

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

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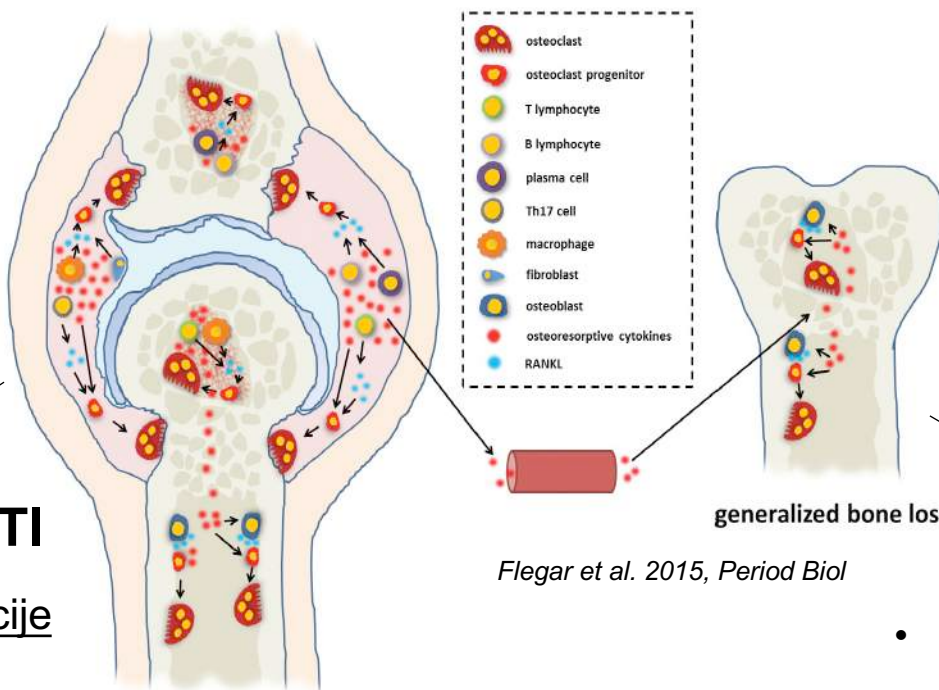
HRZZ
Hrvatska zaklada
za znanost



