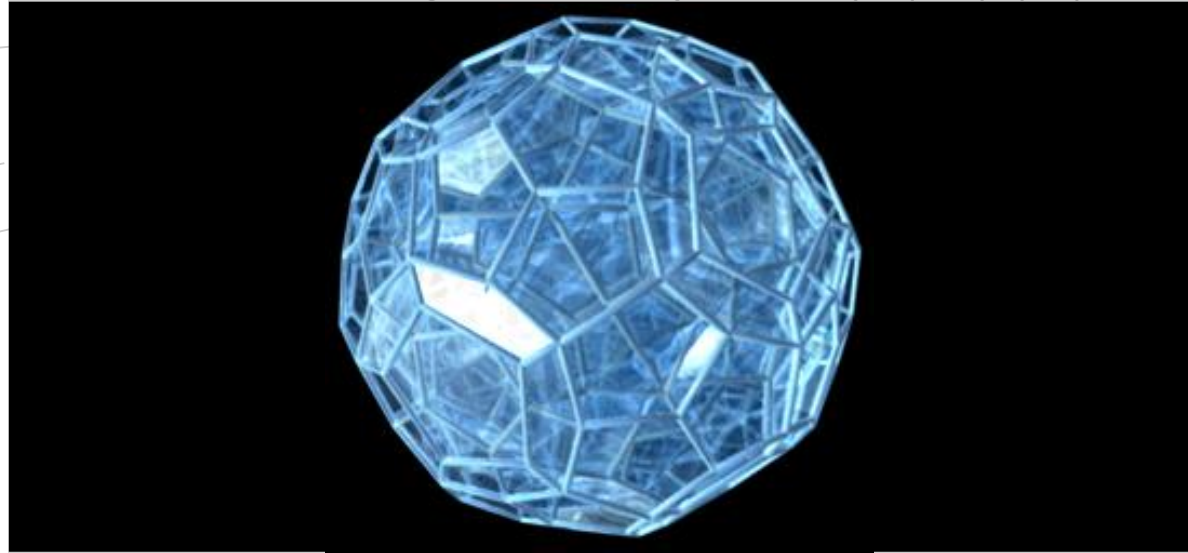




Department of Genetics, Cell- and Immunobiology  
Semmelweis-University, Faculty of Medicine



# BESZÁMOLÓ



2017. 11. 16



- I. KERINGŐ EXTRACELLULÁRIS VEZIKULÁK JELLEMZÉSE
- II. REDOX HOMEOSZTÁZIS JELLEMZÉSE EGÉSZSÉGES ÉS PREECLAMPSIÁS VÁRANDÓSSÁGBAN
- III. EV HATÁSA A BeWo SEJTVONALRA, ILLETVE A BeWo VISSZAHATÁSÁNAK VIZSGÁLATA

# I. KERINGŐ EV JELLEMZÉS (1)



MAGAS FELBONTÁSÚ ÁRAMLÁSI CITOMETRIA  
LÉZER PÁSZTÁZÓ KONFOKÁLIS MIKROSKÓPIA

FUNKCIONÁLIS HATÁSOK



EXOFACIÁLIS MINTÁZAT

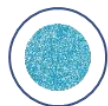
TÖMEGSPEKTROMETRIA

PCR ARRAY – miRNS

DINAMIKUS FÉNYSZÓRÁS

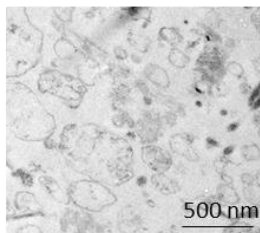
manuscripttrackingsystem		SCIENTIFIC REPORTS
<a href="#">tracking system home</a>	<a href="#">author instructions</a>	<a href="#">reviewer instructions</a>
<a href="#">help</a>	<a href="#">tips</a>	<a href="#">logout</a>
<a href="#">journal home</a>		
<b>Manuscript #</b>	SREP-17-47402	
<b>Current Revision #</b>	0	
<b>Submission Date</b>	30th October 17	
<b>Current Stage</b>	Manuscript Assigned to Editor	
<b>Title</b>	The impact of circulating preeclampsia-associated extracellular vesicles on the migratory activity and phenotype of THP-1 monocytic cells	
<b>Manuscript Type</b>	Original Research	
<b>Manuscript Comment</b>	Figure S7 was drawn by Árpád Ferenc Kovács using Adobe Photoshop and Microsoft Powerpoint.	
<b>Corresponding Author</b>	Dr. Árpád Ferenc Kovács (dr.arpad@yahoo.com) (Semmelweis University)	
<b>Contributing Authors</b>	Dr. Orsolya Láng, Dr. Lilla Tunik, Mr. András Ács, László Kóhidai, Ms. Nóra Fekete, Dr. Alasztes Bálint, Dr. Tamás Mészáros, Prof. Edit Buzás, Prof. János Rigo Jr., Dr. Eva Pallinger	
<b>Authorship</b>	Yes	
<b>Abstract</b>	Intercellular communication via extracellular vesicles (EVs) is a highly dynamic and specific process. The interaction between EVs and their target cells, especially immune cells, results in functional and phenotype changes that consequently may play a significant role in various physiological states and the pathogenesis of immune-mediated disorders. Monocytes are the most prominent environment-sensing immune cells in circulation, skilled to shape their microenvironments via cytokine secretion and further differentiation. Both the circulating monocyte subset distribution and the blood plasma EV pattern are characteristic for preeclampsia, a pregnancy induced immune-mediated hypertensive disorder. We hypothesized that preeclampsia-associated EVs (PE-EVs) induced functional and phenotypic alterations of monocytes. First we proved EV binding and uptake by THP-1 cells. Cellular origin and protein cargo of circulating PE-EVs were characterized by flow cytometry and mass spectrometry. An altered phagocytosis-associated molecular pattern was found on PE-EVs: an elevated CD47 "don't eat me" signal and decreased exofacial phosphatidylerine "eat-me" signal were found along with decreased uptake of PE-EVs. PE-EVs induced significantly lower chemotaxis and cell motility, but accelerated cell adhesion of THP-1 cells. PE-EVs caused sustained TNF production of THP-1 cells. PE-EVs induced altered monocyte functions suggest that circulating PE-EVs may have a role in the pathogenesis of preeclampsia.	
<b>Techniques</b>	Life sciences techniques, Cell/tissue technologies [Flow cytometry]; Life sciences techniques, Structural biology [Mass spectrometry]; Life sciences techniques, Protein techniques [Protein expression];	
<b>Subject Terms</b>	Biological sciences/Cell biology/Cell migration Health sciences/Molecular medicine	
<b>Competing Financial Interest</b>	There is <b>NO</b> Competing Interest.	
<b>Applicable Funding Source</b>	Országos Tudományos Kutatási Alprogramok (Hungarian Scientific Research Fund) - PD121187 [Tunik] Országos Tudományos Kutatási Alprogramok (Hungarian Scientific Research Fund) - 11958 [Buzás] Országos Tudományos Kutatási Alprogramok (Hungarian Scientific Research Fund) - 120237 [Buzás] Országos Tudományos Kutatási Alprogramok (Hungarian Scientific Research Fund) - K113023 [Rigo Jr]	

# I. KERINGŐ EV JELLEMZÉS (2)

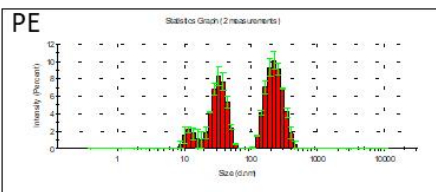
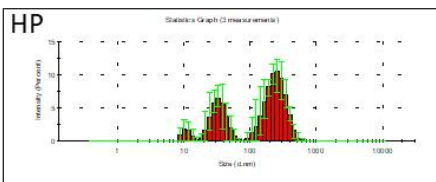


## MIKROVEZIKULA 12.5K pellet

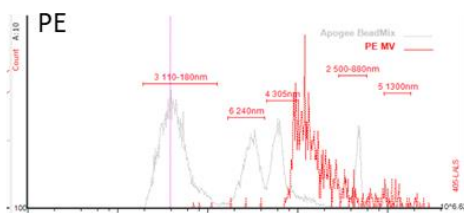
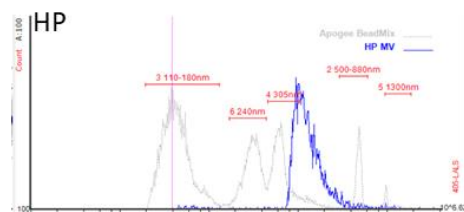
TEM



DLS

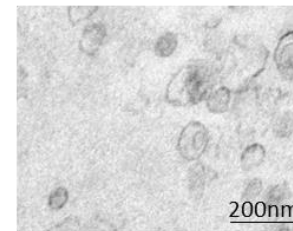


APOGEE

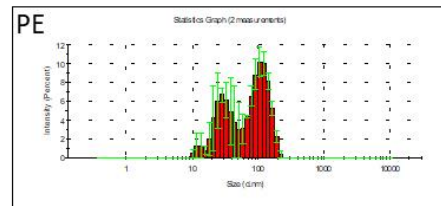
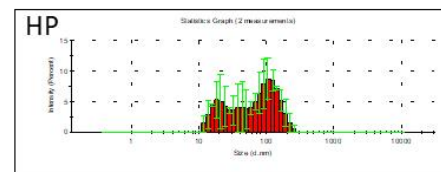


## EXOSZÓMA 100K pellet

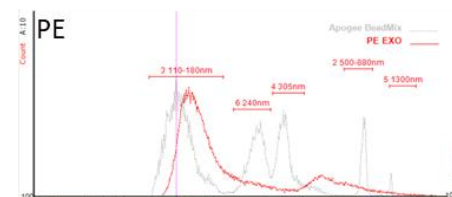
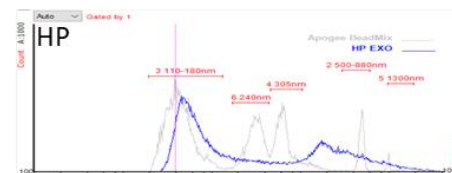
TEM



