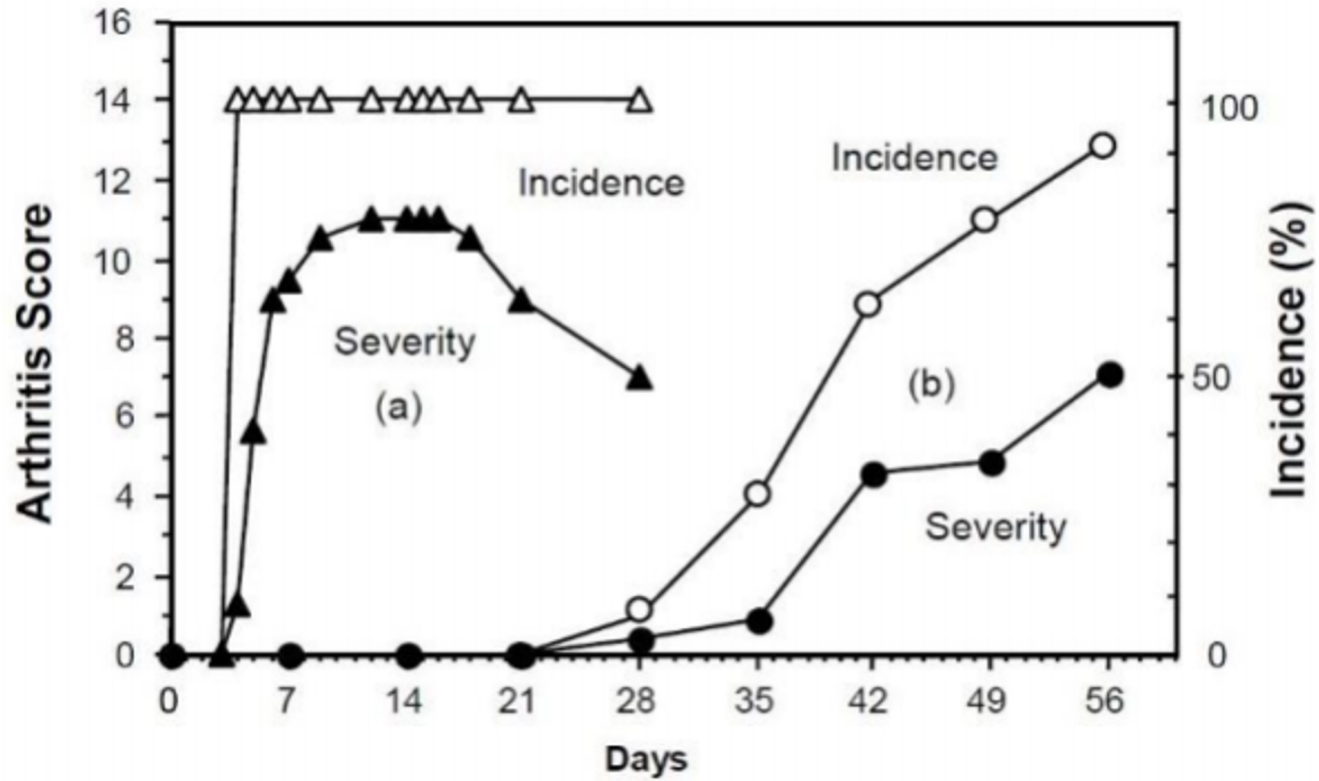
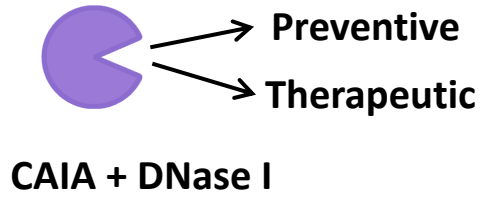


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## Extracellular mitochondrial DNA and oxidatively damaged DNA in synovial fluid of patients with rheumatoid arthritis

Research article | Published: 25 June 2003

Volume 5, article number R234, (2003) [Cite this article](#)

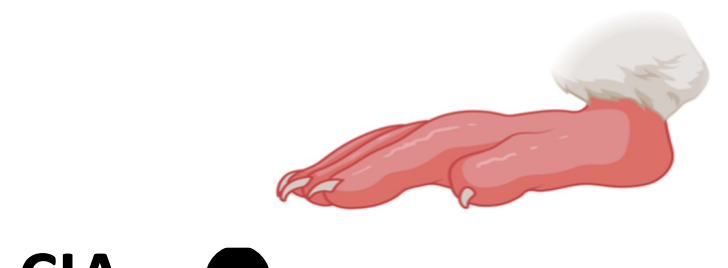


CAIA ▲

Collagen antibody-  
induced arthritis

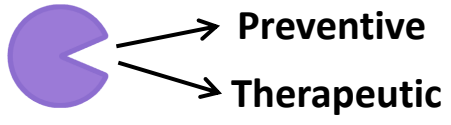


vs.



CIA ●

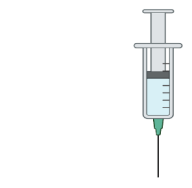
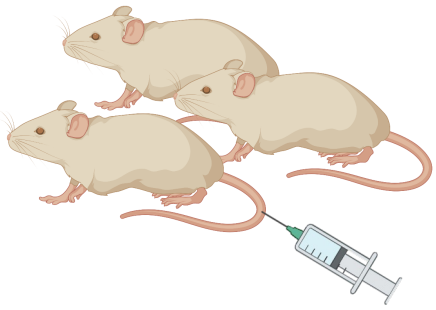
Collagen-induced  
arthritis



# DNase application - in CIA model

CAIA + DNase I

DNase I 10mg/kg (i.p.)      DNase I 10mg/kg (i.p.)