Reumatoidni artritis

LOKALNI GUBITAK KOSTI

- Ireverzibilne deformacije



Flegar et al. 2015, Period Biol

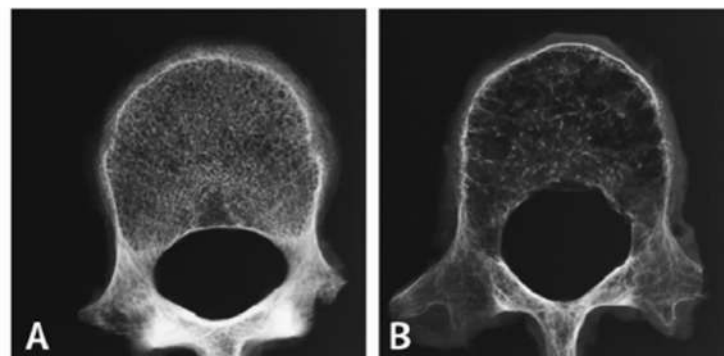
SUSTAVNI GUBITAK KOSTI

- Osteopenija/osteoporoza

local bone loss

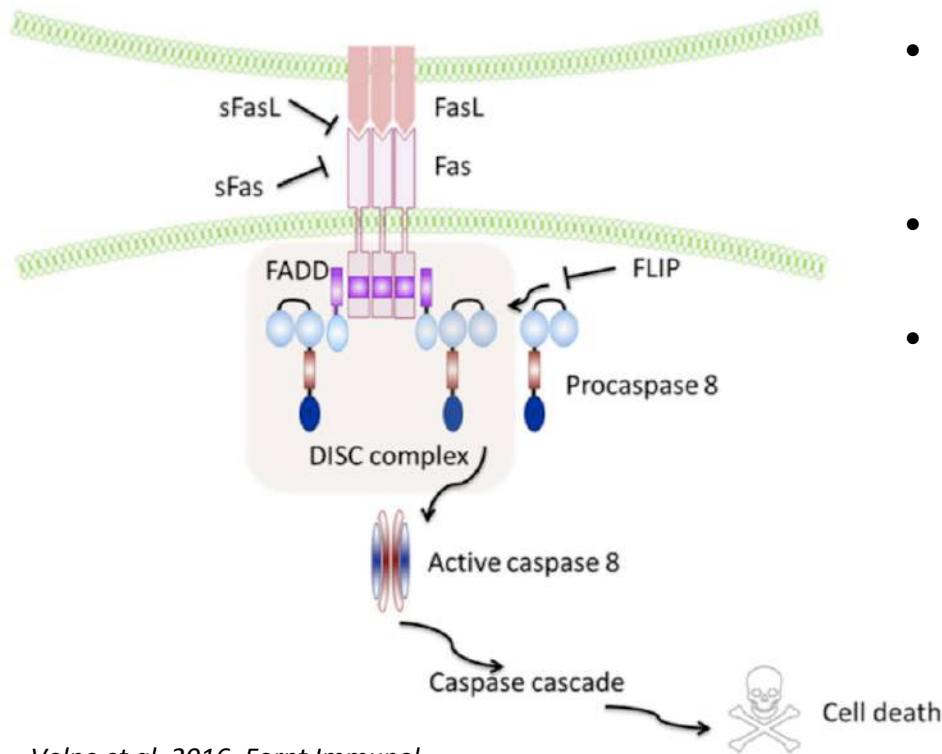


Schett et al. 2007, Arthritis Res Ther



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)

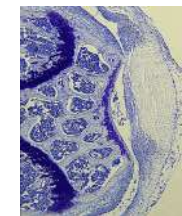
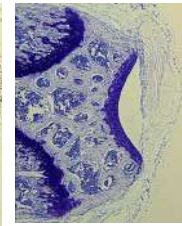
1. 50 µg mBSA
u 10 µL PBS,
intra-artikularno
(i.a., AIA)
2. 10 µL PBS i.a.
(ctrl, NI)



Dan 31
(dan 10 poslije indukcije)
UZIMANJE UZORAKA

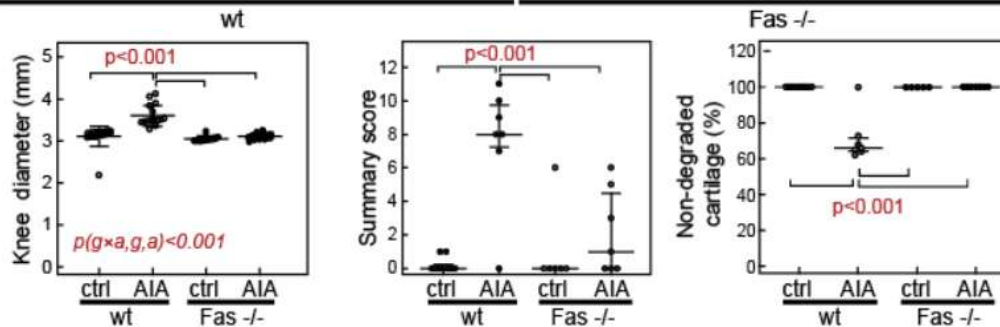
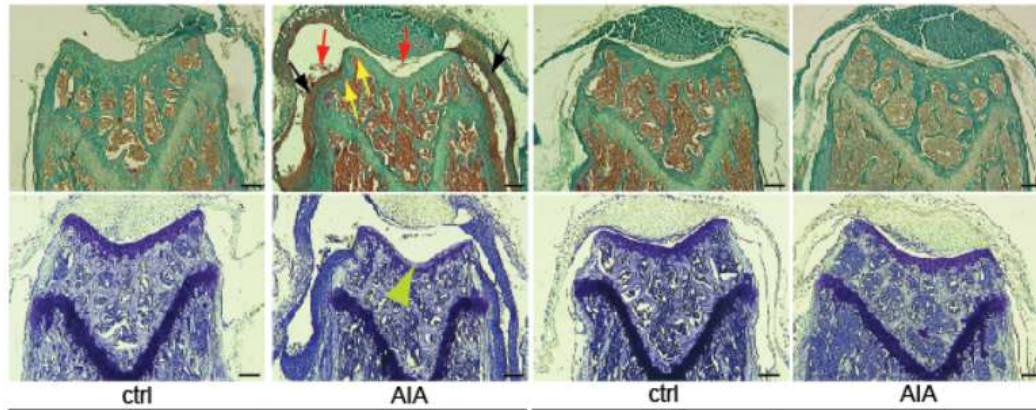
POKUSNE SKUPINE:

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)

