

# **Environmental-pollutant-induced pathologies of pregnancy: Modeling the mechanistic role of fetal extracellular vesicles using organ-on-a-chip**

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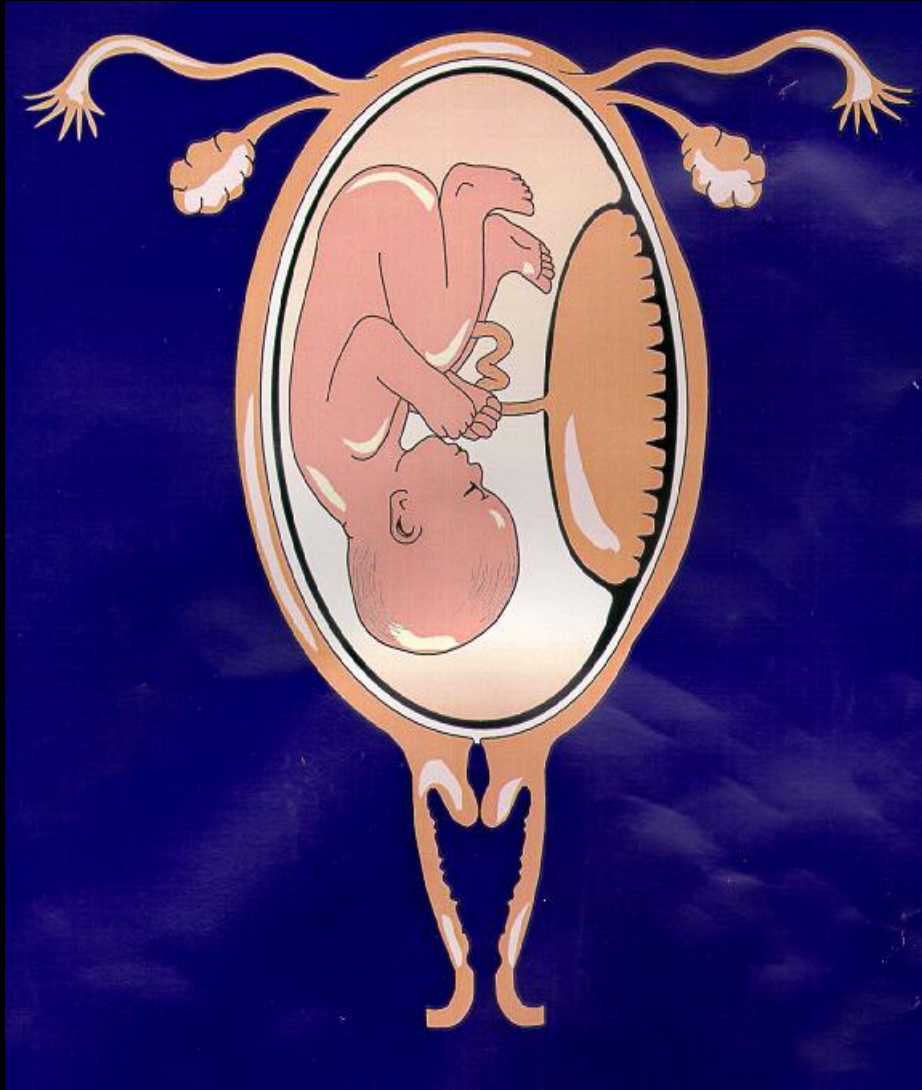
Executive Director, Preterm Birth International Collaborative (PREBIC, Inc.)

Workshop on Extracellular Vesicles, Exosomes, and Cell Cell Signaling in Response to Environmental Stress

NIH/NIEHS

September 27 28, 2021

# Pregnancy and Childbirth



Happy & Healthy Baby!



**Definition of preterm birth:**  
***Babies born alive before 37 completed weeks of pregnancy***

***15 million/year***

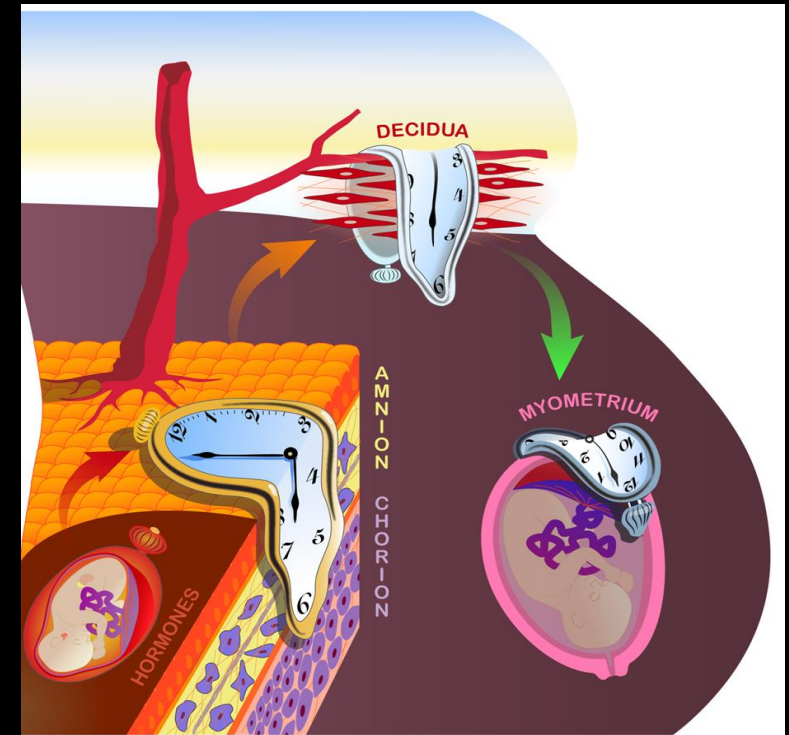
- ***1 million neonatal deaths***
- ***Life-long disabilities***
- ***Adult-onset diseases***