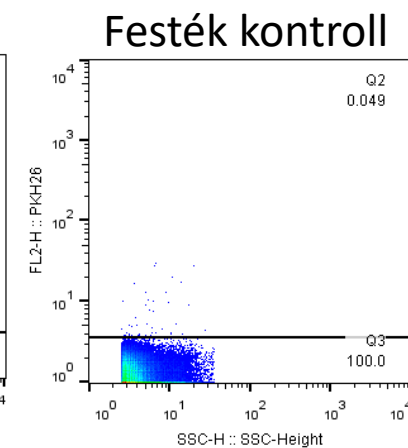
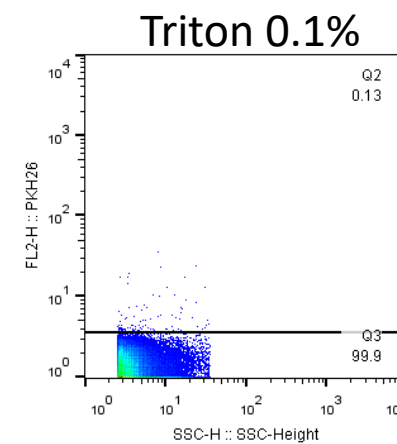
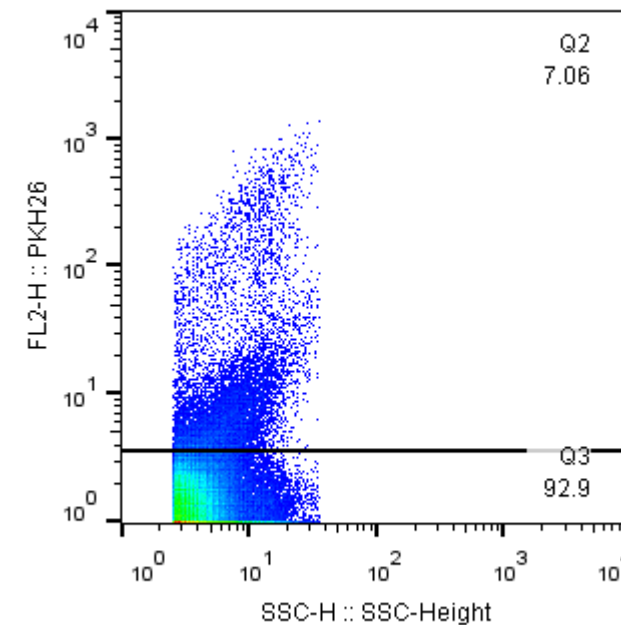
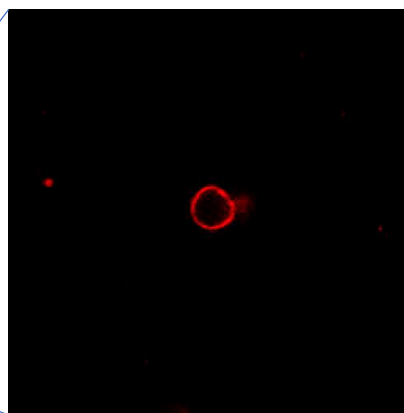
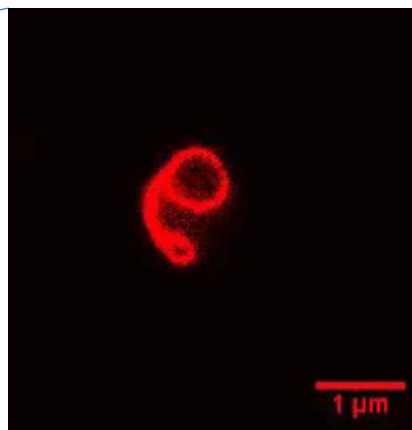
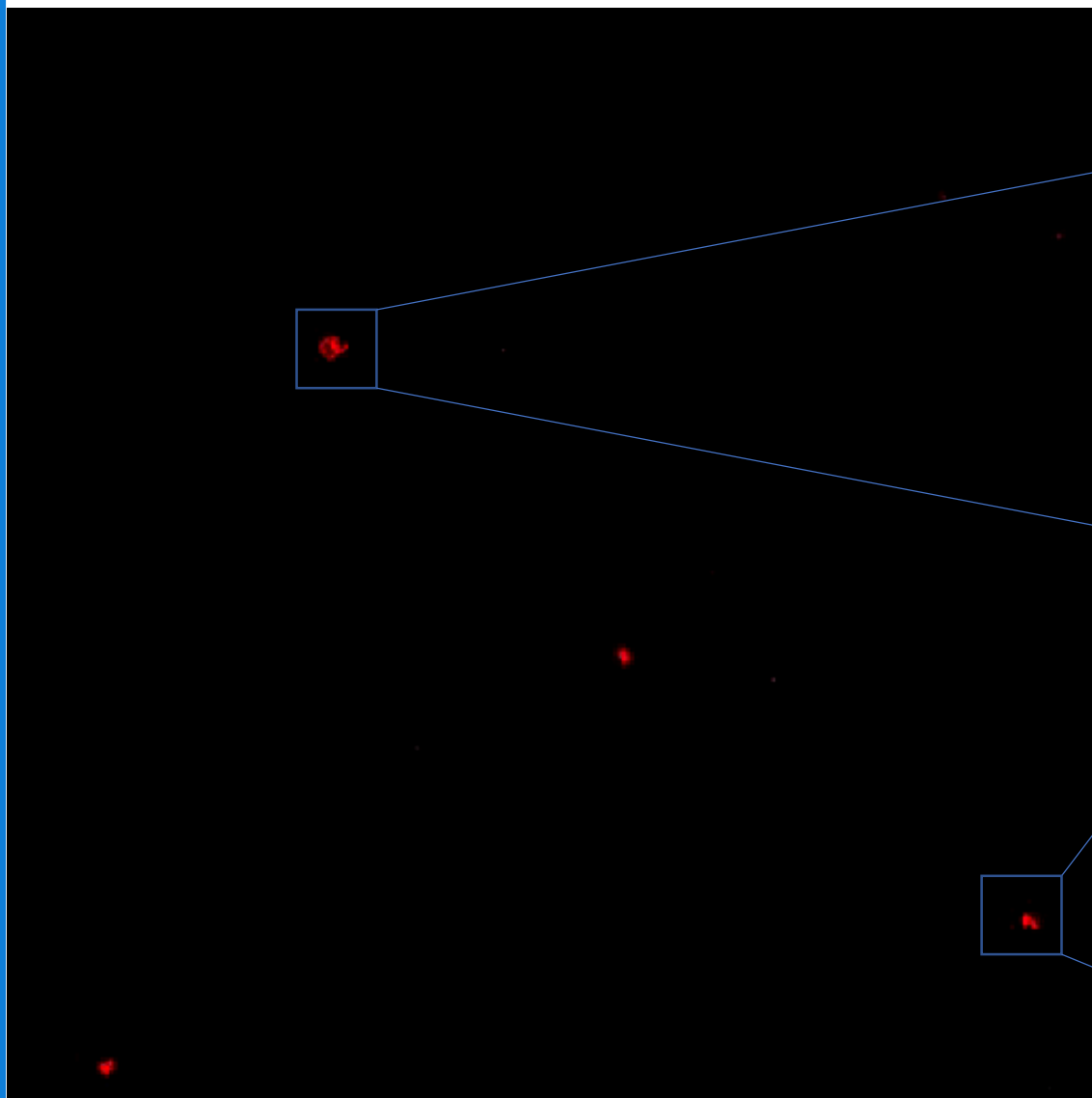
DLS



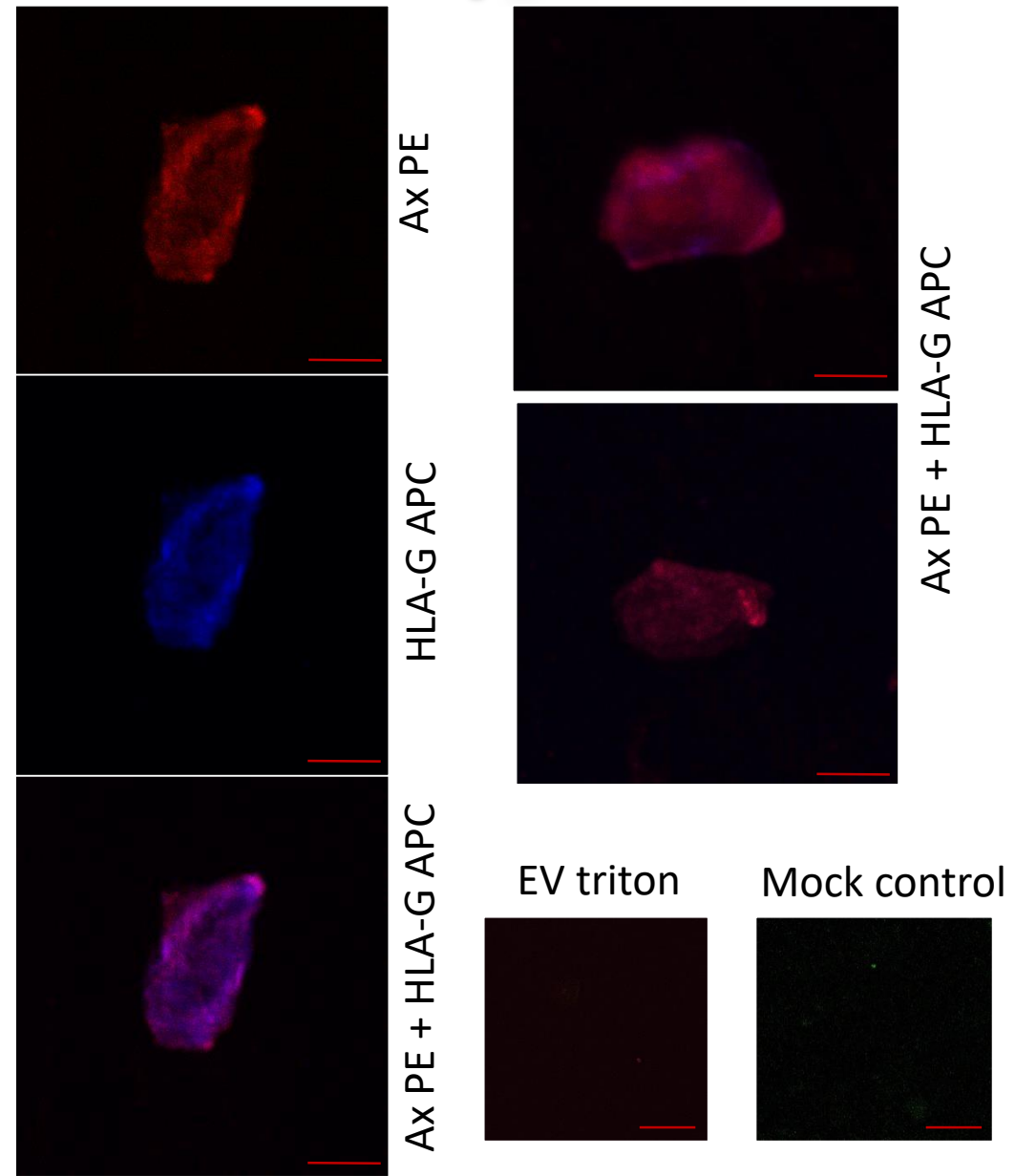
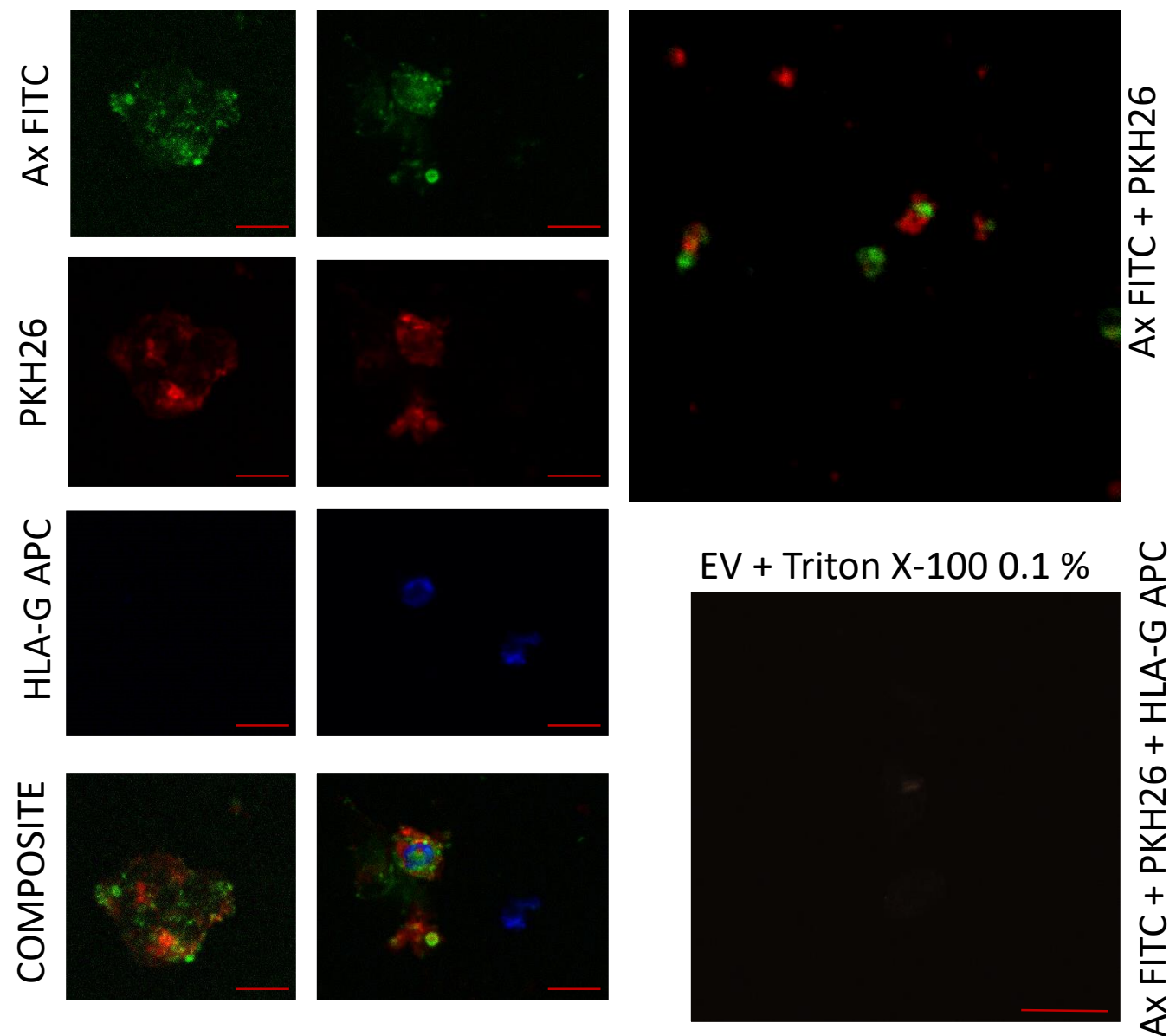
APOGEE