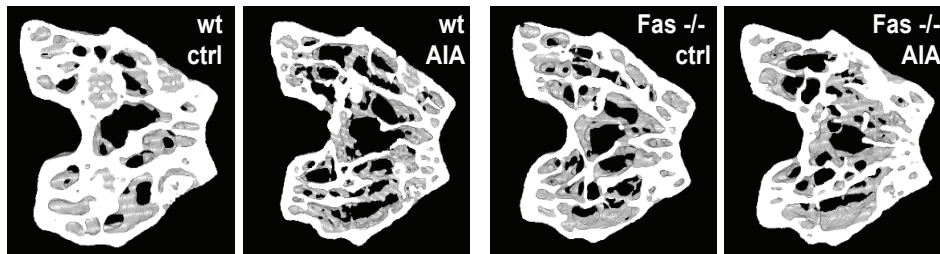


Artritis potaknut antigenom (AIA) u Fas-/- miševa

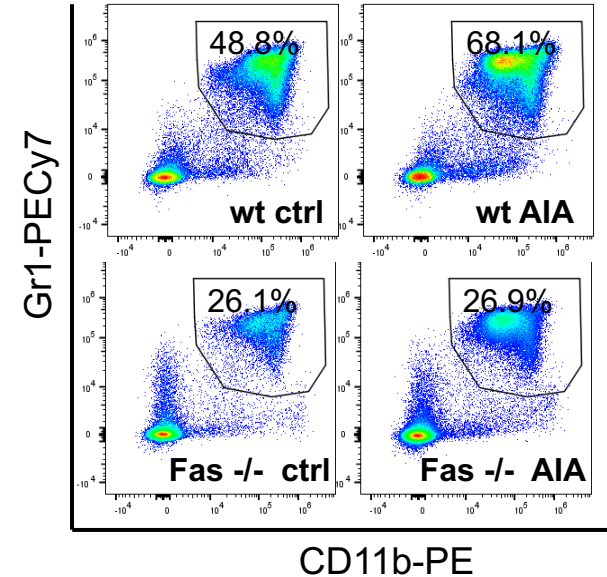
Smanjena sinovijalna upala i oštećenje hrskavice



Izostatak lokalnog koštanog razaranja



Smanjeno nakupljanje mijeloidnih stanica u sinoviji



Analiza transkriptoma sinovijalnih mijeloidnih stanica



B6 & Fas $-/-$
miševi,
AIA, d31

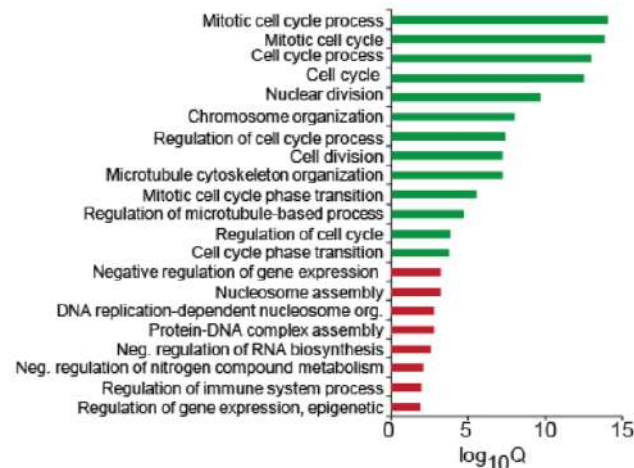
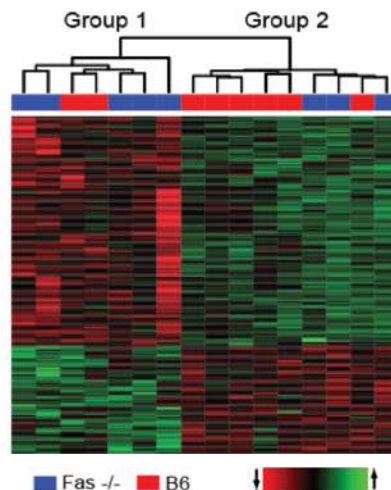
opuštanje
sinovijalnih stanica
pomoću
kolagenaze