# Signaling Human Parturition

## Timing of birth

**Who decides?** The Mother or the fetus??

- Pregnancy - a balanced inflammatory state
  - Required for feto-placental growth
- Overwhelming inflammation - A trigger for human parturition at term and preterm



Human Reproduction Update, Vol.22, No.5 pp. 535-560, 2016  
Advanced Access publication on June 30, 2016 doi:10.1093/humupd/dmw022

human  
reproduction  
update

GRAND THEME REVIEW

**Novel concepts on pregnancy clocks  
and alarms: redundancy and synergy  
in human parturition**

Ramkumar Menon<sup>1,\*</sup>, Elizabeth A. Bonney<sup>2</sup>, Jennifer Condon<sup>3</sup>,  
Sam Mesiano<sup>4</sup>, and Robert N. Taylor<sup>5</sup>

# Inflammation is Associated with Term and Preterm Labor

## Physiologic Activation

Fetal signals of organ maturity  
Feto-maternal endocrine factors

## Pathologic Activation

Risk exposures

Increased inflammation

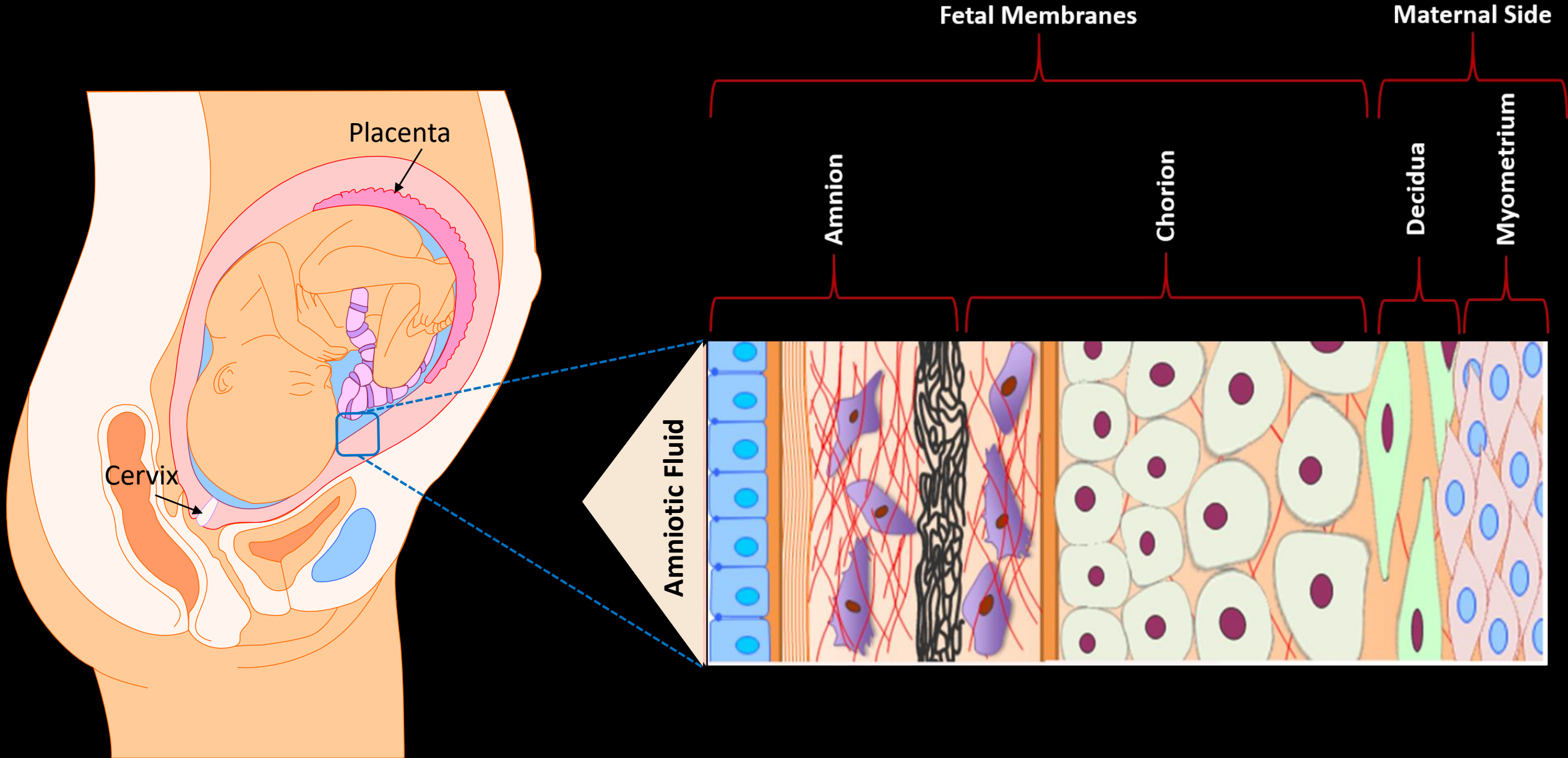
Cervical Ripening

Myometrial activation/contractions  
(Labor)

