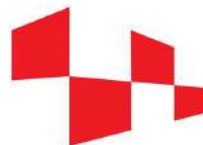


Mijeloidno-specifični molekularni posrednici subhondralnog koštanog razaranja u mišjem modelu reumatoidnog artritisa

Nina Lukač

Mentorica: prof. dr. sc. Nataša Kovačić

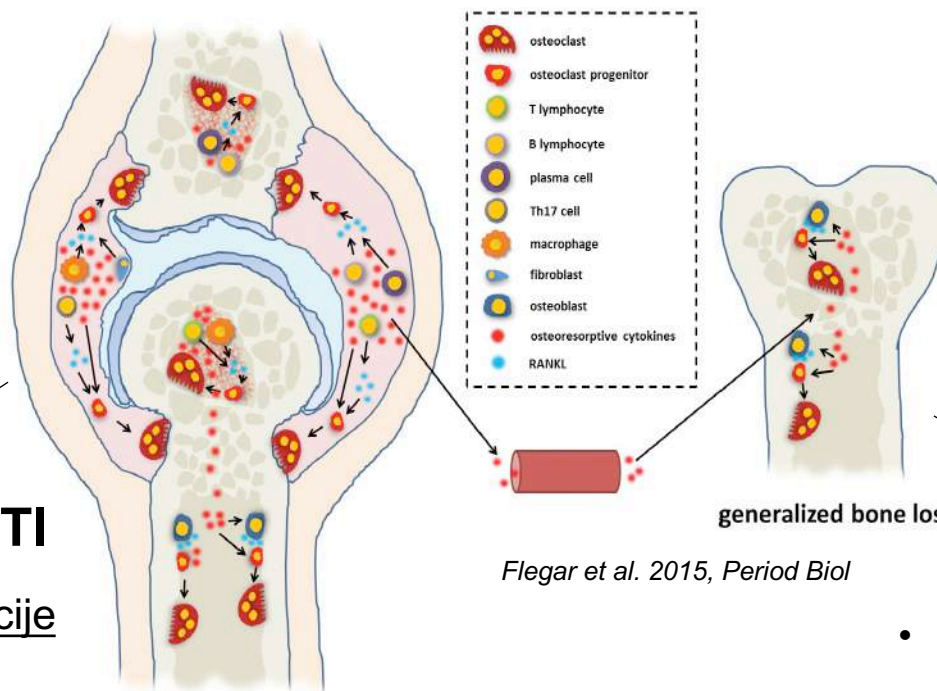
Zavod za anatomiju
Laboratorij za molekularnu imunologiju,
Hrvatski institut za istraživanje mozga
Medicinski fakultet Sveučilišta u Zagrebu