FASC izdvajanje
CD11b+Gr-1+
stanica

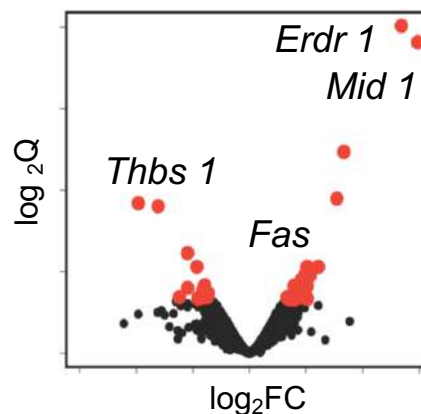


Affymetrix ST 2.0
genski čip

A. Hierarhijsko grupiraje



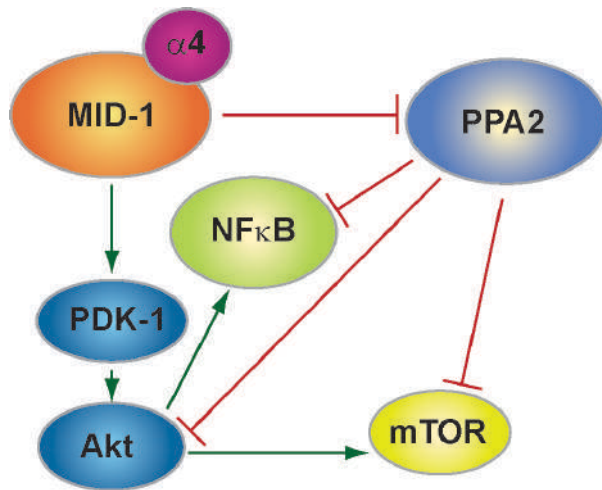
B. Izražaj gena - Fas $-/-$ vs. B6



Različito izraženi geni

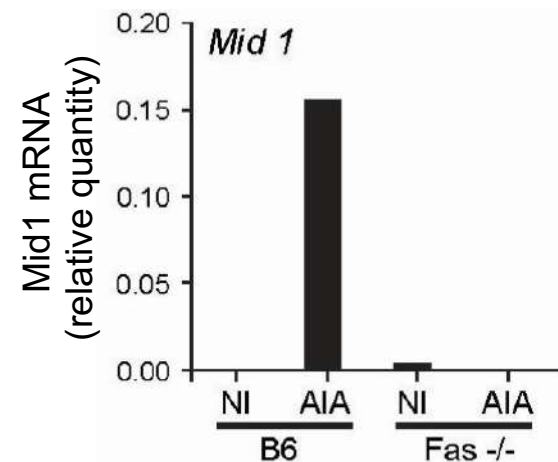
- *Midline 1* (TRIM18)

- 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*).

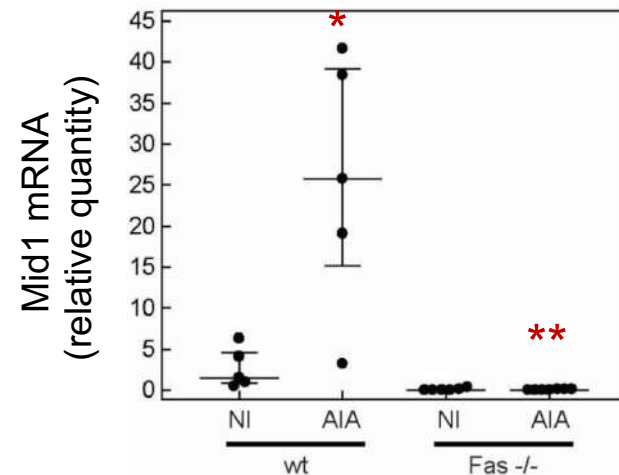


Modificirano prema Koehler A, 2014 ,
European Journal of Cancer

Sortirane CD11b⁺Gr1⁺ stanice (qPCR)



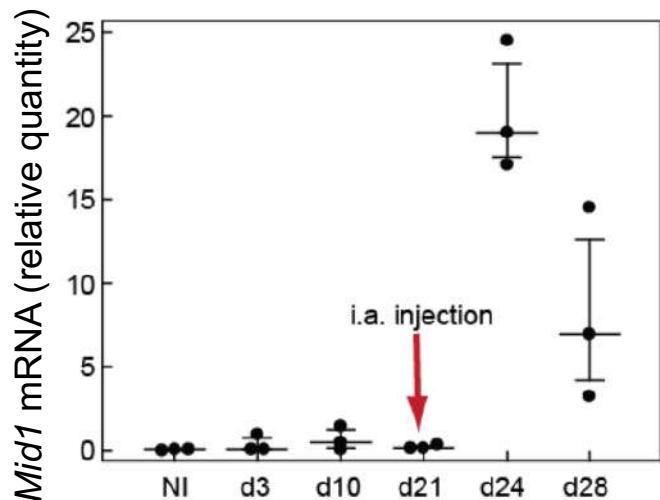
Tkivni ekstrakti koljena (qPCR)