Delivery

Intervention strategies

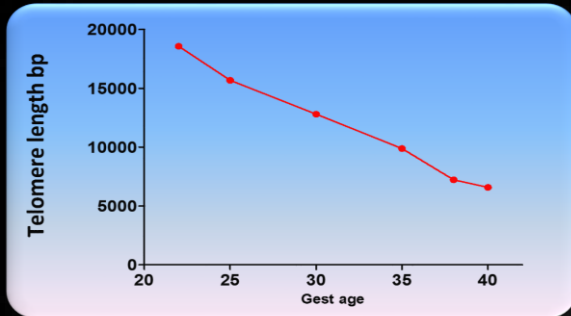
# Basic Anatomy Of The Maternal-Fetal Interface



# Senescence of Fetal Membranes at Term : A Factor Associated with Parturition

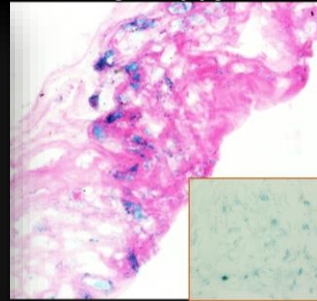
## Human

### Telomere length

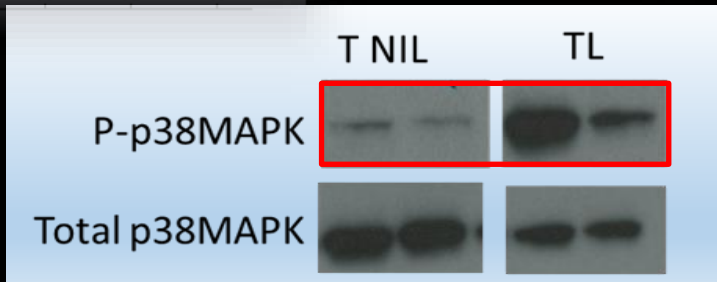
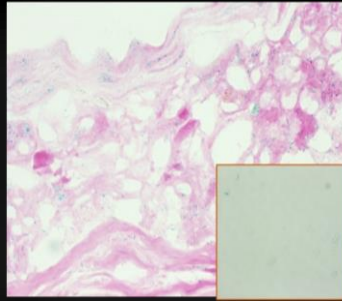


### SA-β-Galactosidase

Term labor



Term not in labor

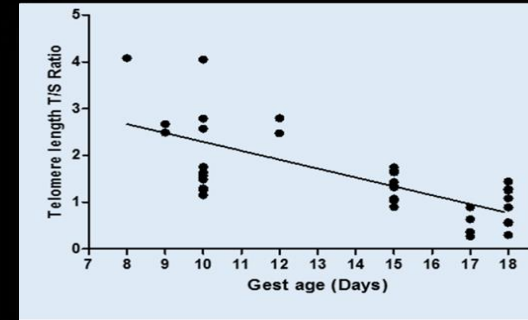


Term not in labor – T NIL

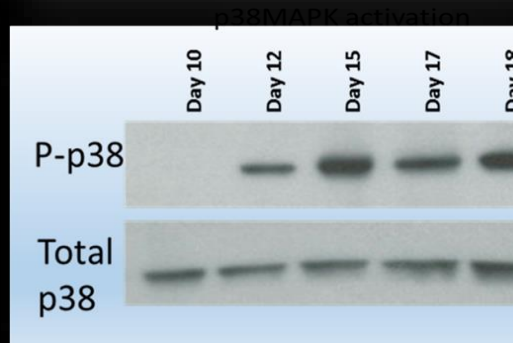
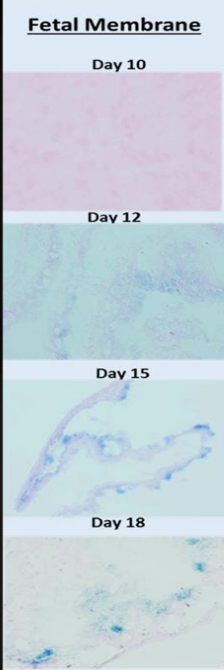
Term labor – TL