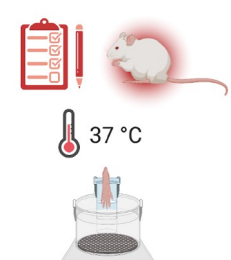
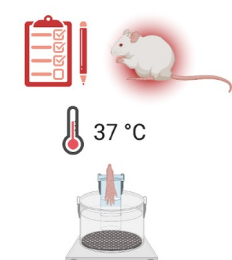
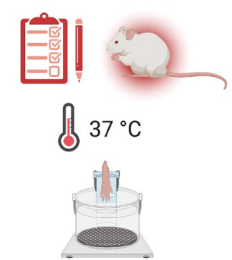
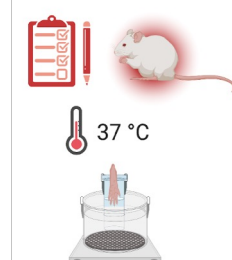
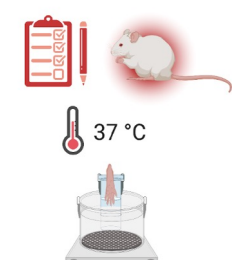
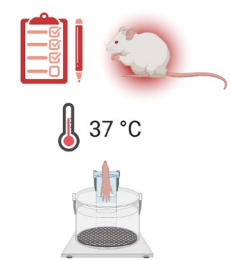
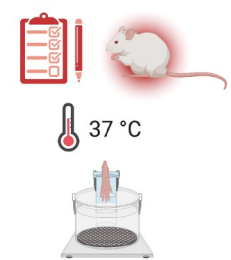
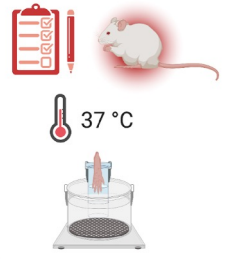
Daily injection

“PREVENTIVE”

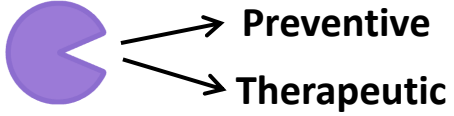
“THERAPEUTIC”



Baseline measuring  
Bovine collagen type II (s.c.)