# I. KERINGŐ ÉV JELLEMZÉS (3)



# I. KERINGŐ EV JELLEMZÉS (4)



# I. KERINGŐ EV JELLEMZÉS (5)



Folyamatban

Endotél és monocita eredetű MV kvantitatív felmérése

LDL asszociált vezikulák felmérése

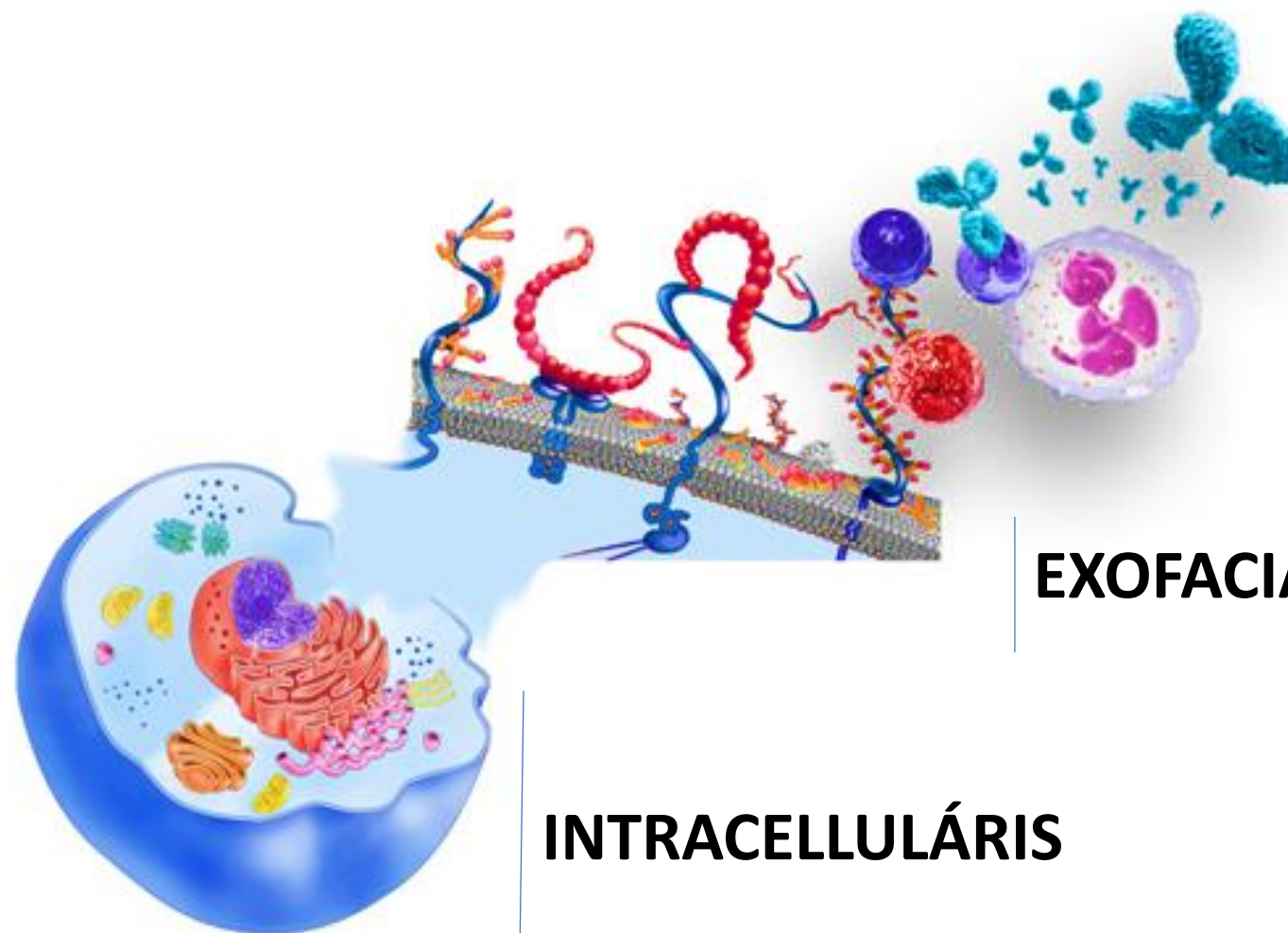
miRNS cargo azonosítása



HP-EV

PE-EV

# II. REDOX HOMEOSZTÁZIS (1)



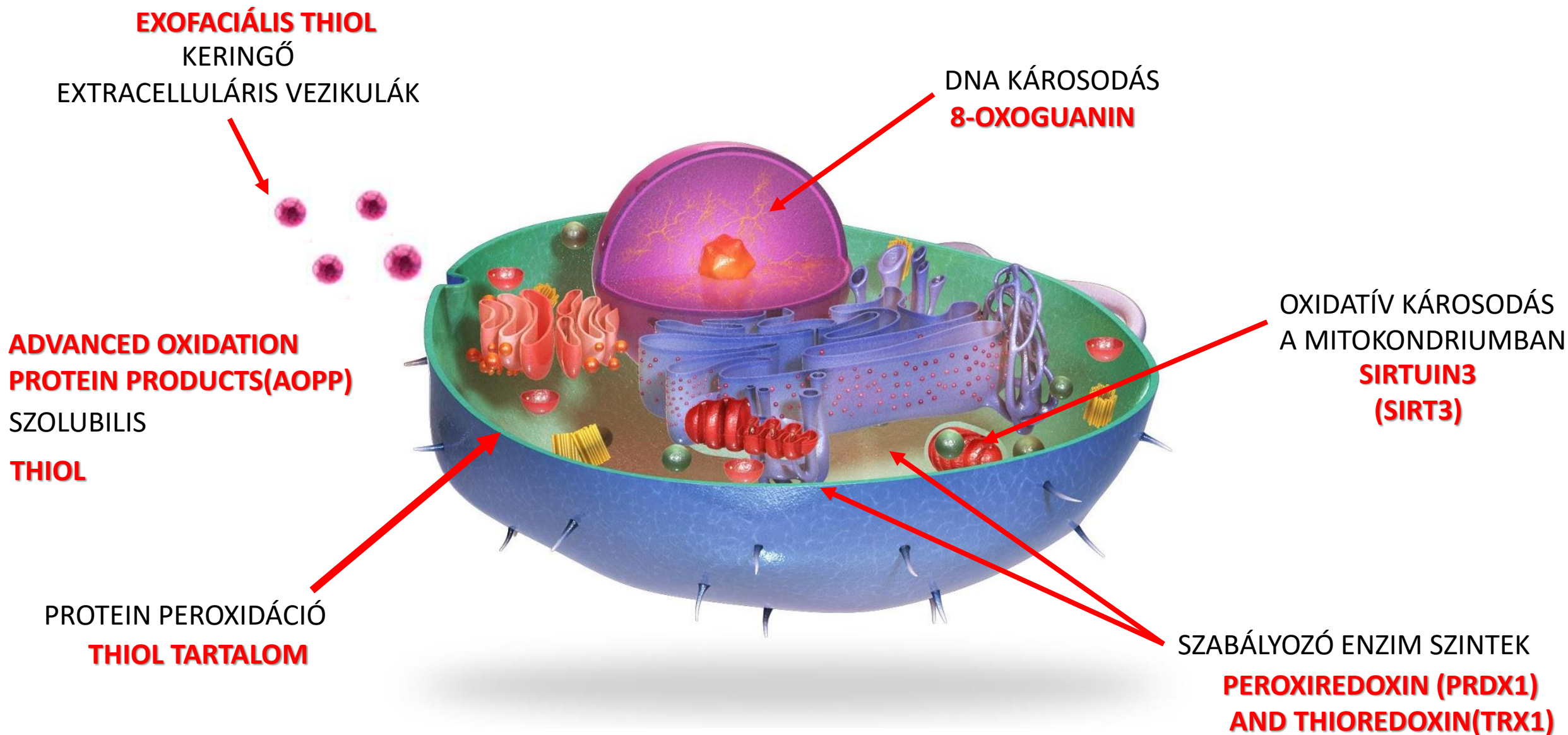
**INTRACELLULÁRIS**

**EXOFACIÁLIS**

**EXTRACELLULÁRIS**



# II. REDOX HOMEOSZTÁZIS (2)

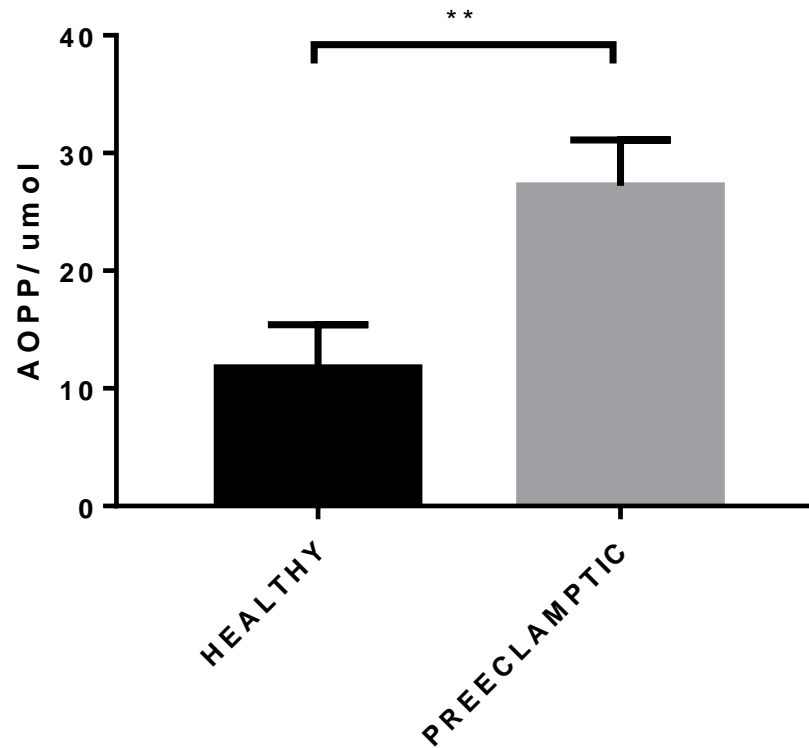
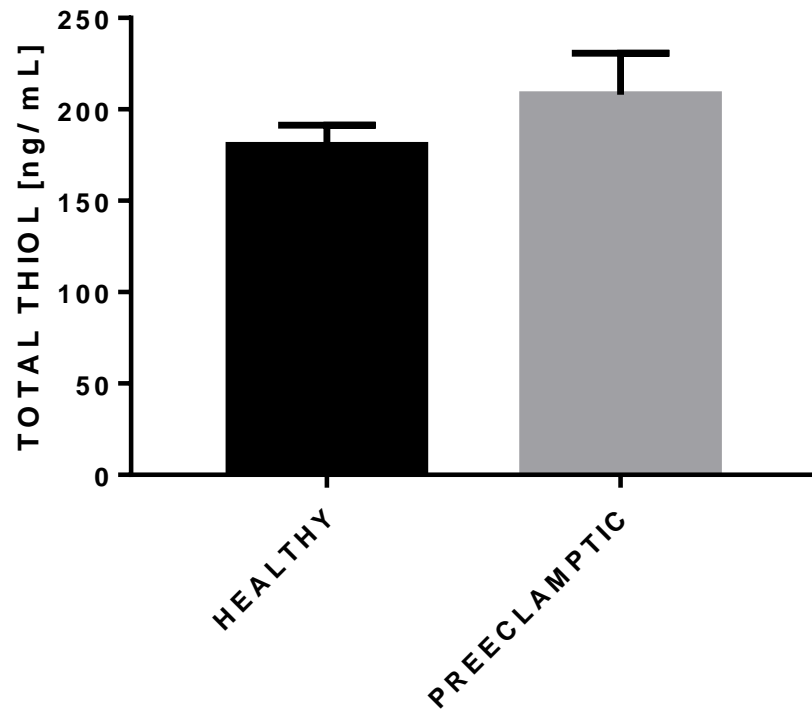