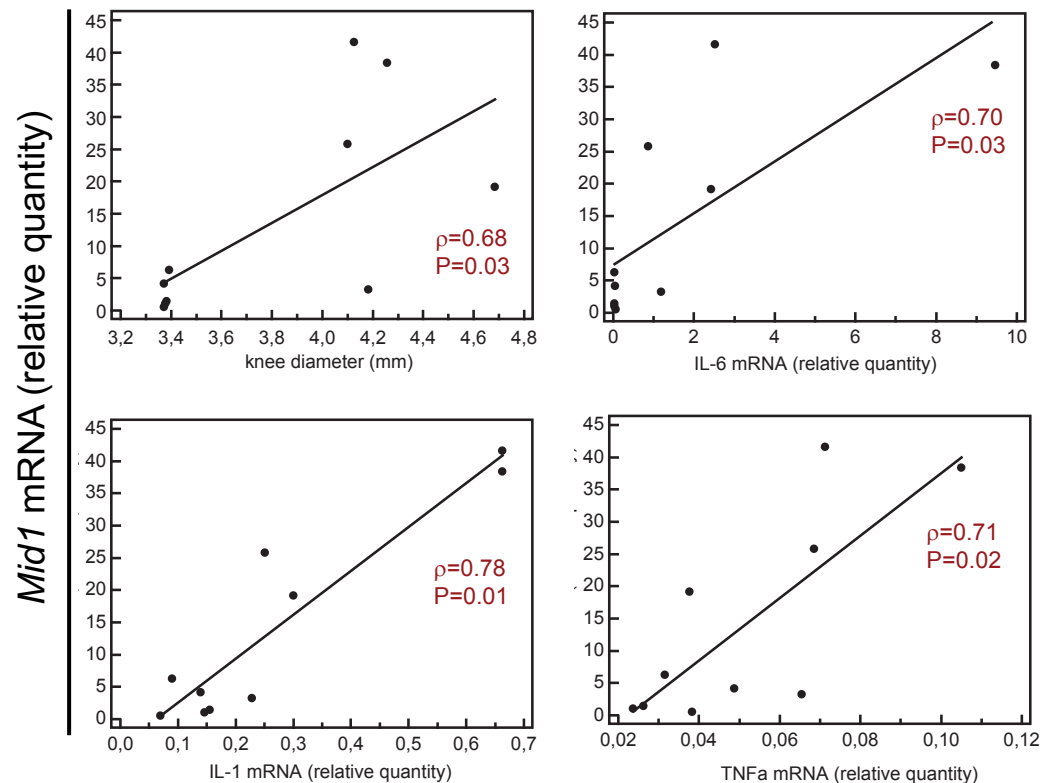
* p<0.05 vs. NI,

** p<0.005 vs. wt

Pojačan izražaj *Mid 1* nakon nastupa AIA



Povezanost s lokalnim izražajem pro-upalnih citokina i jakosti artritisa



Mid1 siRNA (intra-artikularna primjena)