HRZZ
Hrvatska zaklada
za znanost



Reumatoidni artritis



**LOKALNI
GUBITAK KOSTI**

- Irreverzibilne deformacije

generalized bone loss

**SUSTAVNI
GUBITAK KOSTI**

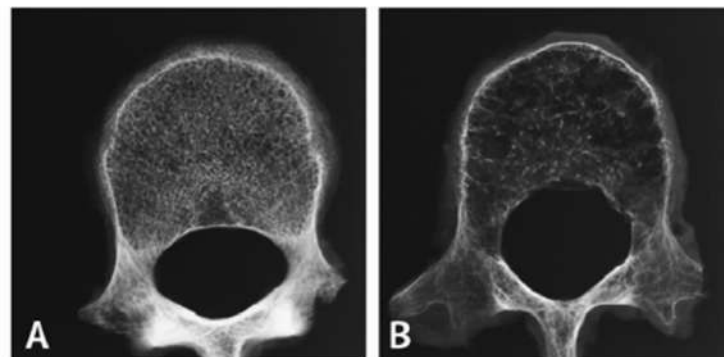
- Osteopenija/osteoporoza

Flegar et al. 2015, Period Biol

local bone loss

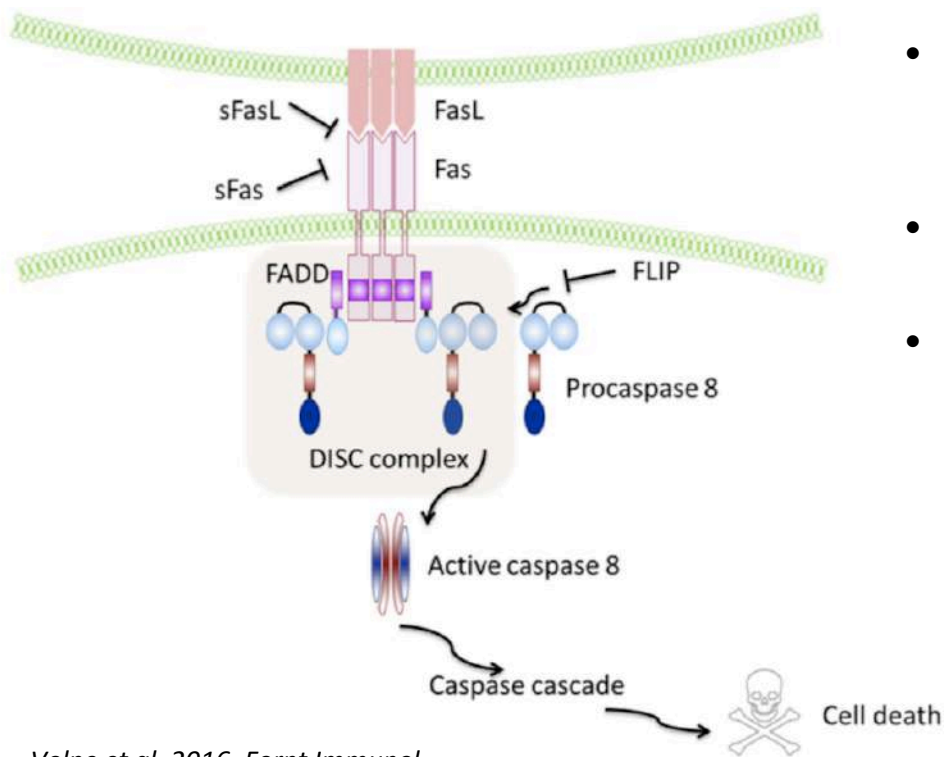


Schett et al. 2007, Arthritis Res Ther



Dougherty et al. 2010 Biomed Imaging Interv J

Fas/FasL sustav



Volpe et al. 2016, *Front Immunol*

- TNF obitelj
- **Apoptoza** + Neapoptotične uloge sustava
- Homeostaza imunskog sustava
- **Homeostaza koštanog sustava**
 - Djelovanje na koštane stanice (Wu 2003, Park 2005, Kovacic 2007)
 - Posrednik postmenopauzalne osteoporoze (Katavic 2003, Nakamura 2007, Kovacic 2010)
 - **Reumatoidni artritis**

Artritis potaknut antigenom (AIA)



C57BL6 (B6/wt) i Fas^{-/-} miševi
ženke, dob - 12 tjedana

↑
Dan 0
(primarna
imunizacija)

1. 200 µg mBSA u kompletnom Freundovom adjuvansu (CFA), s.c. (AIA, ctrl)
2. PBS (NI)

↑
Dan 7
("booster")

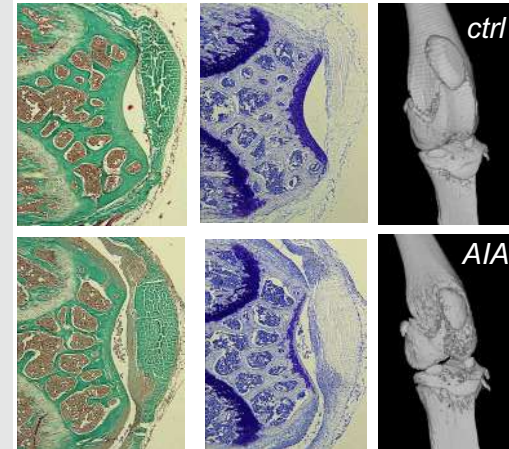
1. 100 µg mBSA u CFA, s.c. (AIA, ctrl)
2. PBS (NI)

↑
Dan 21
(indukcija artritisa,
procjena imunizacije)

1. 50 µg mBSA u 10 µL PBS, intra-artikularno (i.a., AIA)
2. 10 µL PBS i.a. (ctrl, NI)