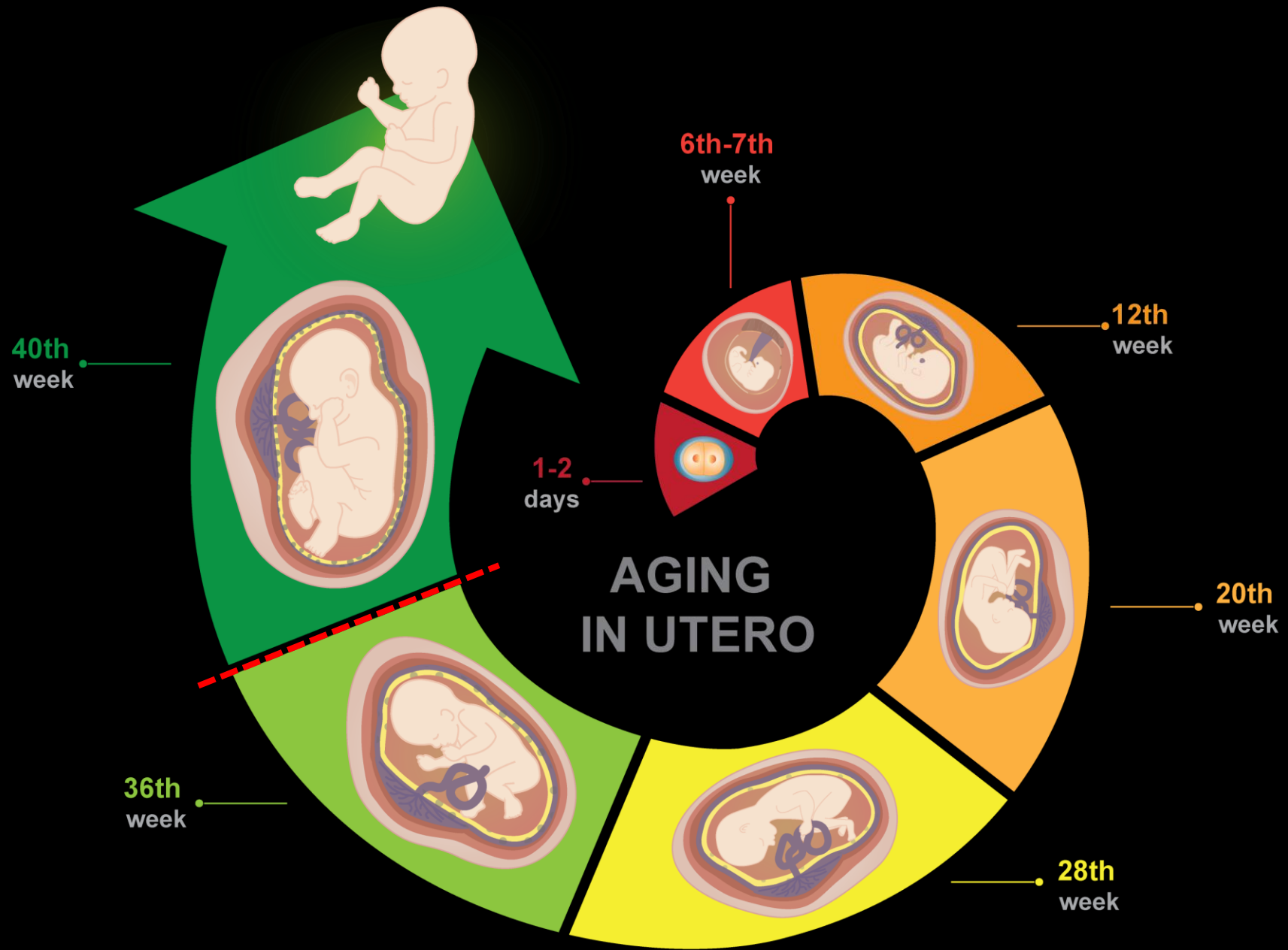
## Mouse

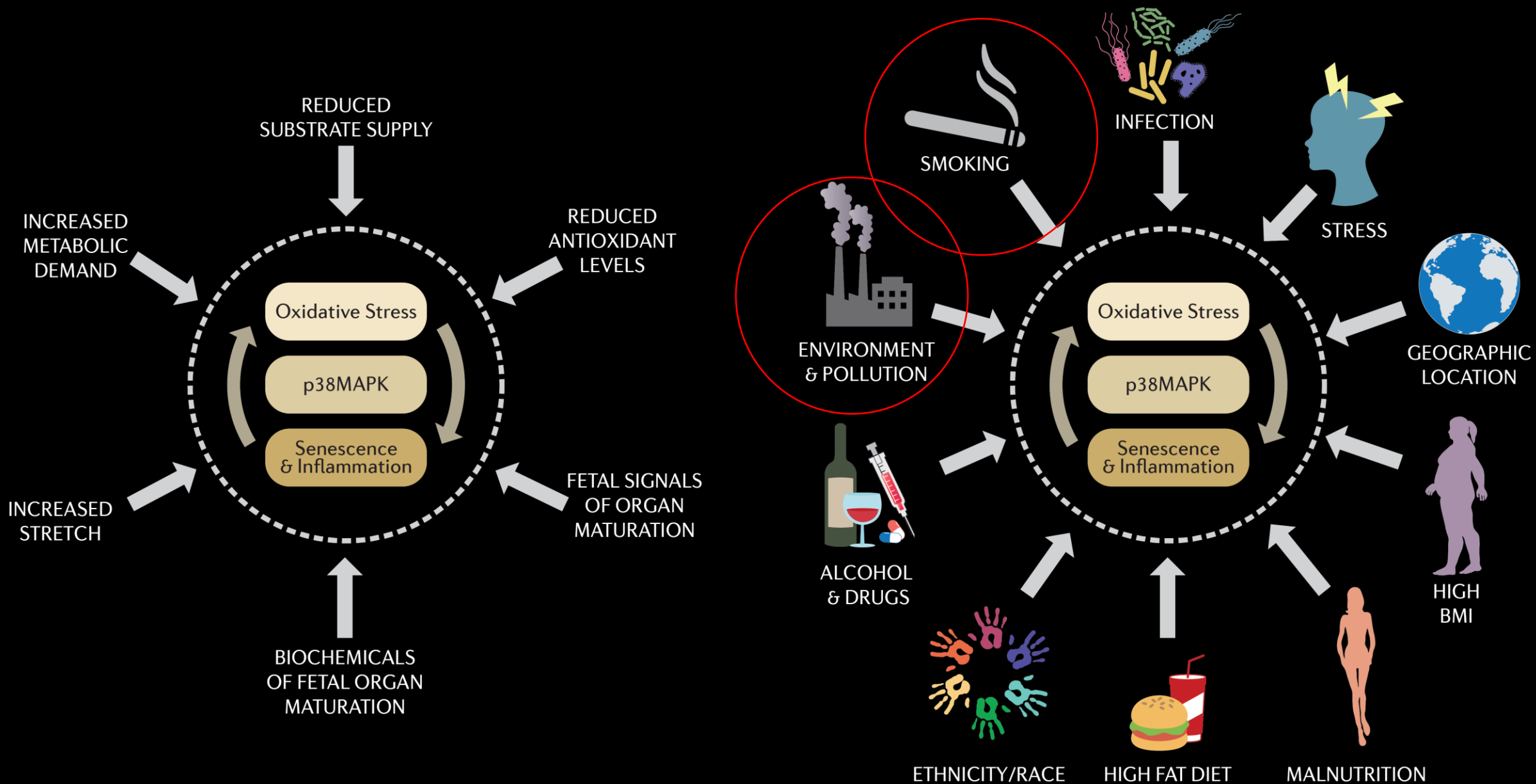
### Telomere