# II. REDOX HOMEOSZTÁZIS (3)

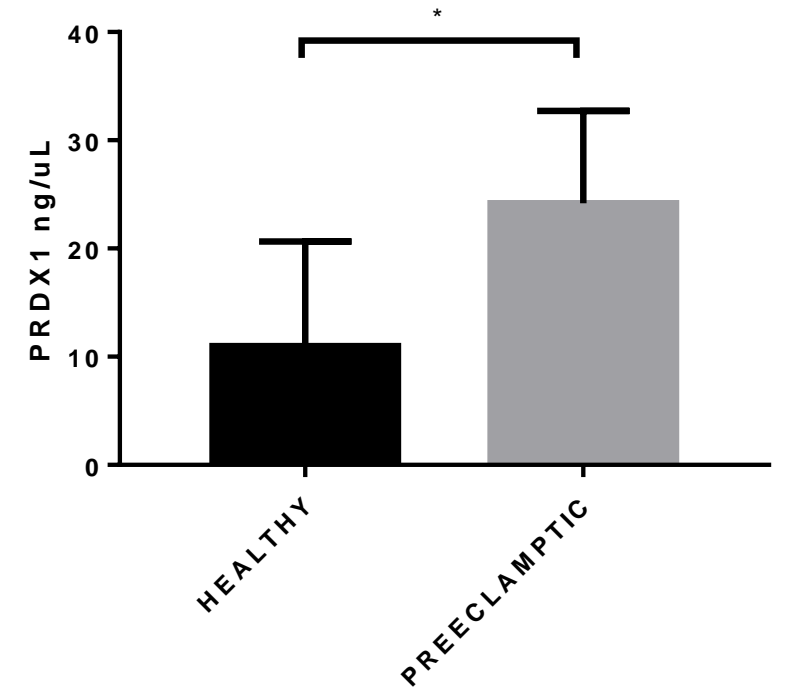
## I. PLAZMA TOTÁL THIOL, AOPP AND PRDX1

DTNB assay,

ELISA



p<0.005, Mann-Whitney test

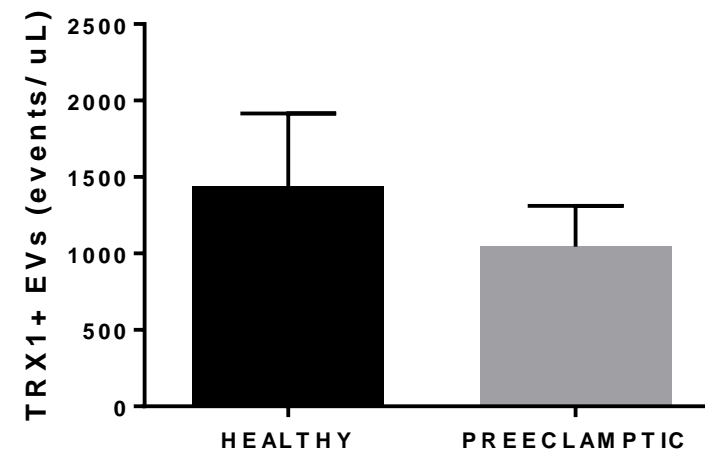
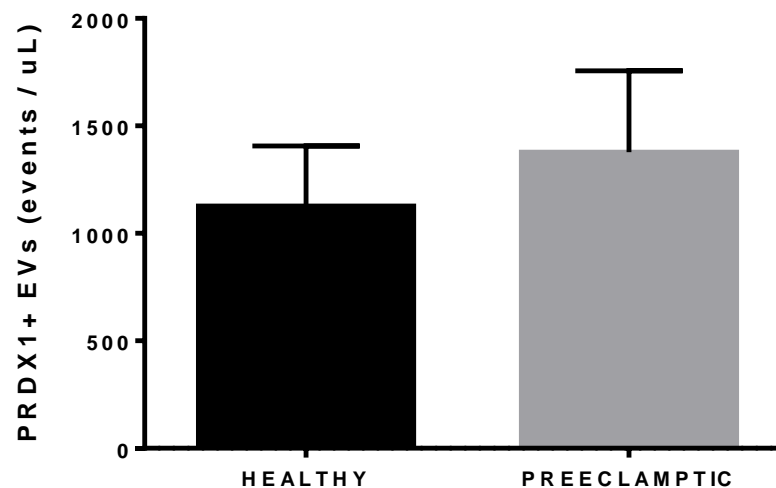
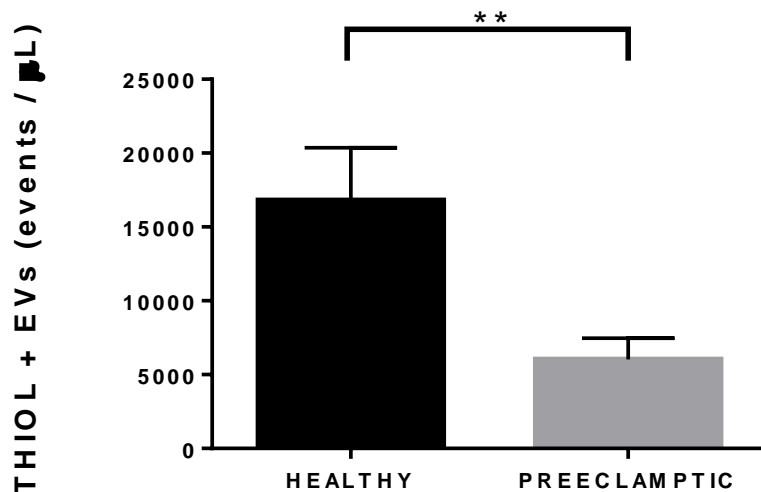


p<0.05, Mann-Whitney test

# II. REDOX HOMEOSZTÁZIS (4)

## II. EXOFACIÁLIS THIOL, PRDX1 ÉS TRX1 A KERINGŐ EXTRACELLULÁRIS VEZIKULÁKON

### FACS

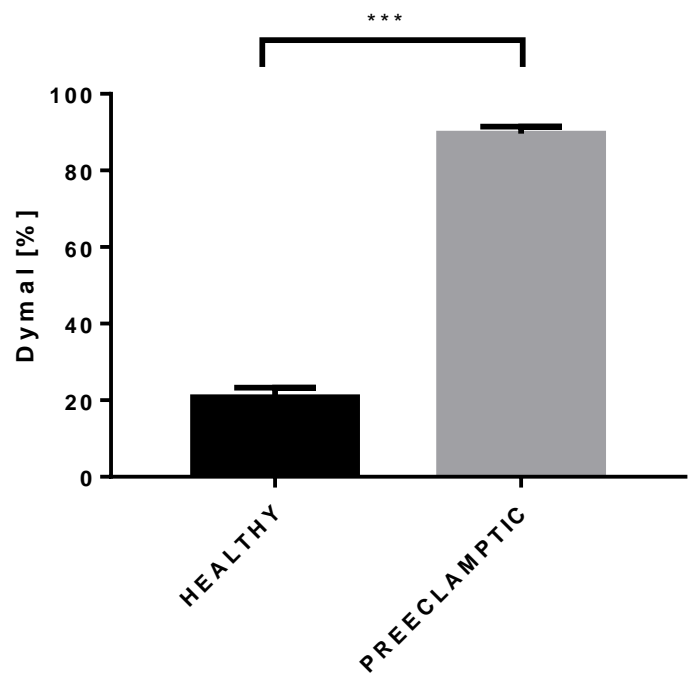


Unpaired t-test;  $p < 0.05$

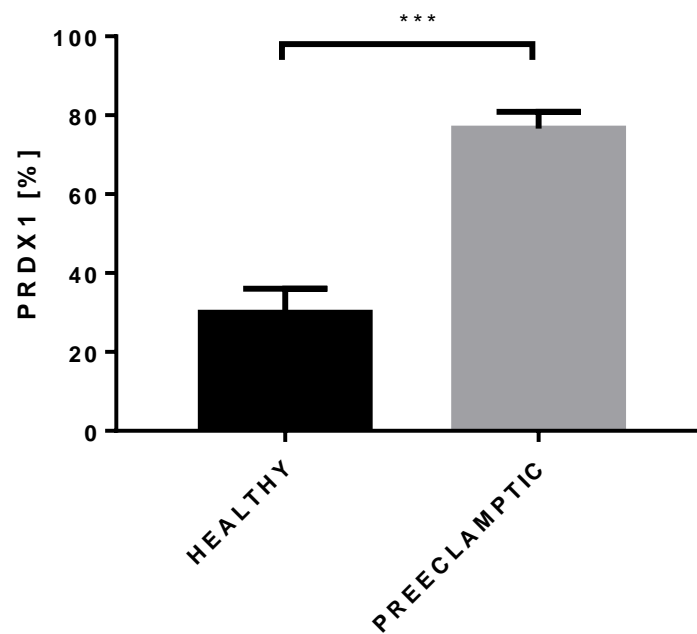
# II. REDOX HOMEOSZTÁZIS (5)