Scoring every 7 days



CAIA + DNase I

CTRL



CIA



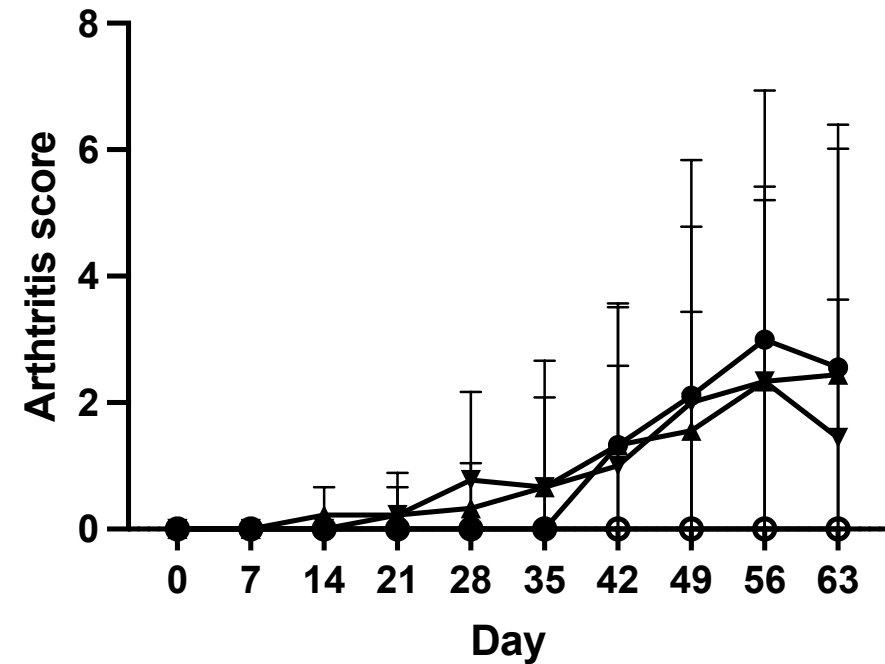
Therapeutic

CIA +

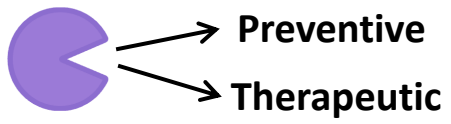


Preventive

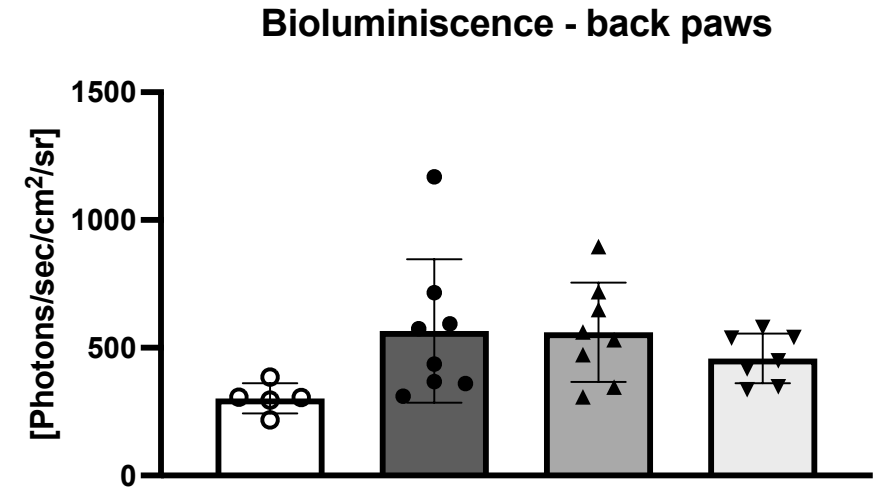
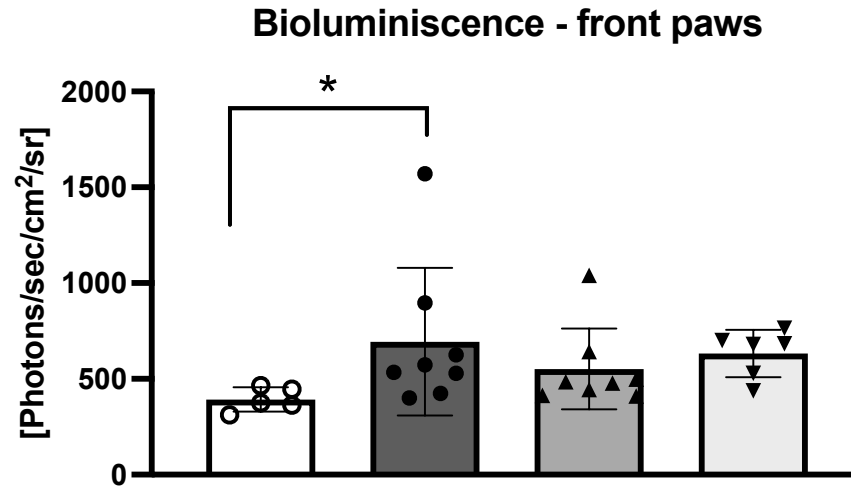
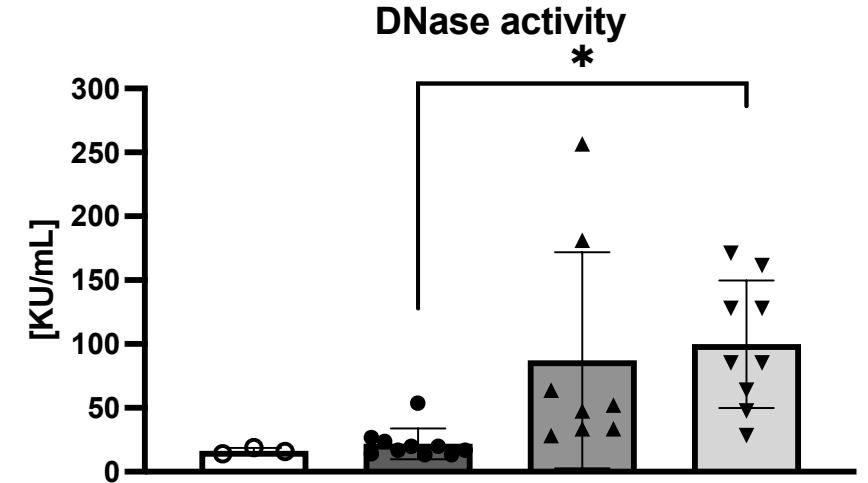
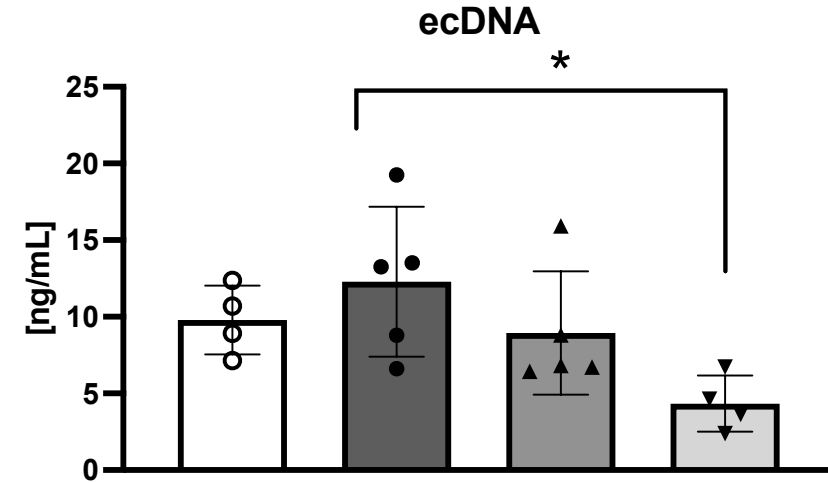
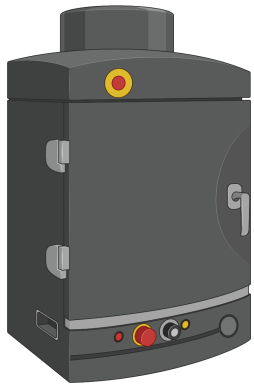
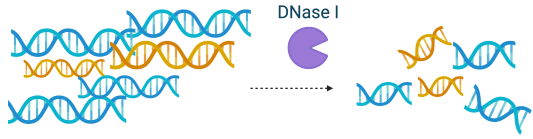
CIA +



○ CTRL - females    ● CIA - females    ▲ CIA - females - therapeutic    ▼ CIA - females - preventive



CAIA + DNase I

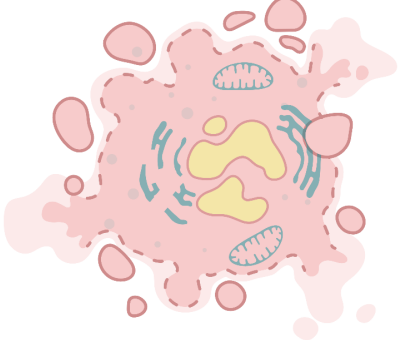


○ CTRL - females    ● CIA - females    ▲ CIA - females - therapeutic    ▼ CIA - females - preventive

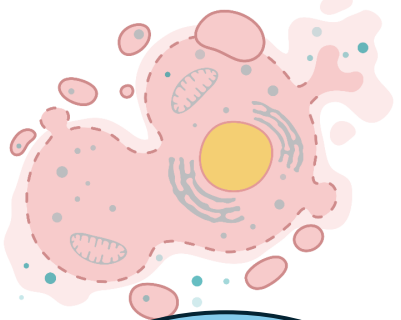




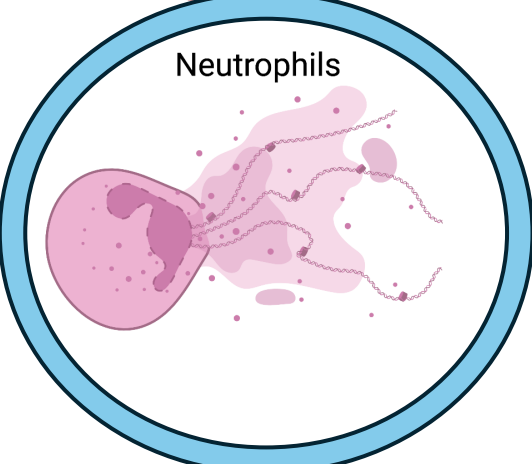
Necrosis



Apoptosis



Neutrophils



## NETosis as Source of Autoantigens in Rheumatoid Arthritis

[Elisa Corsiero](#)<sup>1,†</sup> [Federico Pratesi](#)<sup>2,†</sup> [Edoardo Prediletto](#)<sup>1</sup> [Michele Bombardieri](#)<sup>1,‡</sup> and [Paola Migliorini](#)<sup>2,\*‡</sup>