length



### SA-β-Gal



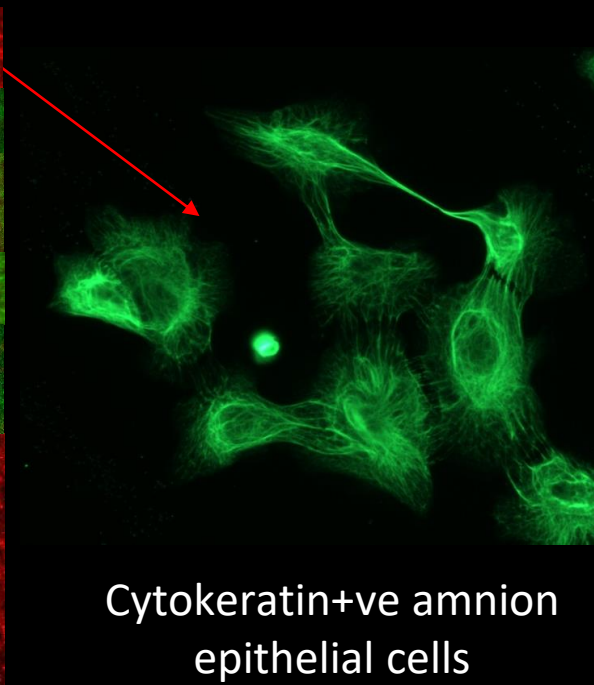
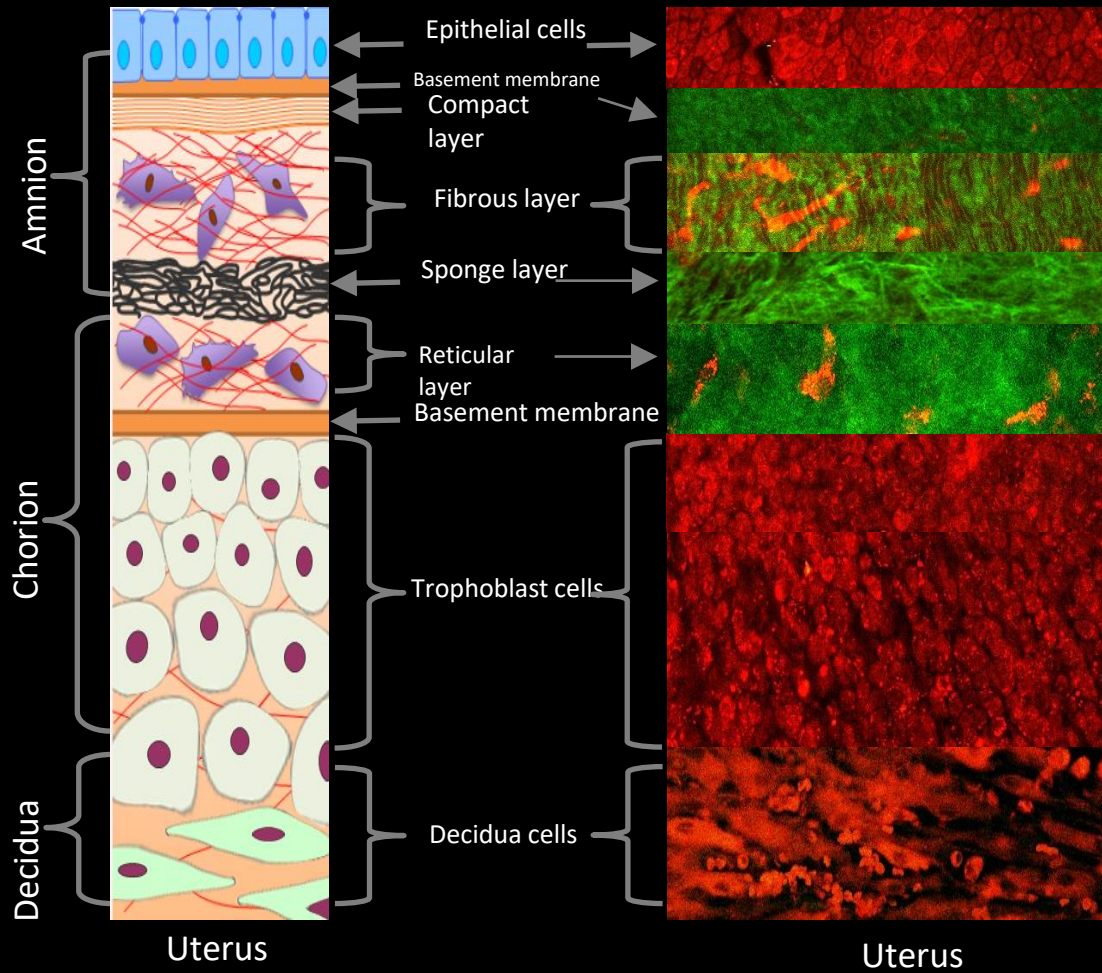