## III. EXOFACIÁLIS THIOL , PRDX1 ÉS TRX1 LYMPHYCYTÁN

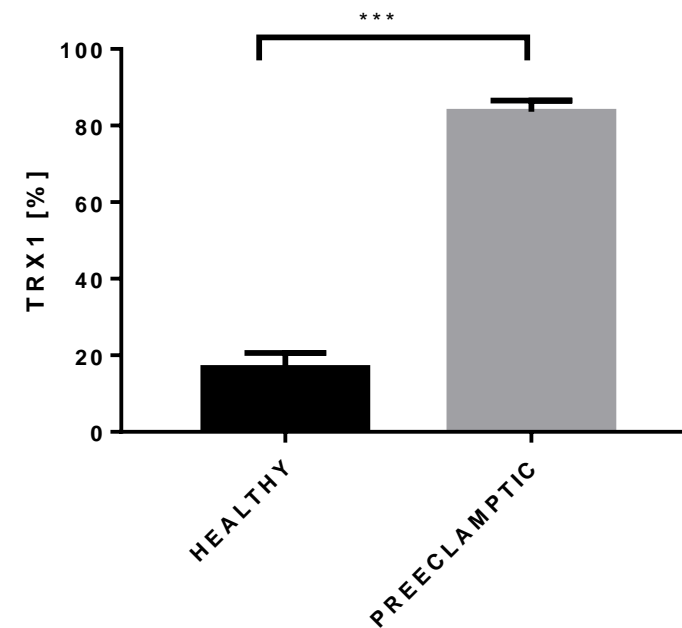
### FACS



p<0.0001, Mann-Whitney test



p<0.0005, Mann-Whitney test

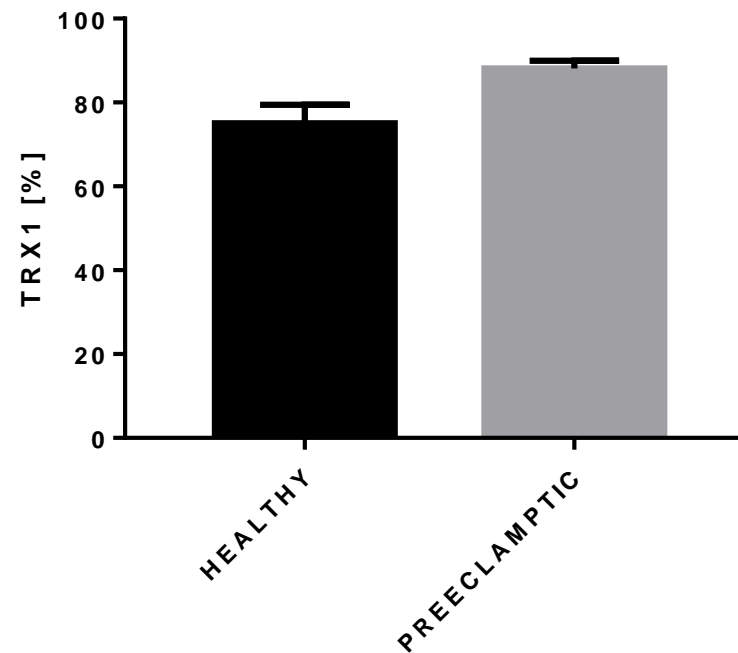
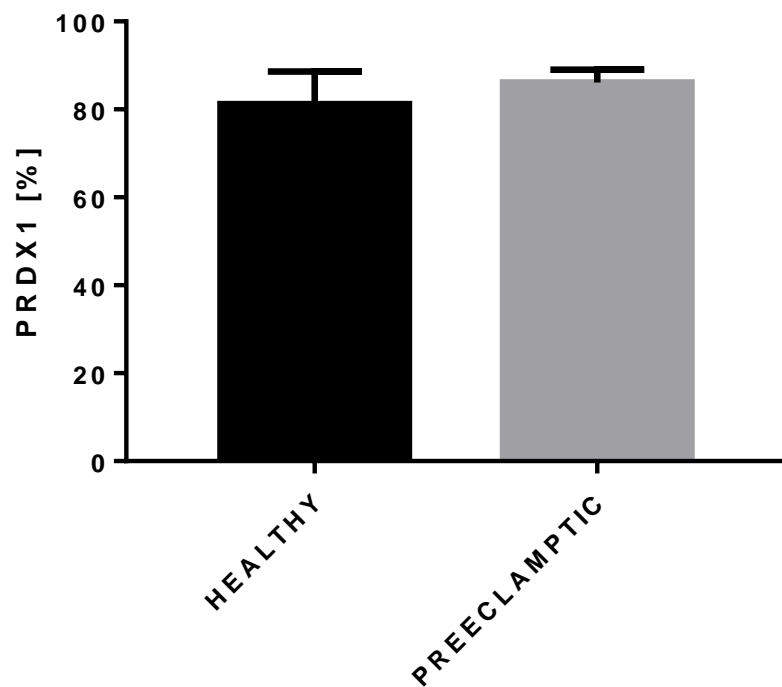


p<0.0001, Mann-Whitney test

# II. REDOX HOMEOSZTÁZIS (6)

## IV. INTRACELLULÁRIS PRDX1 ÉS TRX1 LYMPHOCYTA SEJTEKBEN

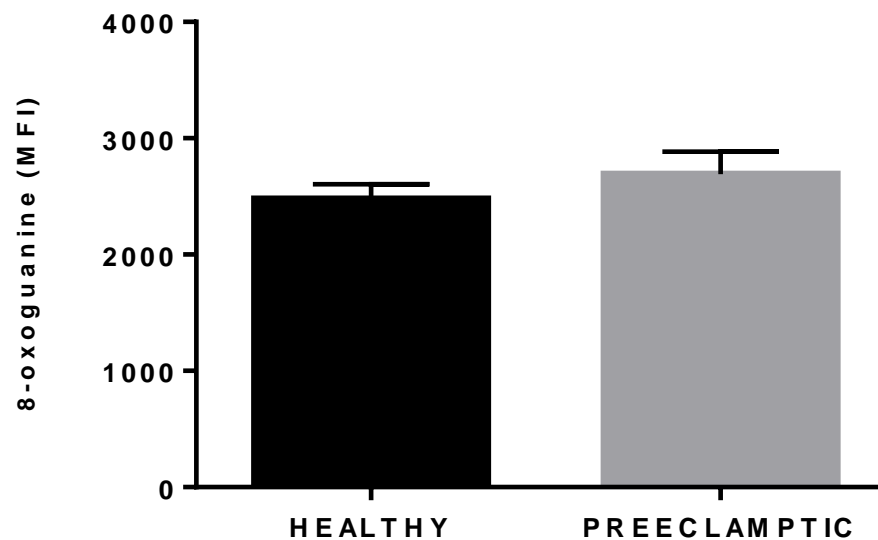
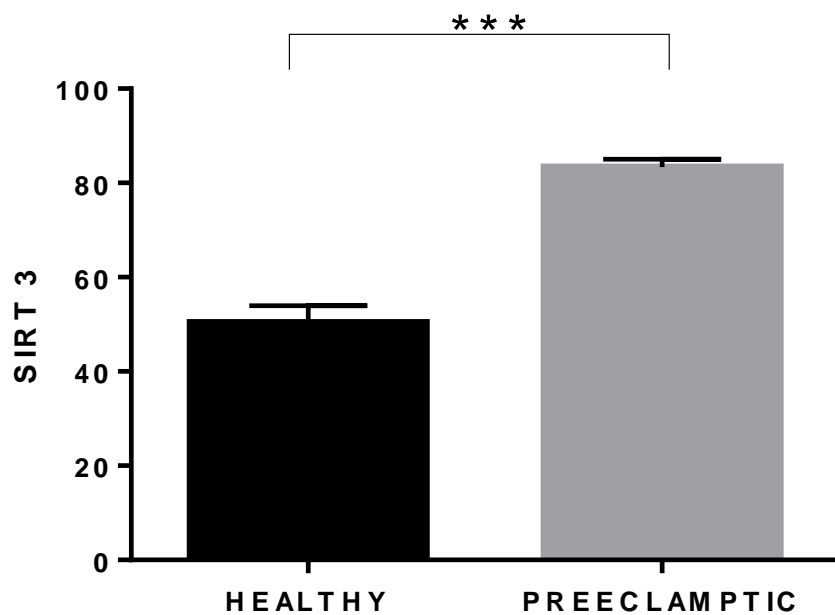
### FACS



# II. REDOX HOMEOSZTÁZIS (7)

## V. INTRACELLULÁRIS SIRT3 ÉS OXOGUANIN-8 LYMPHOCYTA SEJTEKBEN

### FACS



$p < 0.0001$ , Mann-Whitney test

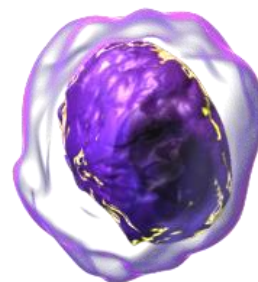
# II. REDOX HOMEOSZTÁZIS (8)