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Rheumatoid arthritis

## Neutrophil extracellular trap-associated carbamylation and histones trigger osteoclast formation in rheumatoid arthritis

[Liam J O'Neil](#)<sup>1, 2</sup>, [Christopher B Oliveira](#)<sup>1</sup>, [Xinghao Wang](#)<sup>1</sup>, [Mario Navarrete](#)<sup>2</sup>, [Ana Barrera-Vargas](#)<sup>3</sup>, [Javier Merayo-Chalico](#)<sup>3</sup>, [Rwan Aljahdali](#)<sup>1</sup>, [Eduardo Aguirre-Aguilar](#)<sup>3</sup>,  [Philip Carlucci](#)<sup>1</sup>,  [Mariana J Kaplan](#)<sup>1</sup>,  [Carmelo Carmona Rivera](#)<sup>1</sup>

Research Highlight | [Published: 29 June 2020](#)

RHEUMATOID ARTHRITIS

## NETs directly injure cartilage in RA

[Joanna Clarke](#) 

[Nature Reviews Rheumatology](#) **16**, 410 (2020) | [Cite this article](#)

**1819** Accesses | **6** Citations | **5** Altmetric | [Metrics](#)

Rheumatoid arthritis

## Carbamylated NETs promote bone erosion in RA

[Jessica McHugh](#) 

[Nature Reviews Rheumatology](#) **19**, 193 (2023) | [Cite this article](#)

**505** Accesses | **1** Citations | **7** Altmetric | [Metrics](#)



Neutrophils infiltrate the synovial joints in rheumatoid arthritis (RA) and can release neutrophil extracellular traps (NETs) that are implicated in RA pathogenesis. However, the role of neutrophils in bone destruction remains unclear. New findings link carbamylated NET proteins to bone erosion in RA.



Journal of Autoimmunity  
Volume 113, September 2020, 102484



## Autoantibodies to neutrophil extracellular traps represent a potential serological biomarker in rheumatoid arthritis

[Cynthia M. de Bont](#)<sup>a</sup>, [Marloes E.M. Stokman](#)<sup>a</sup>, [Priscilla Faas](#)<sup>a</sup>, [Rogier M. Thurlings](#)<sup>b</sup>, [Wilbert C. Boelens](#)<sup>a</sup>, [Helen L. Wright](#)<sup>c</sup>, [Ger J.M. Pruijn](#)<sup>a</sup>  

[Show more](#) 

## Alleviation of arthritis through prevention of neutrophil extracellular traps by an orally available inhibitor of protein arginine deiminase 4

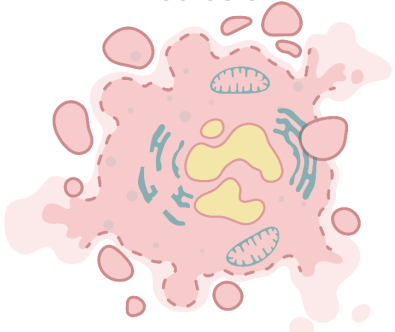
[Chandru Gajendran](#), [Shoichi Fukui](#), [Naveen M. Sadhu](#), [Mohammed Zainuddin](#), [Sridharan Rajagopal](#), [Ramachandraiah Gosu](#), [Sarah Gutch](#), [Saeko Fukui](#), [Casey E. Sheehy](#), [Long Chu](#), [Santosh Vishwakarma](#), [D. A. Jeyaraj](#), [Gurulingappa Hallur](#), [Denisa D. Wagner](#) & [Dhanalakshmi Sivanandhan](#) 

[Scientific Reports](#) **13**, Article number: 3189 (2023) | [Cite this article](#)

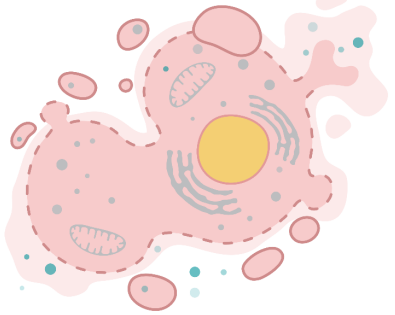
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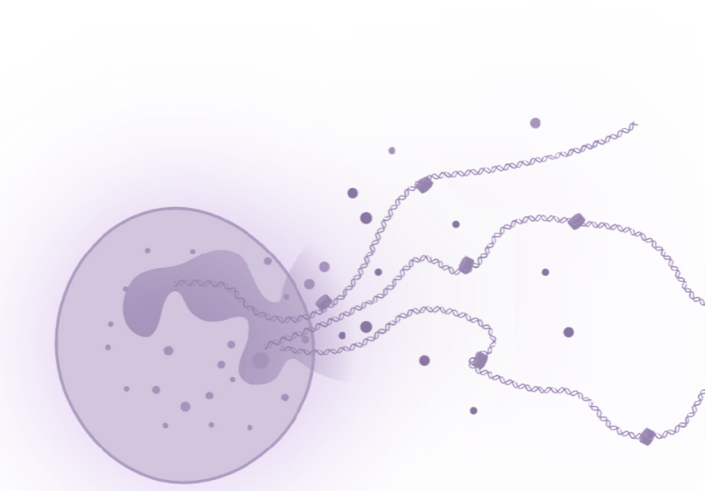
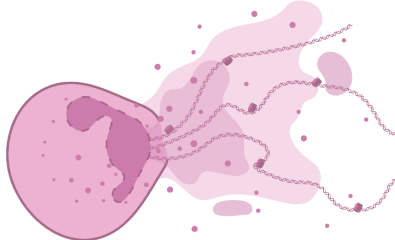
Necrosis