# Term not in labor fetal membranes

Amniotic Cavity



**Environmental toxin (PBDE)  
or  
Cigarette smoke extract (CSE)**

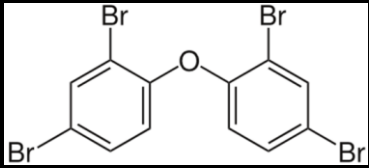
**Reactive Oxygen Species**

**Damage due to OS**

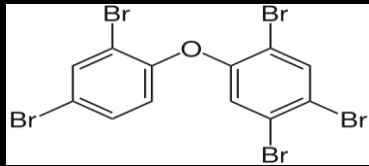
**Development of pathology**

# Polybrominated Diphenyl Ethers (PBDE)

## PBDE-47

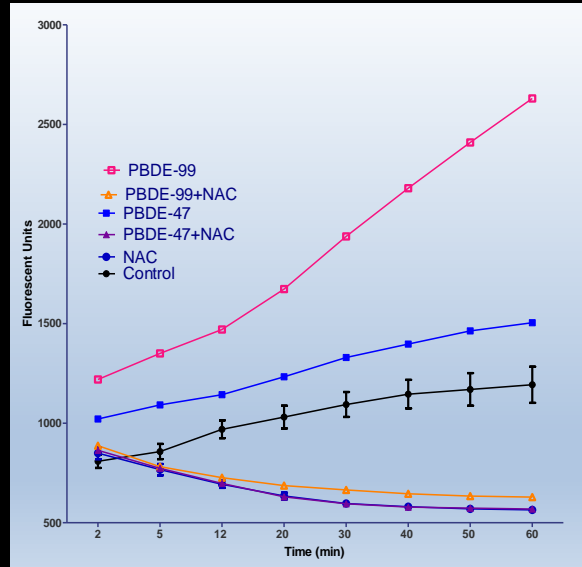


## PBDE-99



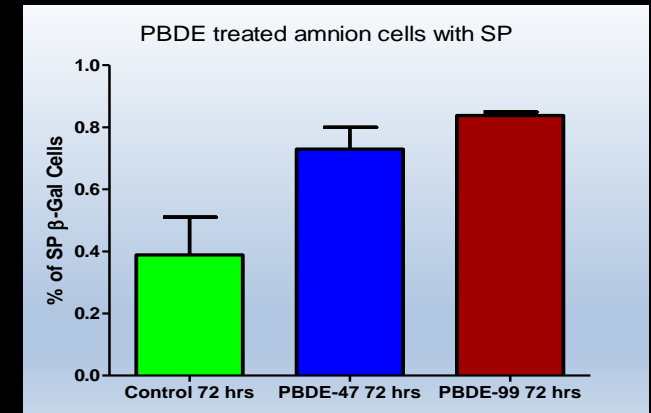
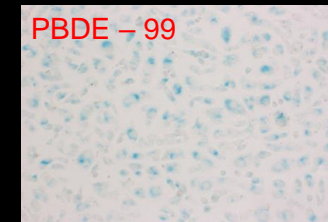
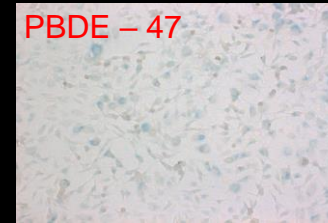
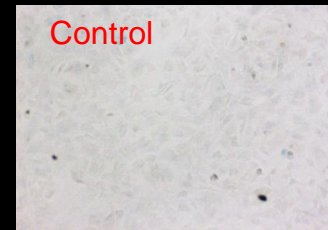
- Commercial PBDE (flame retardant chemical) is a mixture of different PBDE congeners.
- PBDEs exist as mixtures of distinct chemicals called congeners with unique molecular structures based on total number or position of bromine atoms attached to the ether molecule.

### PBDE causes ROS production from human fetal amnion cells



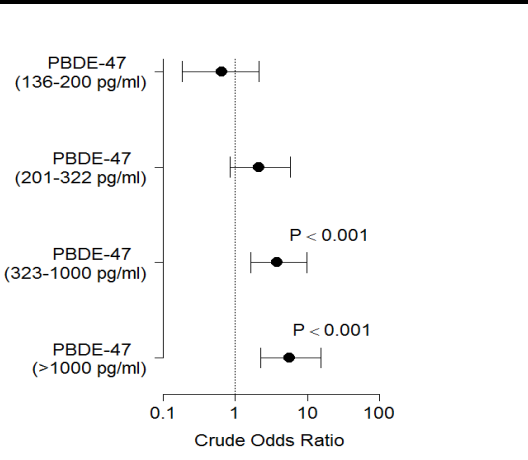
Reactive oxygen species

### Senescence Associated $\beta$ -Galactosidase (SA- $\beta$ -Gal)



### Senescence associated secretory phenotype (SASP)

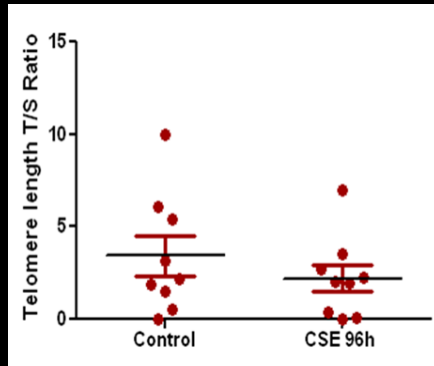
- Increased inflammatory cytokines
- IL 6, IL 8, TNF  $\alpha$ , GM CSF
- MMP9



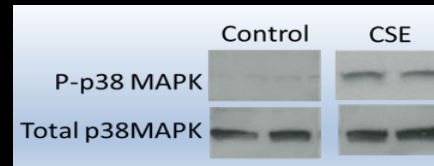
Amniotic fluid PBDE-47 effect on odds of delivering preterm. Crude odds ratios 95% CI.