## OXIDATÍV STRESSZ MARKEREINEK SZINTJE PREECLAMPSIÁBAN



EXOFACIÁLIS THIOL ↓

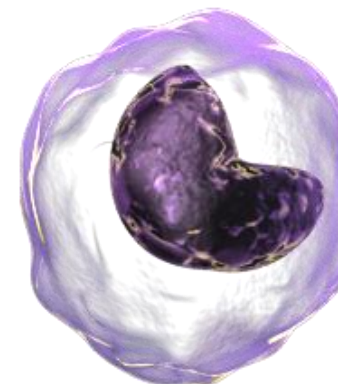
EXOFACIÁLIS THIOL ↑↑



ic SIRT3 ↑

EXOFACIÁLIS SZABÁLYOZÓ ENZIMEK

↑↑ PRDX1 ÉS ↑↑ TRX1

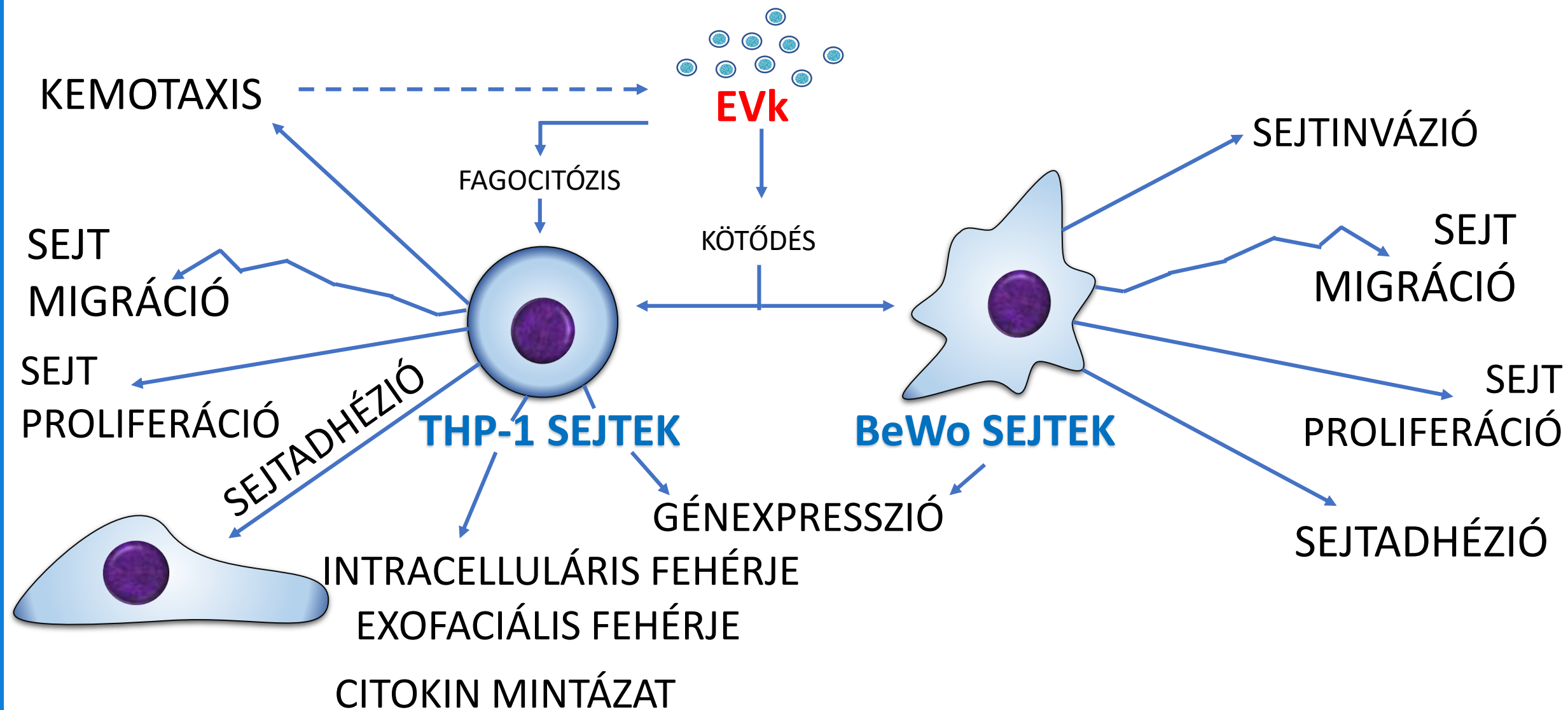


EXOFACIÁLIS SZABÁLYOZÓ ENZIMEK

↑↑ PRDX1 ÉS ↑↑ TRX1

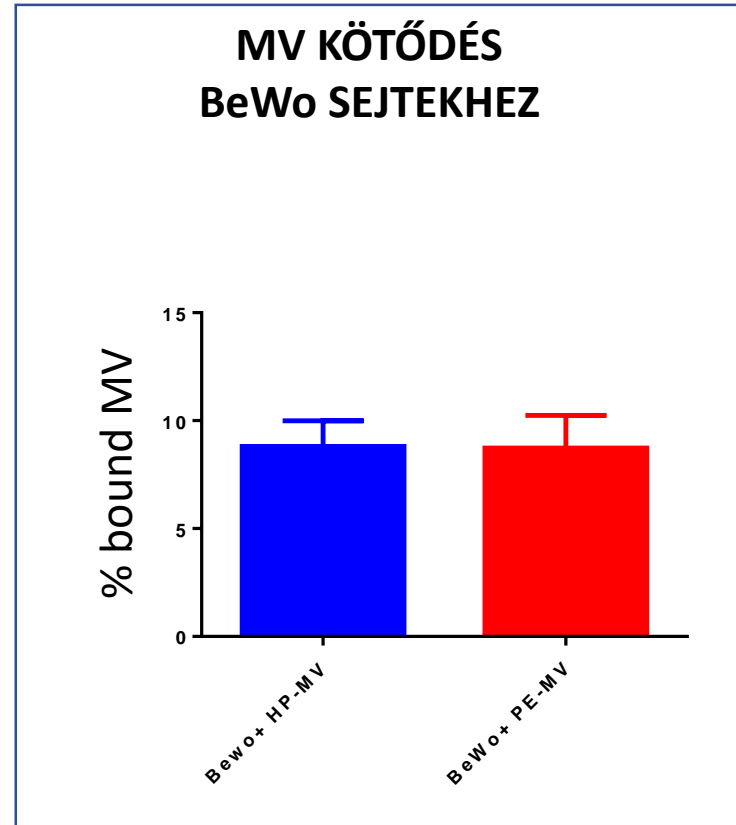
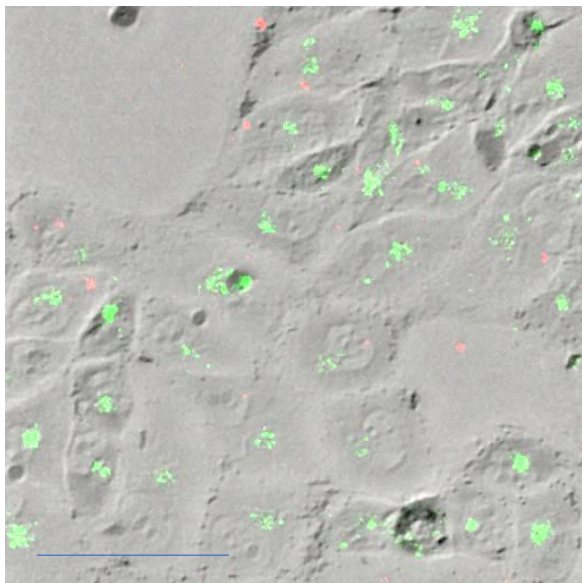
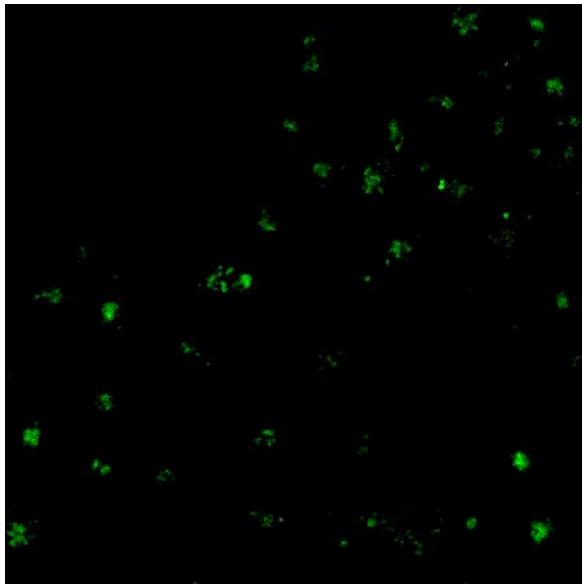
**PLAZMA:** ↑ PRDX1; ↑↑ AOPP; THIOL ↔

# III. EV - BeWo (1)

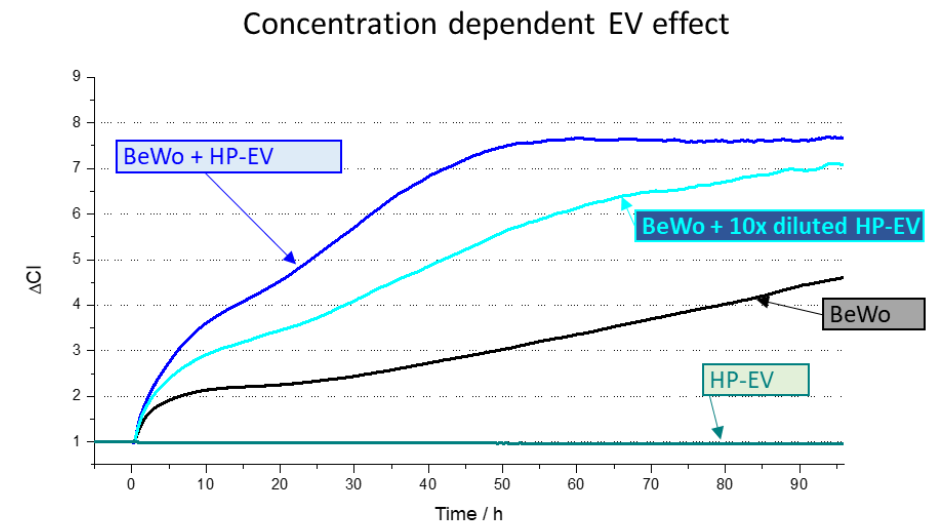
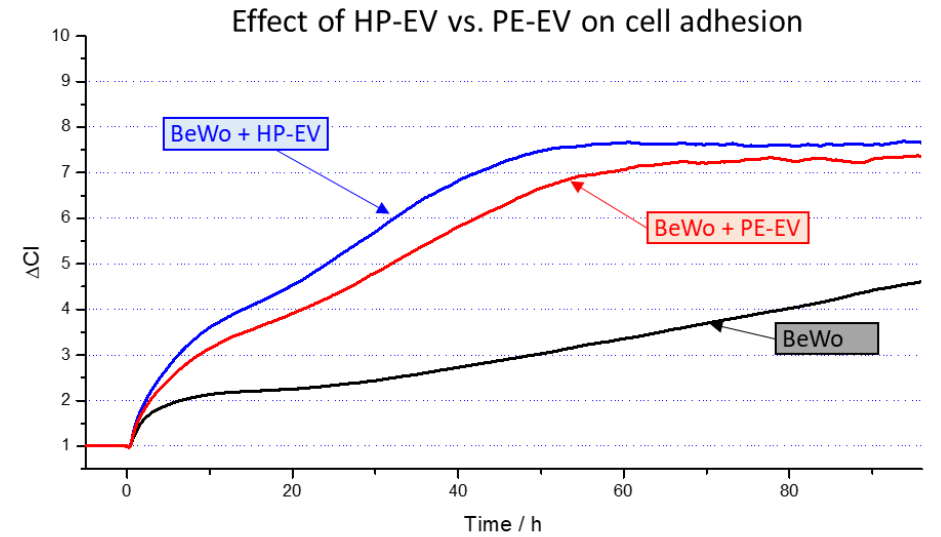