Apoptosis



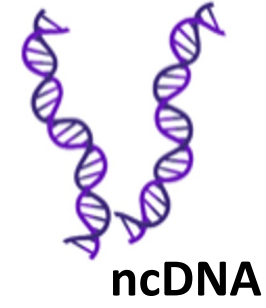
Neutrophils



Neutrophil

NETs

NETosis



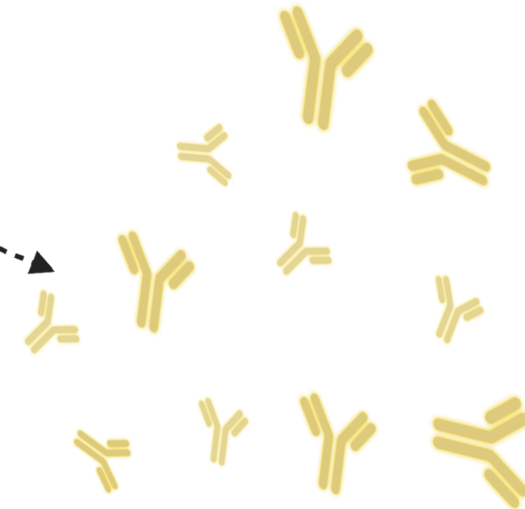
ncDNA



mtDNA



Citrullinated histones



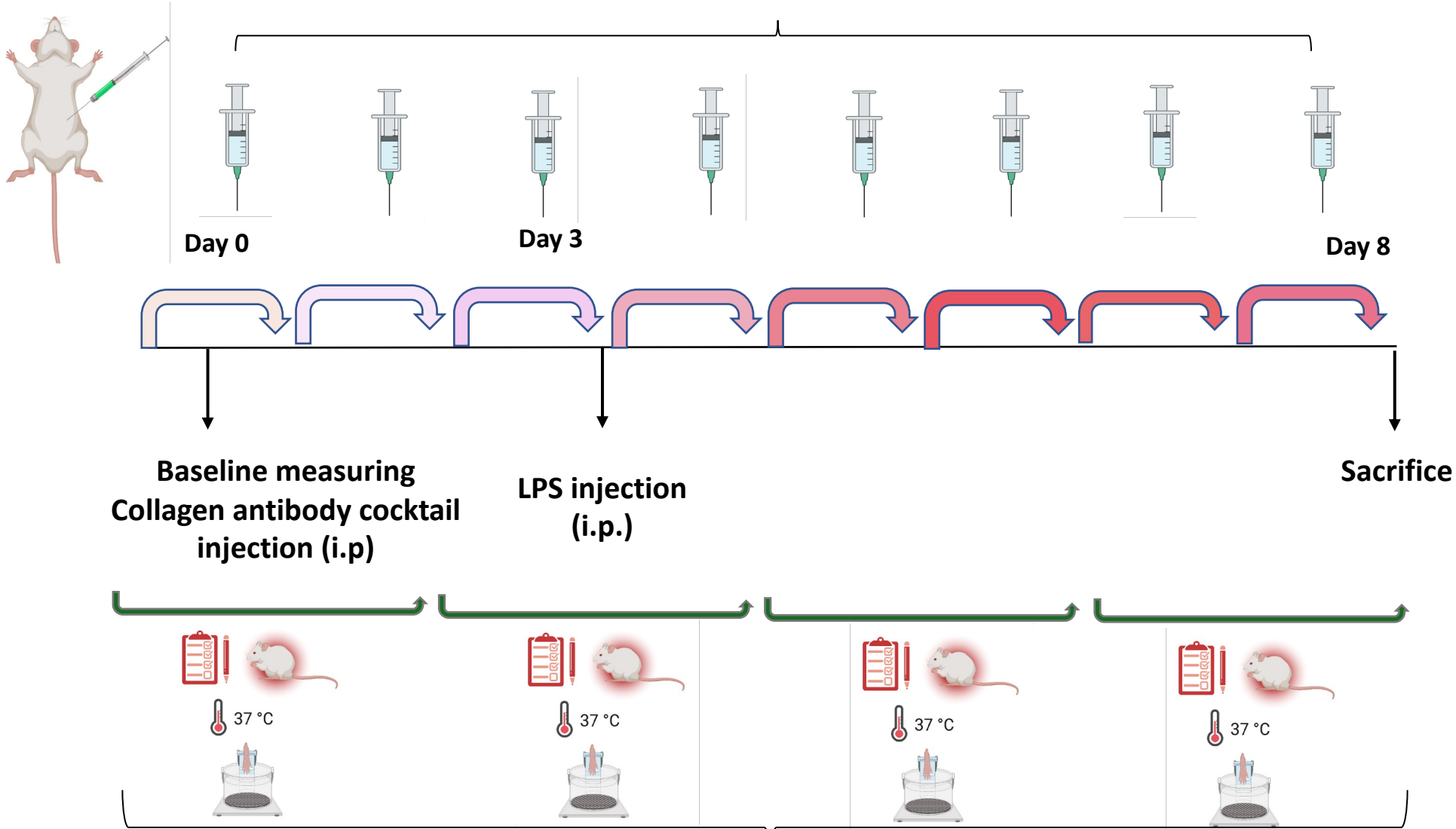
Anti-citrullinated protein antibodies





Cl-amidine

# Application of Cl-amidine in CAIA model - DBA/1J mice



RA scoring, measuring of body temperature and temperature of paws. measuring of paws volume with plethysmometer (every 2 day)