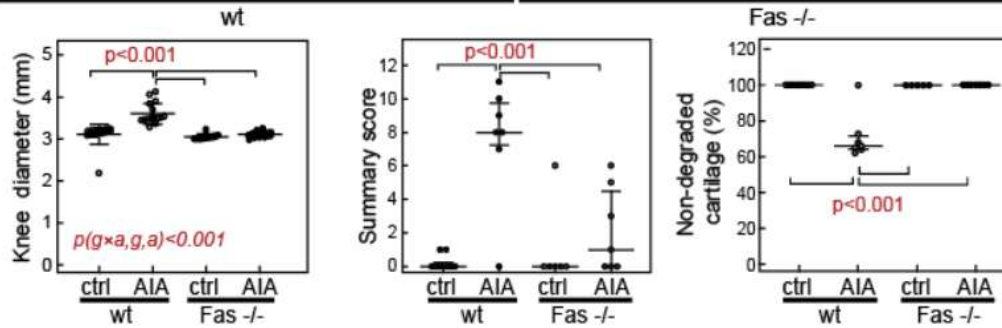
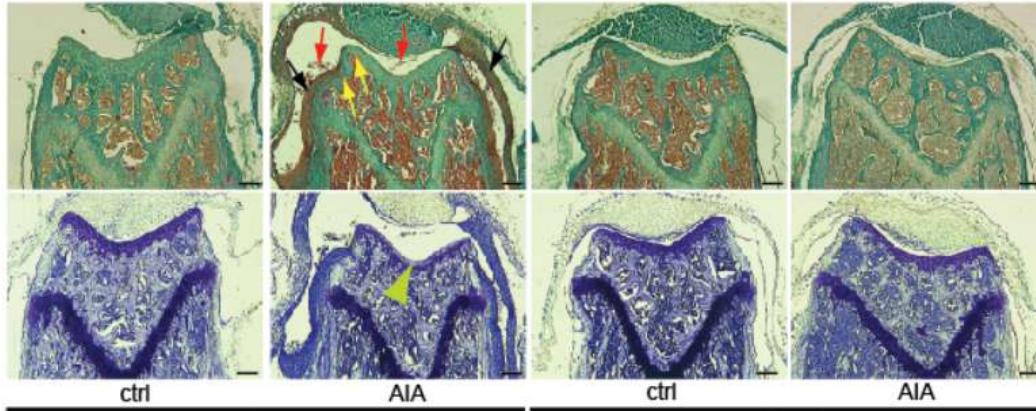
↑
Dan 31
(dan 10 poslije indukcije)
UZIMANJE UZORAKA

- POKUSNE SKUPINE:**
1. WT AIA (n = 8-10)
 2. WT ctrl/NI (n = 8-10)
 3. Fas^{-/-} AIA (n = 8-10)
 4. Fas^{-/-} ctrl/NI (n = 8-10)