# III. EV - BeWo (2)

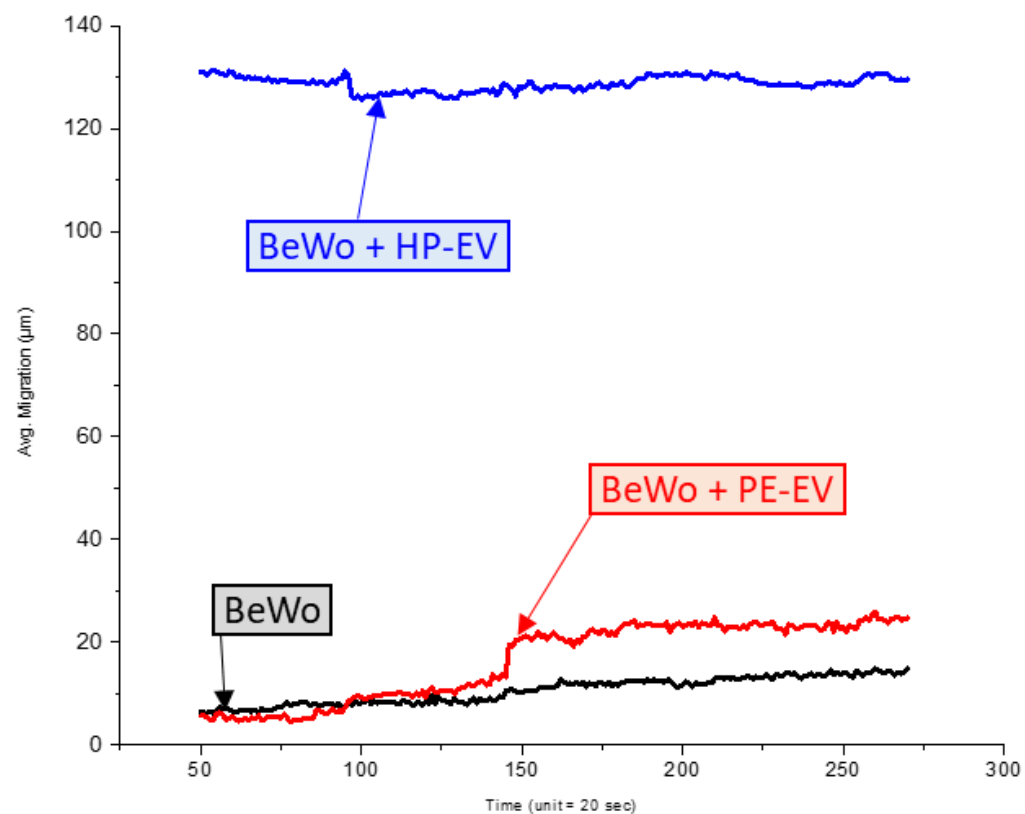


EV-PKH26 BeWo – Hoechst 33342

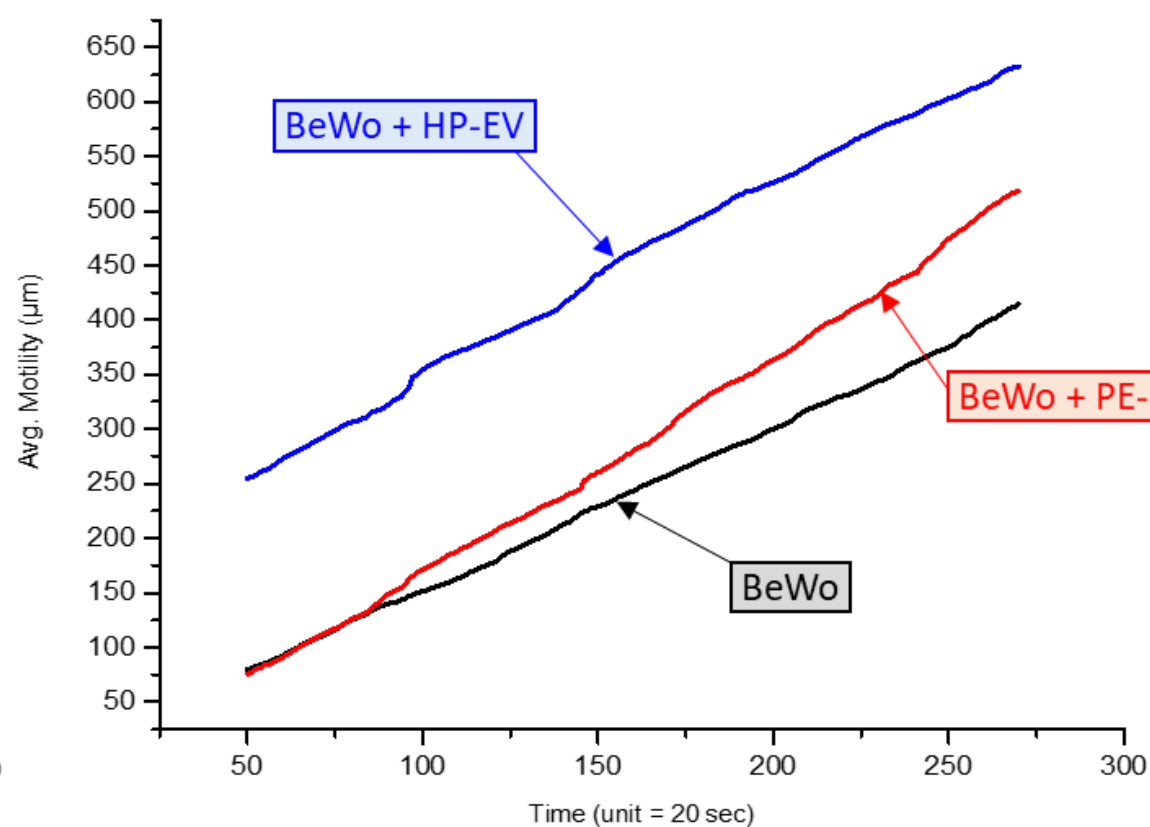


# III. EV - BeWo (3)

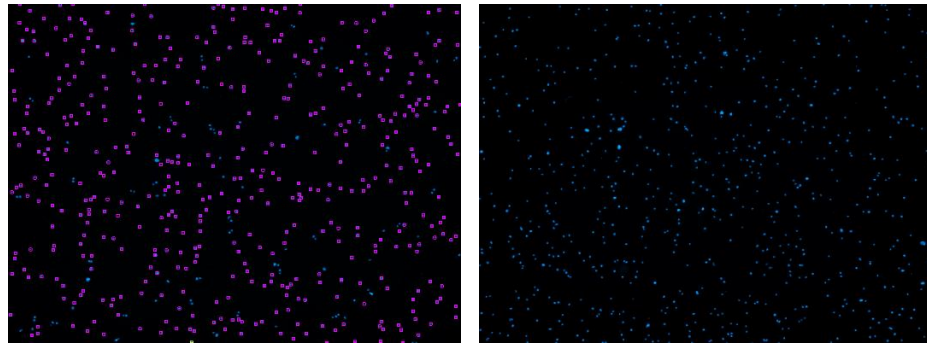
## MIGRÁCIÓ



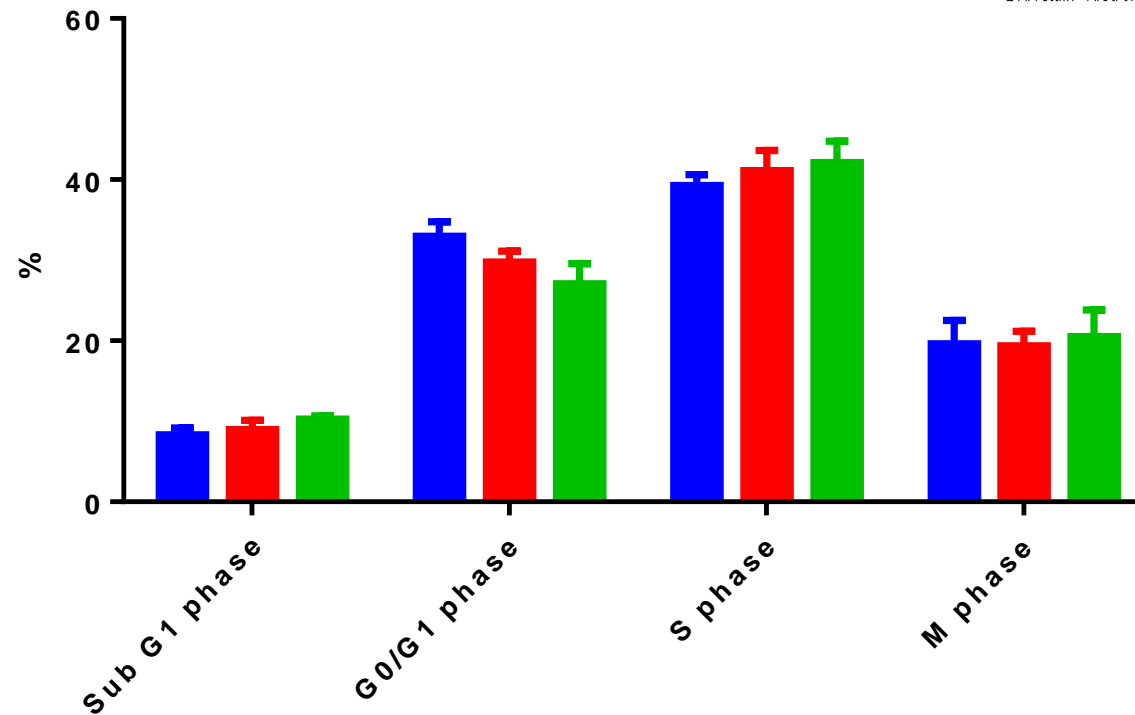
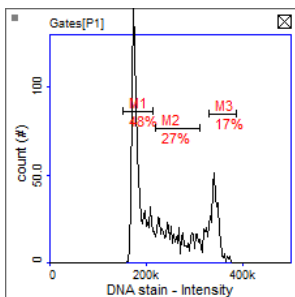
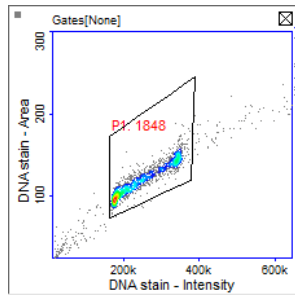
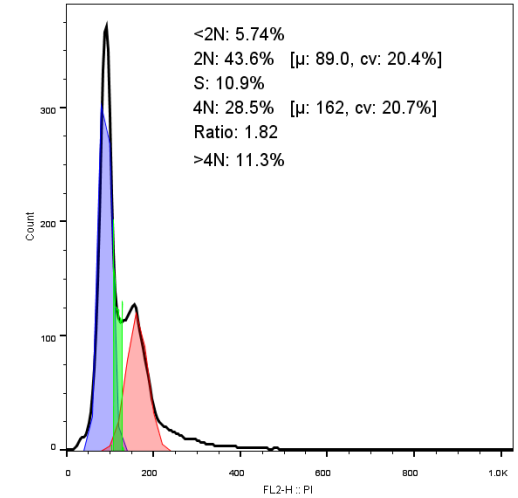
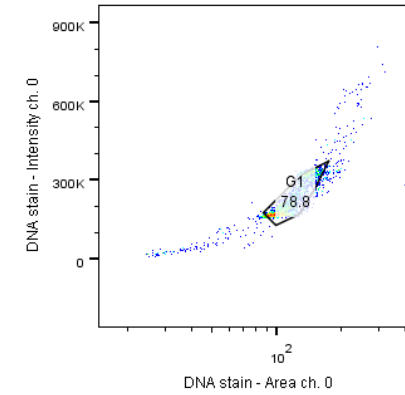
## MOTILITÁS