Cl-amidine

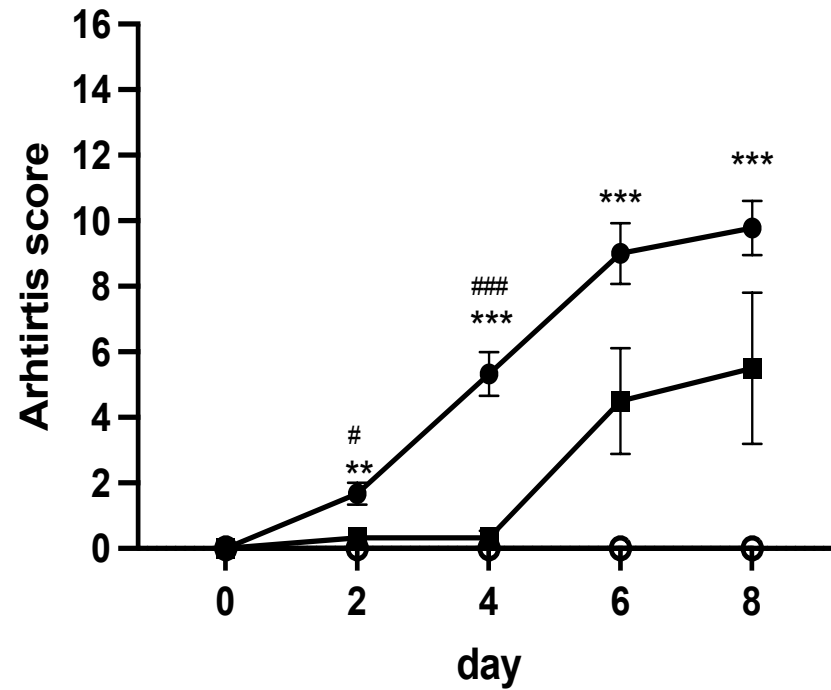
CTRL



CAIA



CAIA +  
Cl amidine



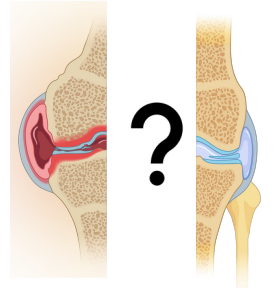
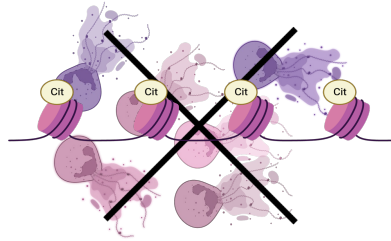
○ CTRL

● CAIA

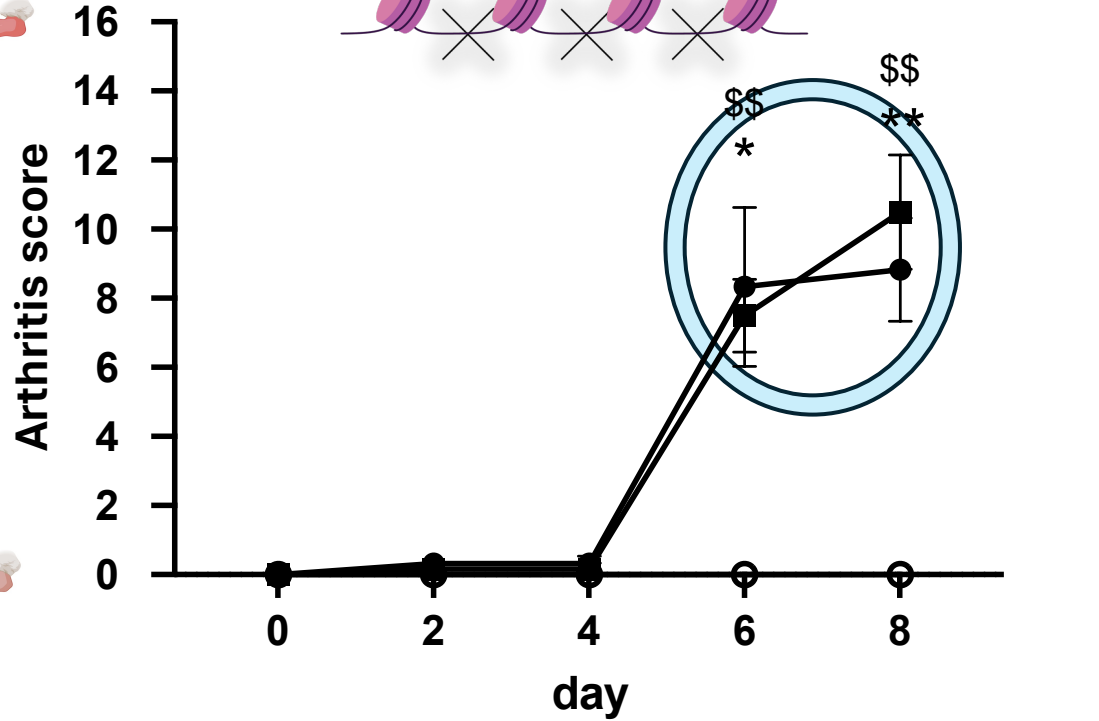
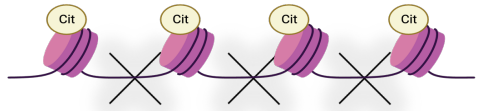
■ CAIA + Cl amidine