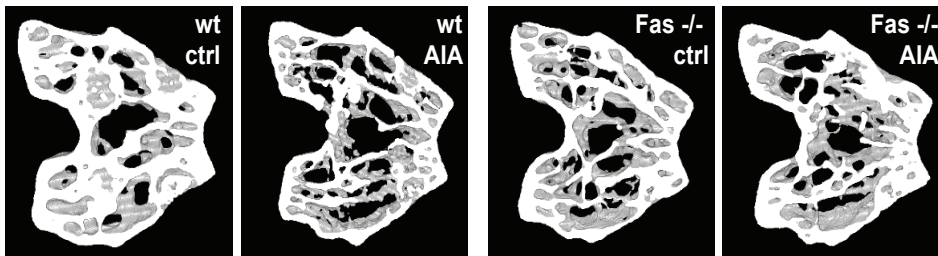


Artritis potaknut antigenom (AIA) u Fas^{-/-} miševa

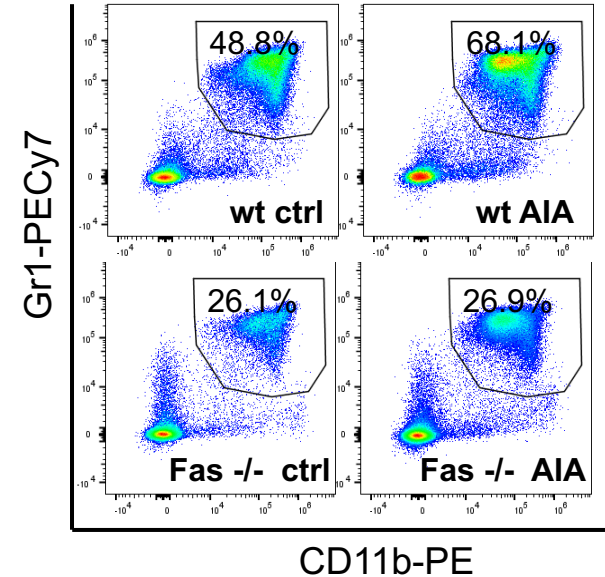
Smanjena sinovijalna upala i oštećenje hrskavice



Izostatak lokalnog koštanog razaranja



Smanjeno nakupljanje mijeloidnih stanica u sinoviji



Analiza transkriptoma sinovijalnih mijeloidnih stanica



B6 & Fas $-/-$
miševi,
AIA, d31

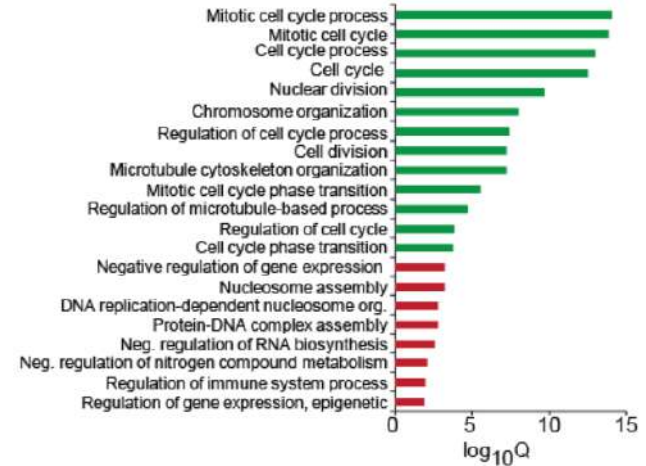
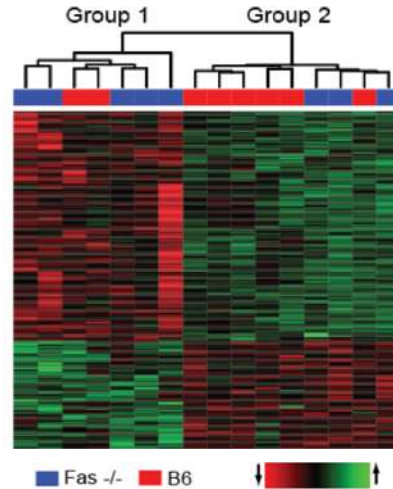
↓
opuštanje
sinovijalnih stanica
pomoću
kolagenaze