# III. EV - BeWo (4)



NC-250 and FC  
 ← DAPI stain | PI stain →

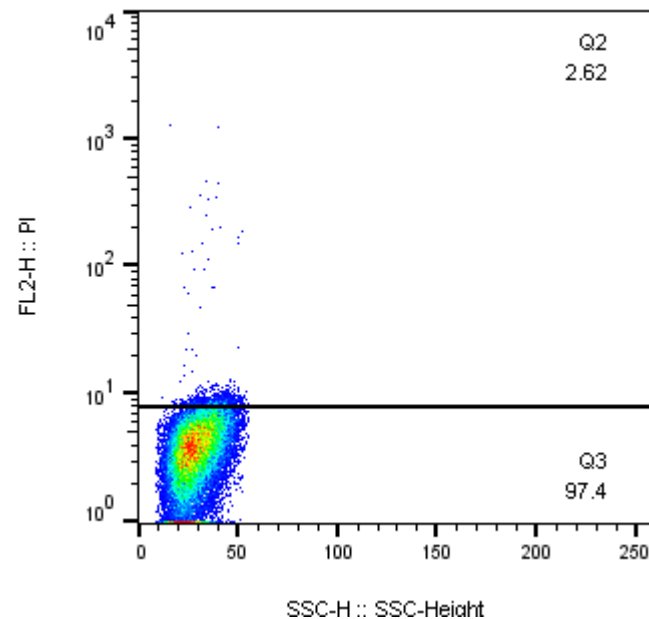
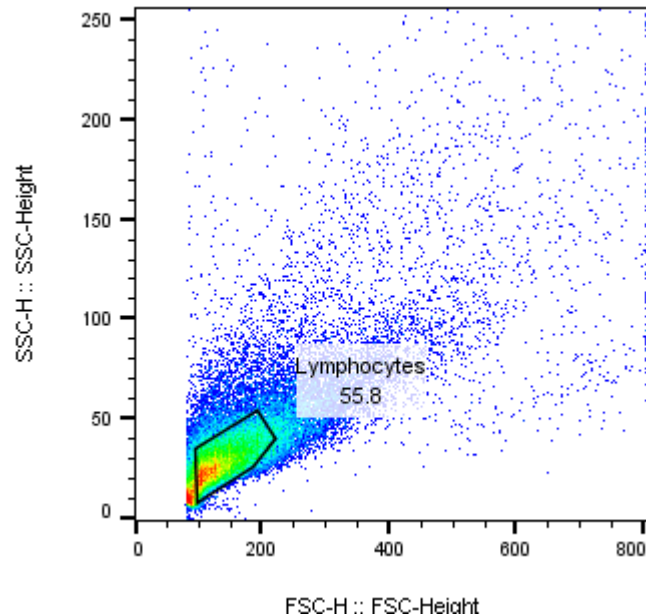


■ BeWo + HP-MV    
 ■ BeWo + PE-MV    
 ■ BeWo

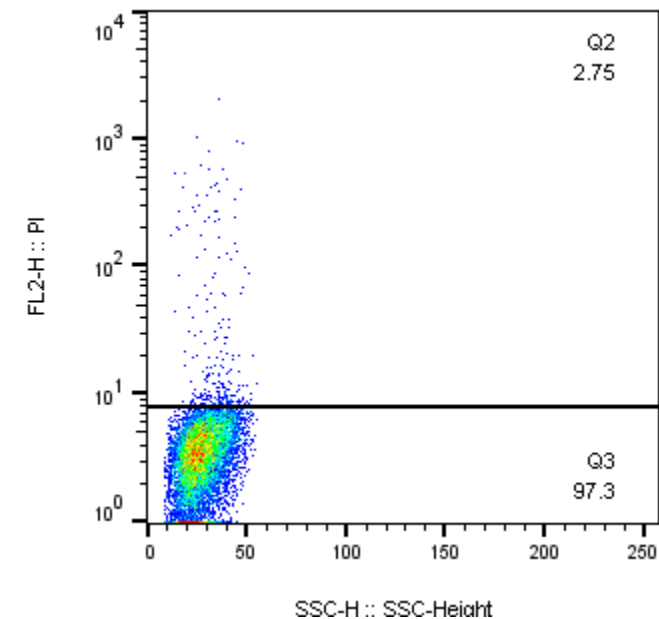
# III. BeWo – LYMPHOCYTA

## Előkísérlet

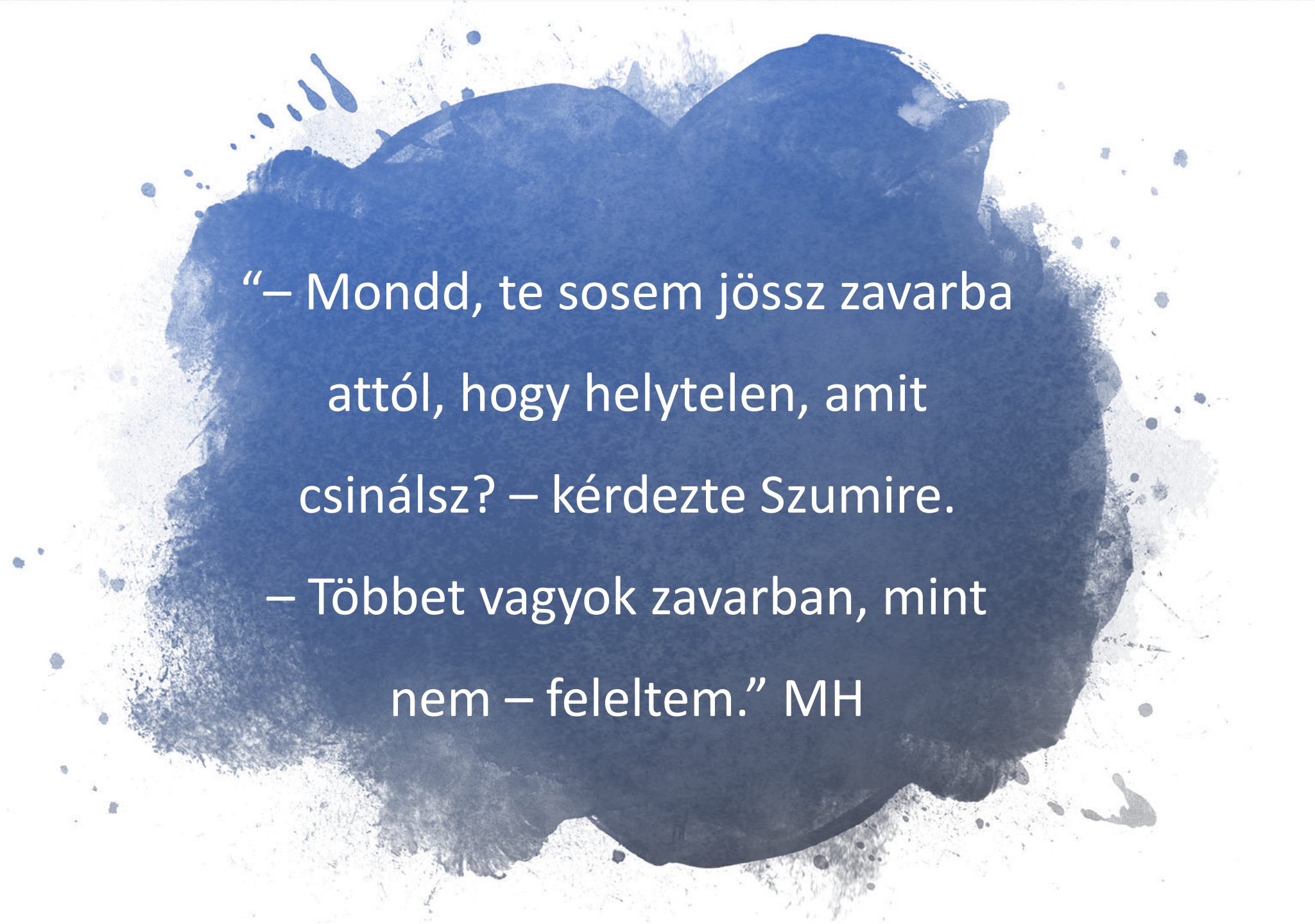
1. LYMPHOCYTA IZOLÁLÁS
2. LYMPHOCYTA BeWo kokultúrák rendszer 48h át (500.000 LY: 50.000 BeWo)
3. LYMPHOCYTA AKTIVÁCIÓ CD3/CD28



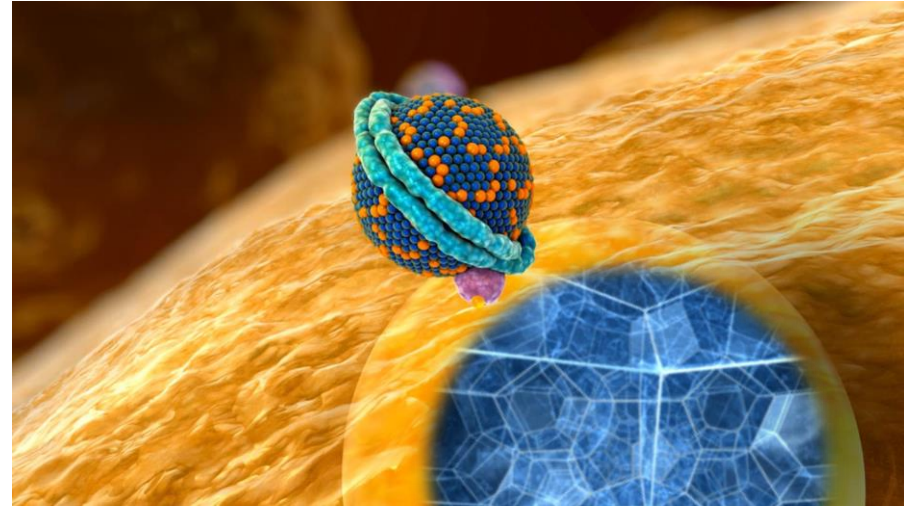
LYMPHOCYTA



LYMPHOCYTA + BeWo



“– Mondd, te sosem jössz zavarba  
attól, hogy helytelen, amit  
csinálsz? – kérdezte Szumire.  
– Többet vagyok zavarban, mint  
nem – feleltem.” MH



KÖSZÖNÖM SZÉPEN A FIGYELMET!