\* CAIA vs CTRL

# CAIA vs CAIA + CL amidine



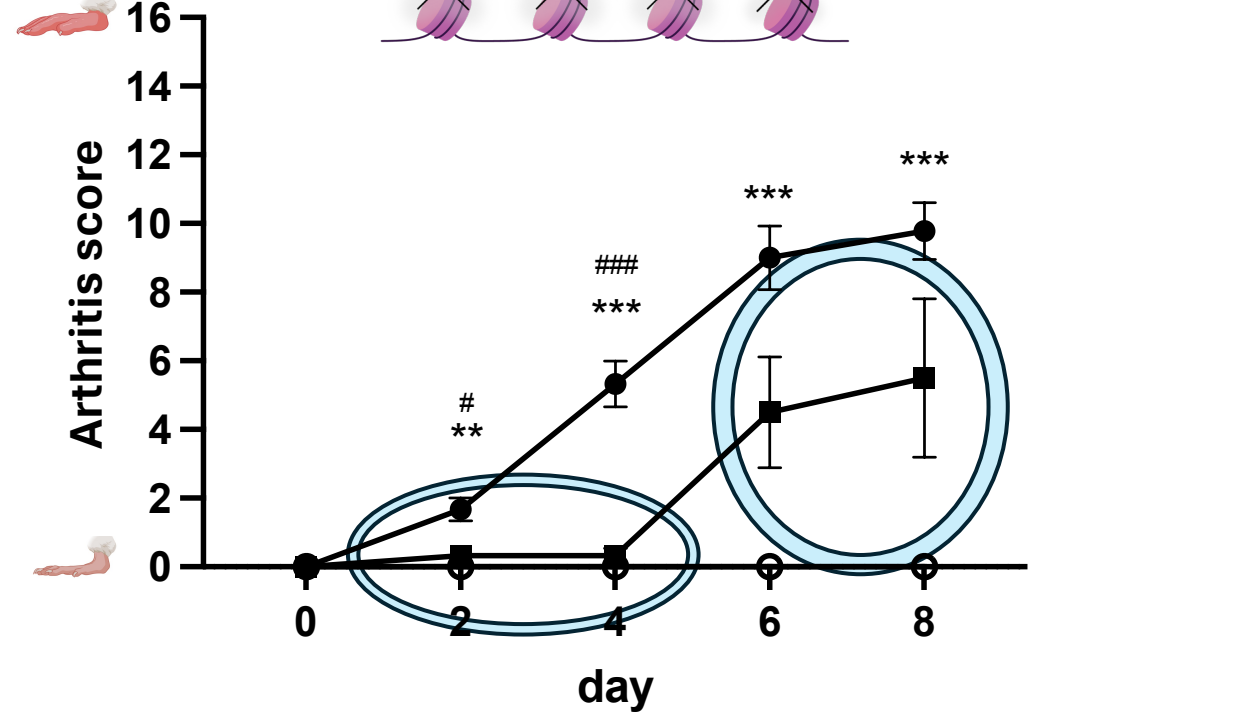
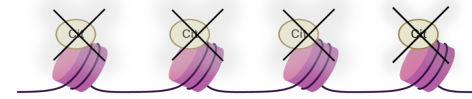
### DNase I



○ Control ● Arthritis ■ Arthritis + DNase I

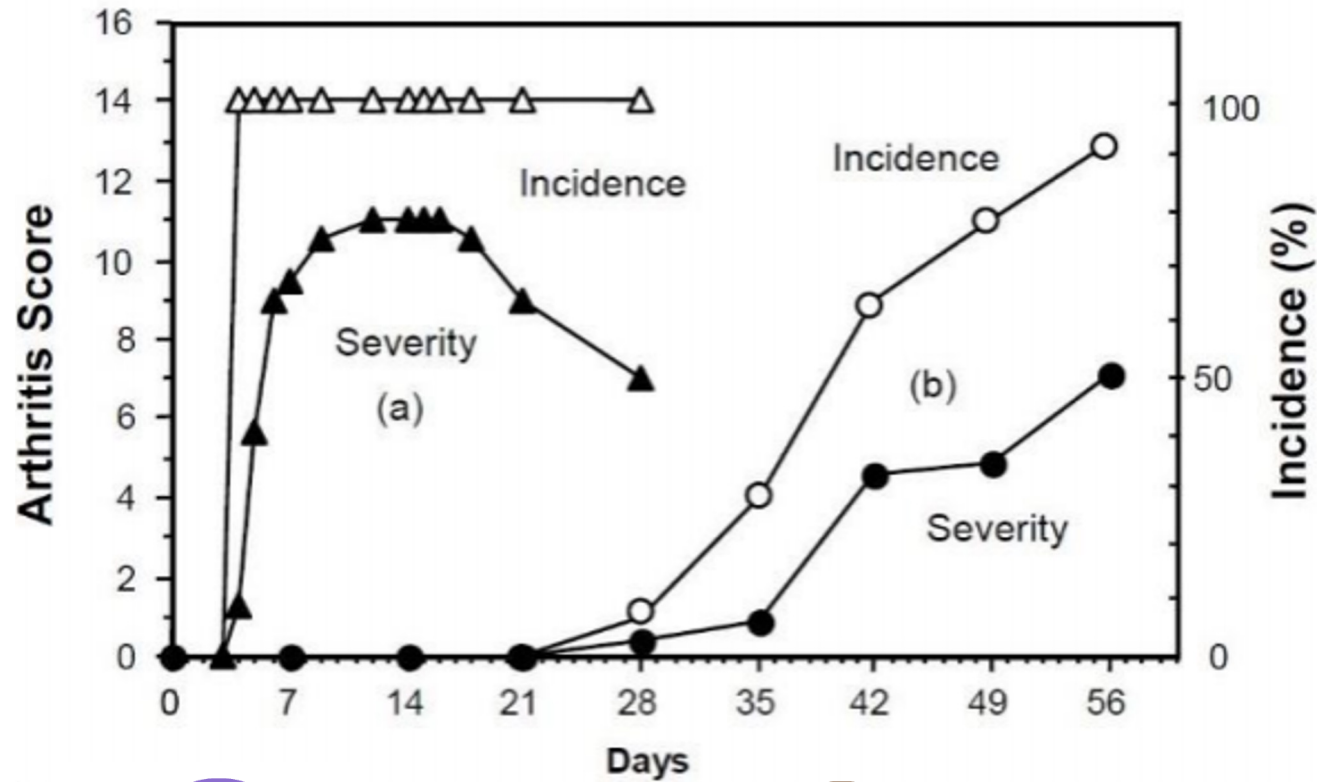
\* Arthritis vs. Control, \$ Arthritis + DNase I vs. Control