↓
FASC izdvajanje
CD11b+Gr-1+
stanica

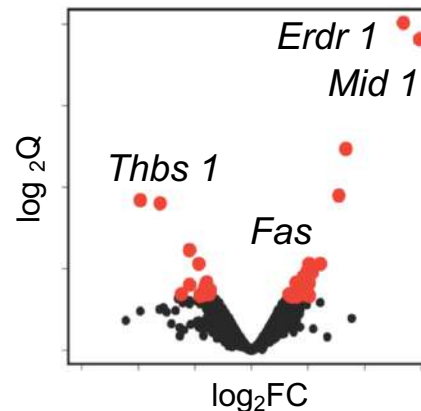


Affymetrix ST 2.0
genski čip

A. Hierarhijsko grupiranje



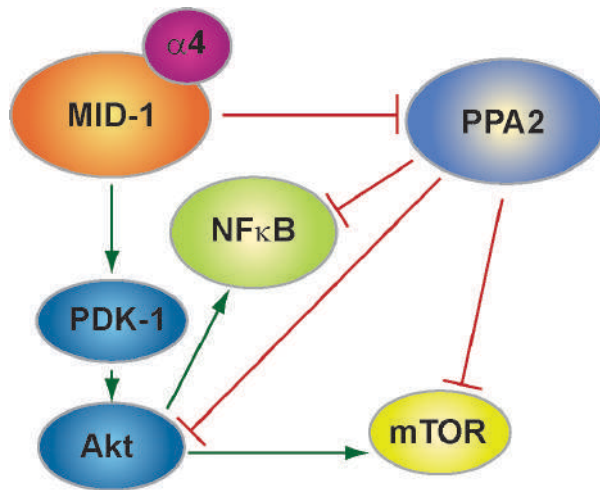
B. Izražaj gena - Fas $-/-$ vs. B6



Različito izraženi geni

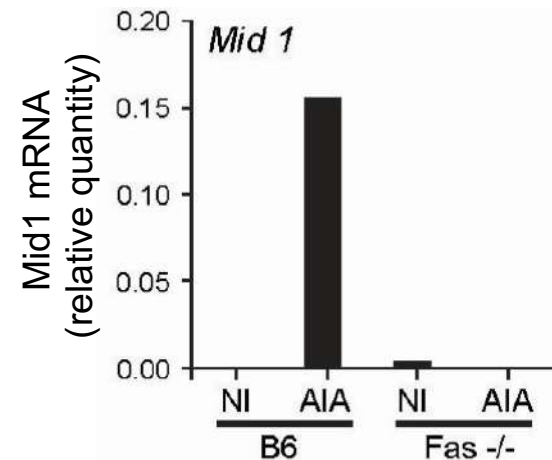
- *Midline 1* (TRIM18)

- E3 ubikvitinska ligaza, potiče razgradnju **proteinske fosfataze 2A (PP2A)** (*Trockenbacher A, 2001 Nat Genetics*)
- Uloga u **alergijskoj upali dišnog sustava** (*Collison A, 2013 Nat Med, 2015 J Allergy Clin Immunol*).

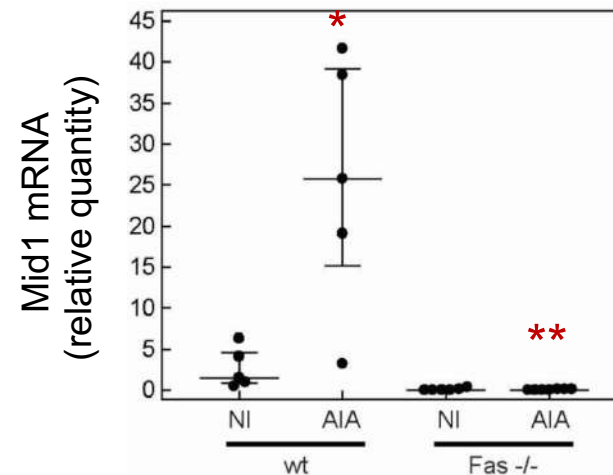


Modificirano prema Koehler A, 2014, *European Journal of Cancer*

Sortirane CD11b⁺Gr1⁺ stanice (qPCR)



Tkivni ekstrakti koljena (qPCR)