# Cigarette smoke Induced Fetal Cell Senescence

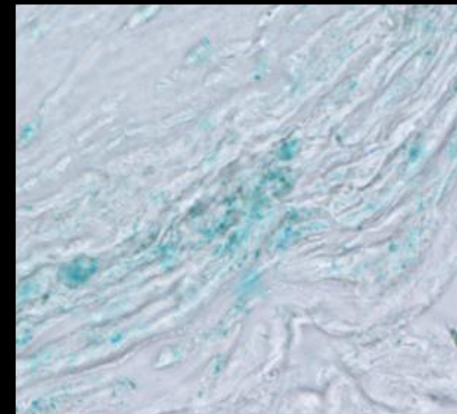
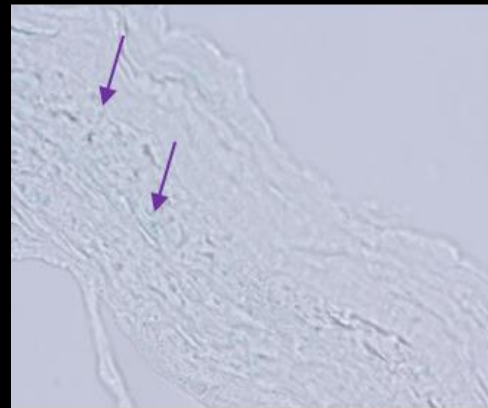
## Telomere length