### NETs inhibitor



○ Control ● Arthritis ■ Arthritis + NETs inhibitor

\* Arthritis vs. Control, # Arthritis vs. Arthritis + NETs inhibitor



Dnase I



Dnase IL3

Article | March 30 2021

## Autoantibody-mediated impairment of DNASE1L3 activity in sporadic systemic lupus erythematosus

In Special Collection: JEM Immunology Collection 2021

Johannes Hartl <sup>ID</sup>, Lee Serpas <sup>ID</sup>, Yueyang Wang <sup>ID</sup>, Ali Rashidfarrokhi <sup>ID</sup>, Oriana A. Perez <sup>ID</sup>, Benjamin Sally <sup>ID</sup>, Vanja Sisirak <sup>ID</sup>, Chetna Soni <sup>ID</sup>, Alireza Khodadadi-Jamayran <sup>ID</sup>, Aristotelis Tsirigos <sup>ID</sup>, Ivan Caiello <sup>ID</sup>, Claudia Bracaglia <sup>ID</sup>, Stefano Volpi <sup>ID</sup>, Gian Marco Ghiggeri <sup>ID</sup>, Asiya Seema Chida <sup>ID</sup>, Ignacio Sanz <sup>ID</sup>, Mimi Y. Kim <sup>ID</sup>, H. Michael Belmont <sup>ID</sup>, Gregg J. Silverman <sup>ID</sup>, Robert M. Clancy <sup>ID</sup>, Peter M. Izmirly <sup>ID</sup>, Jill P. Buyon <sup>ID</sup>, Boris Reizis <sup>ID</sup>

+ Author and Article Information



J Exp Med (2021) 218 (5): e20201138. | <https://doi.org/10.1084/jem.20201138> | Article history

## Host DNases prevent vascular occlusion by neutrophil extracellular traps

MIGUEL JIMÉNEZ-ALCÁZAR <sup>ID</sup>, CHANDINI RANGASWAMY <sup>ID</sup>, RACHITA PANDA <sup>ID</sup>, JOSEPHINE BITTERLING <sup>ID</sup>, YASHIN J. SIMSEK <sup>ID</sup>, ANDY T. LONG <sup>ID</sup>, ROSTYSLAV BILYI <sup>ID</sup>

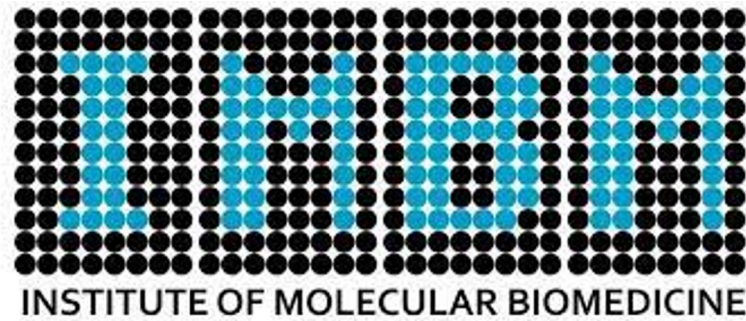
, VEIT KRENN, CHRISTOPH RENNÉ <sup>ID</sup>, [...], AND TOBIAS A. FUCHS <sup>ID</sup>

+8 authors

[Authors Info & Affiliations](#)

6th Central - Eastern European congress  
on cell free DNA and medical practice

# The role of extracellular DNA in pathogenesis of rheumatoid arthritis



**Veronika Borbélyová, Bernard Maximilian Schuh, Peter Celec , Barbora Vlková**  
**kristina.macakova@imbm.sk**



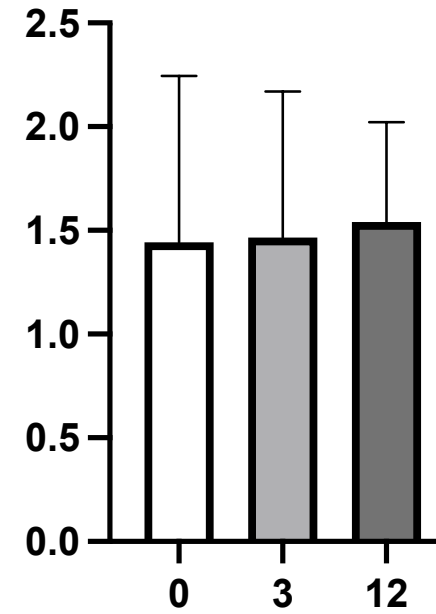
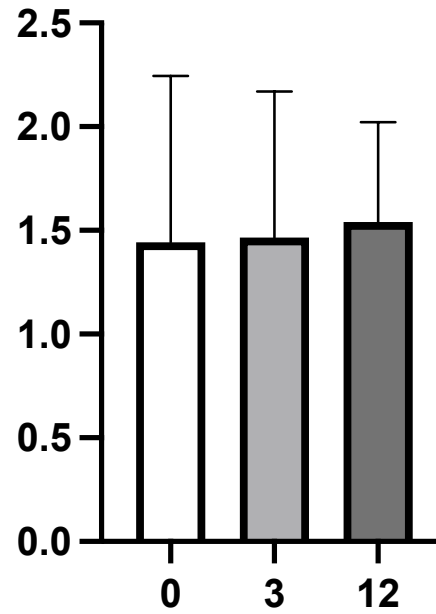
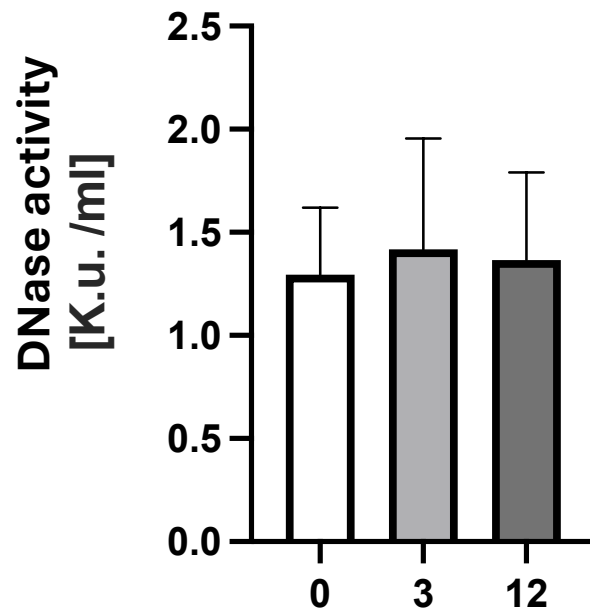
**good responders**



**moderate responders**



**non-responders**



**months**