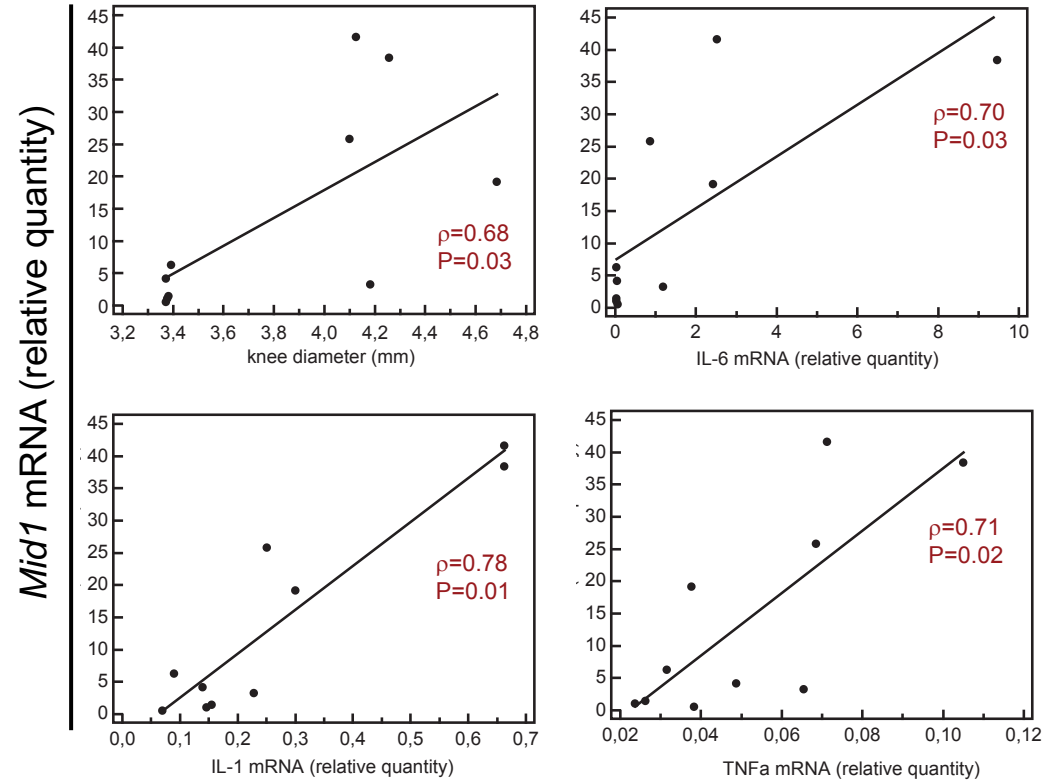
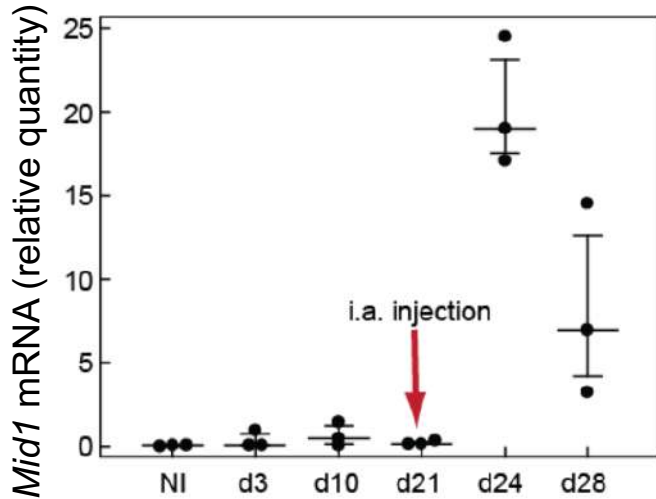


* $p < 0.05$ vs. NI,

** $p < 0.005$ vs. wt

Povezanost s lokalnim izražajem pro-upalnih citokina i jakosti artritisa

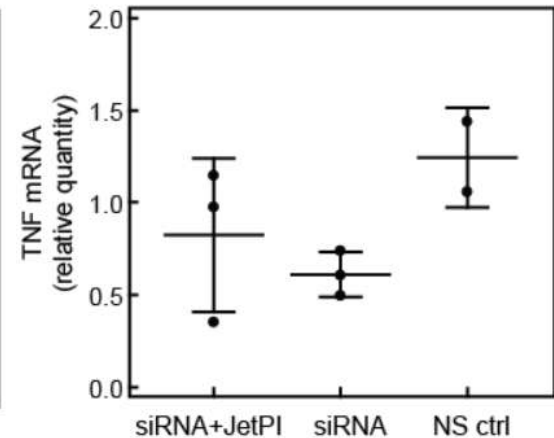
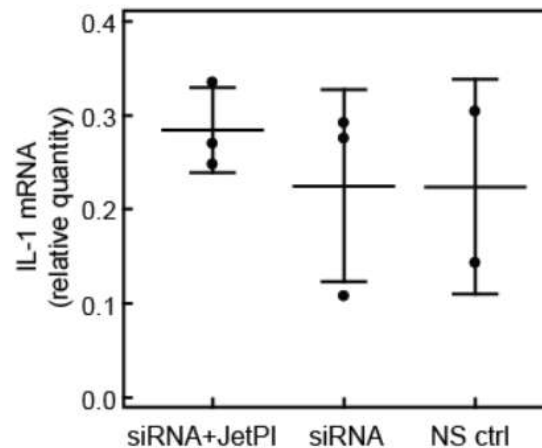
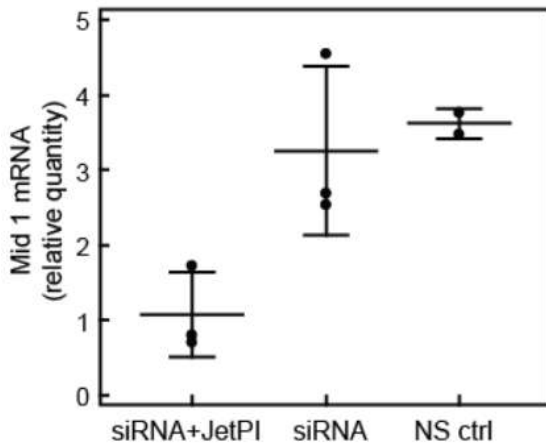
Pojačan izražaj *Mid 1* nakon nastupa AIA