d0

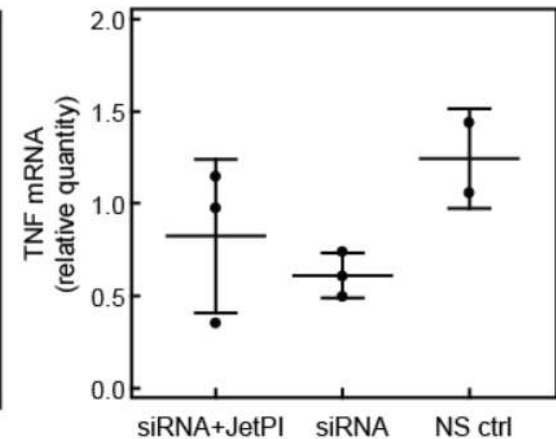
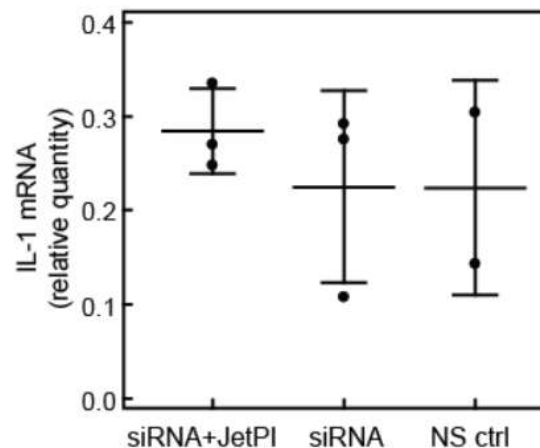
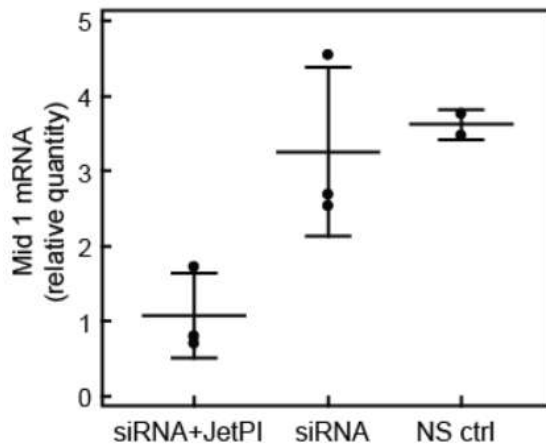
d7

d21

d22/23

d24

siRNA uzorkovanje (qPCR -
ekstrakti zglobnog
tkiva)



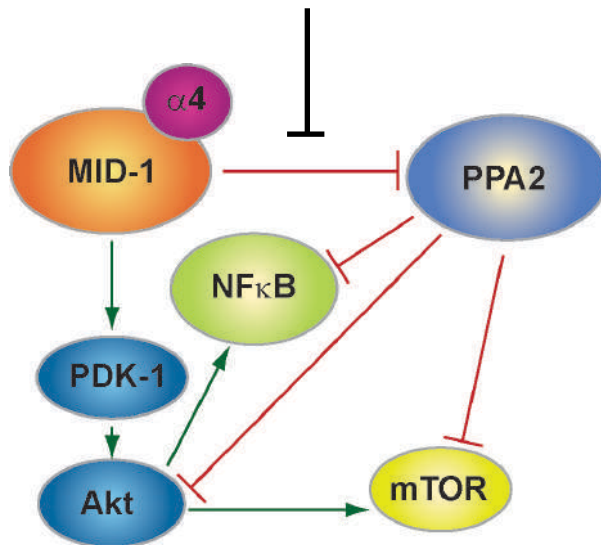
- Bez učinka na artritis

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}

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METFORMIN



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

Cell Death Discovery

ARTICLE

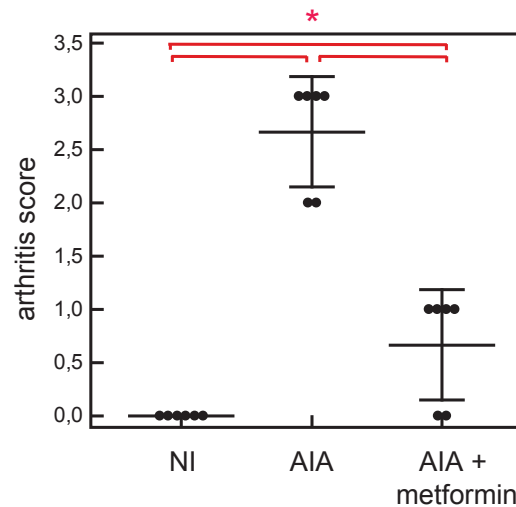
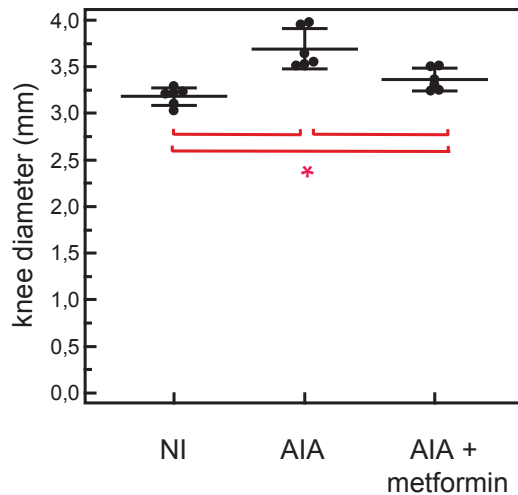
Open Access

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

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

Metformin (inhibicija Mid1-PP2A interakcije)



- 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