Mid1 siRNA (intra-artikularna primjena)



d22/23 siRNA uzorkovanje (qPCR -
ekstrakti zglobnog tkiva)



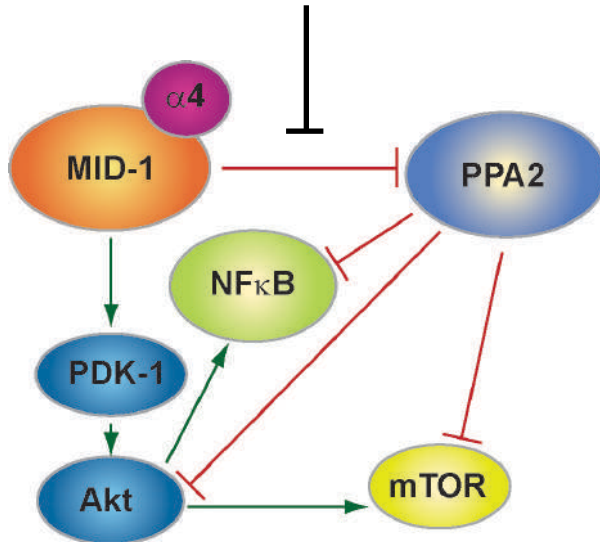
- Bez učinka na artritis

Biguanide metformin acts on tau phosphorylation via mTOR/protein phosphatase 2A (PP2A) signaling

Eva Kickstein^{a,b,1}, Sybille Krauss^{a,b,c,1}, Paul Thornhill^d, Désirée Rutschow^e, Raphael Zeller^e, John Sharkey^d, Ritchie Williamson^d, Melanie Fuchs^{a,b}, Andrea Köhler^{f,9}, Hartmut Glossmann^h, Rainer Schneider^f, Calum Sutherland^d, and Susann Schweiger^{a,b,e,2}

^aMax-Planck Institute for Molecular Genetics, 14195 Berlin, Germany; ^bDepartment for Dermatology, Charité, 10117 Berlin, Germany; ^cGerman Center for Neurodegenerative Diseases (DZNE), 53127 Bonn, Germany; ^dBiomedical Research Institute, Medical School, University of Dundee, DD1 9SY Dundee, United Kingdom; ^eInstitute for Biochemical Pharmacology, University of Innsbruck, 6020 Innsbruck, Austria; ^fInstitute for Biochemistry and Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, 6020 Innsbruck, Austria; ^gDivision of Medical Sciences, Medical School, University of Dundee, DD1 9SY Dundee, United Kingdom; and ⁹Department of Neurology, Medical University Innsbruck, 6020 Innsbruck, Austria

METFORMIN



Modificirano prema Koehler A, 2014, European Journal of Cancer

Matthes et al. *Cell Death Discovery* (2018)4:4
DOI 10.1038/s41420-017-0003-8

Cell Death Discovery

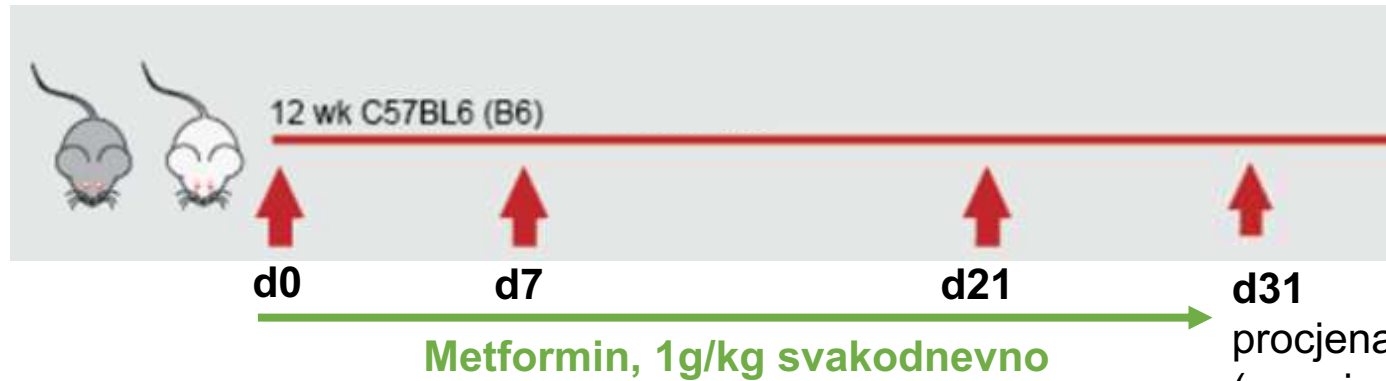
ARTICLE

Open Access

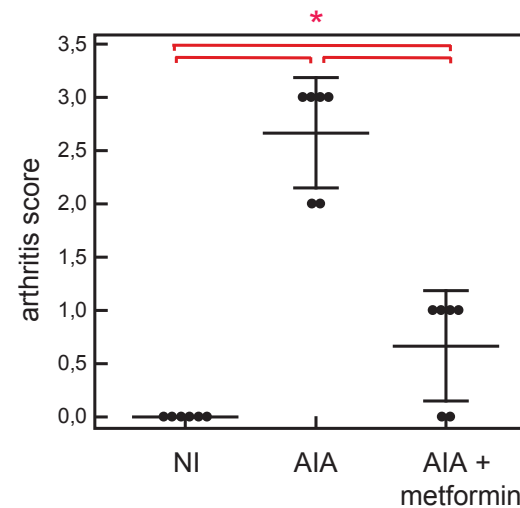
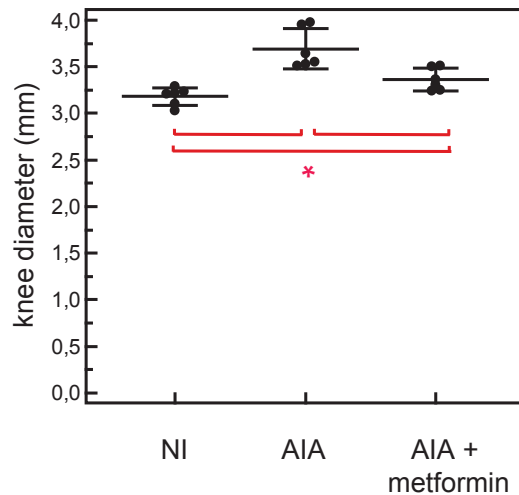
Inhibition of the MID1 protein complex: a novel approach targeting APP protein synthesis

Frank Matthes¹, Moritz M. Hettich¹, Judith Schilling¹, Diana Flores-Dominguez¹, Nelli Blank¹, Thomas Wiggenda², Alexander Buntru², Hanna Wolf¹, Stephanie Weber¹, Ina Vorberg¹, Alina Dagane², Gunnar Dittmar^{2,3}, Erich Wanker², Dan Ehninger¹ and Sybille Krauss¹

Metformin (inhibicija Mid1-PP2A interakcije)



procjena artritisa
(promjer koljena,
vizualna procjena)



- Ublažavanje artritisa
- Mehanizam?

Zaključci

- Neresorptivni AIA u Fas $-/-$ miševa karakterizira smanjeno nakupljanje sinovijalnih mijeloidnih stanica
- Sinovijalne Fas $-/-$ mijeloidne stanice slabije izražavaju gene vezane uz napredovanje staničnog ciklusa i mitozu, što upućuje na njihovu slabiju proliferacijsku sposobnost
- Izražaj *Mid1* je smanjen u Fas $-/-$ sinovijalnim mijeloidnim stanicama
- Mid1 je potencijalna ciljna molekula za terapijsko djelovanje na upalno zglobno oštećenje
- Metformin, koji blokira interakciju Mid1 s PP2A, primjenjen tijekom imunizacijskog protokola, ublažava simptome artritisa