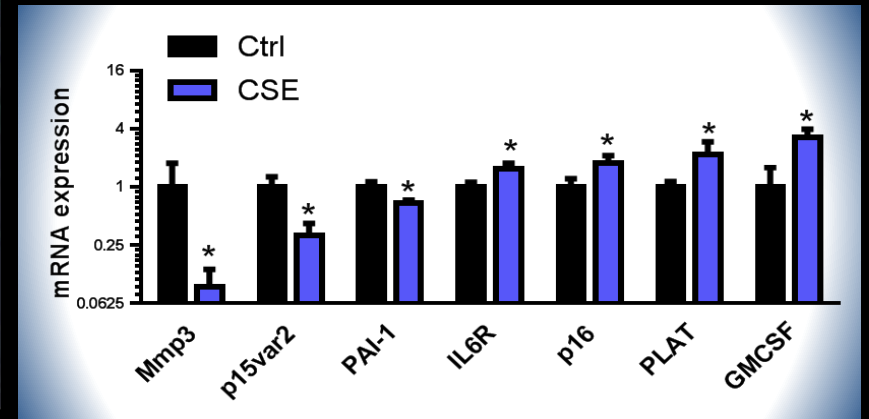
## p38MAPK activation



## Senescence Associated $\beta$ -Galactosidase

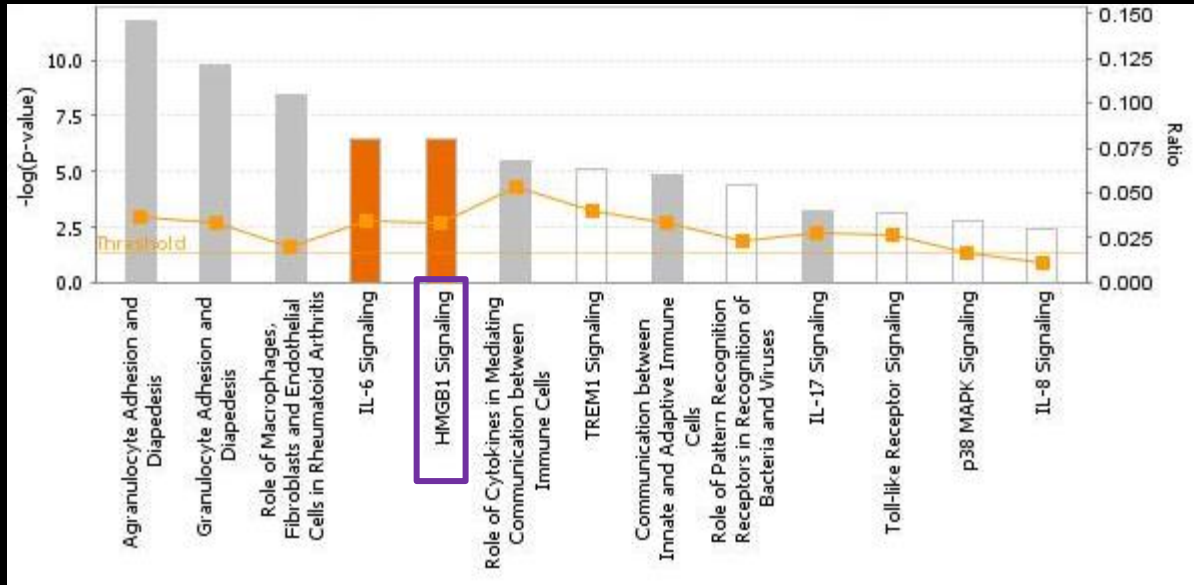


## Senescence Associated Secretory Phenotype - SASP



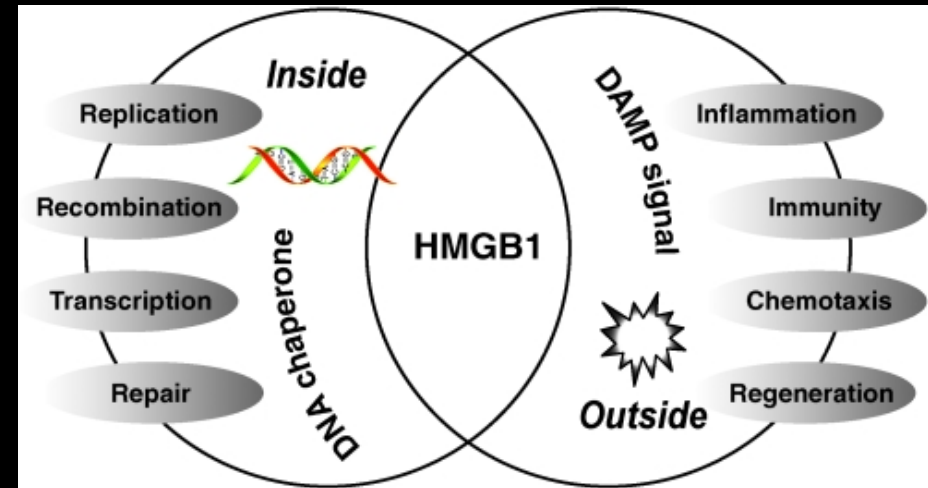
# Senescence Associated Secretory Phenotype - SASP

Control vs CSE treated



Ingenuity pathway analysis

Damage-Associated Molecular Pattern Markers (DAMPs)

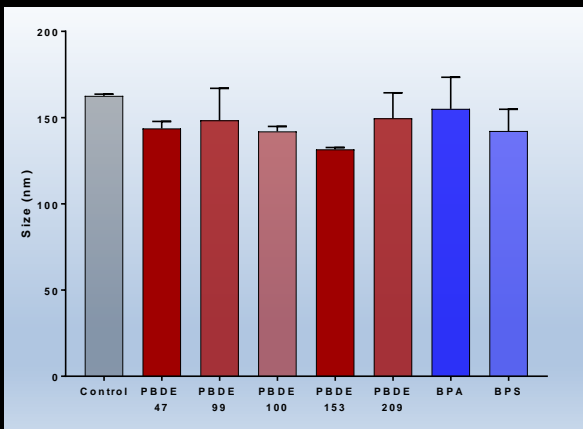


Tang, D., Antioxidants & redox signaling, 14, 1315-1335.

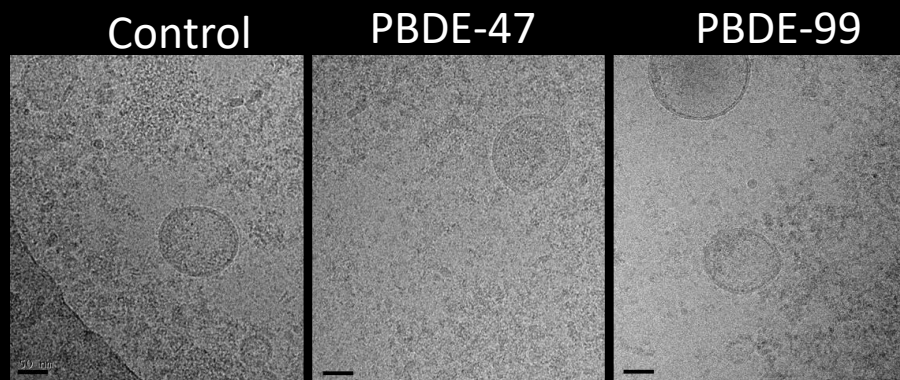
- HMGB1 trafficking
- Short half life
- Protected environment
- EV mediated transport of HMGB1

# Exosomal Characteristics from PBDE Treated Fetal Cells

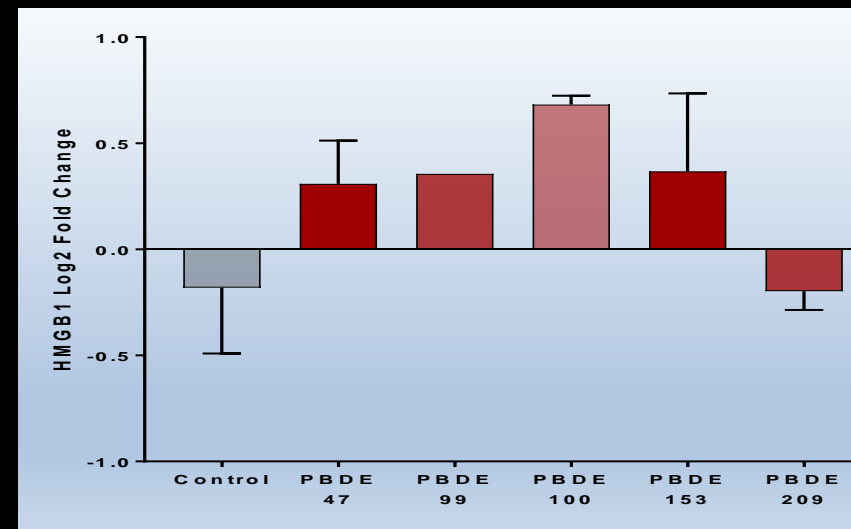
Size



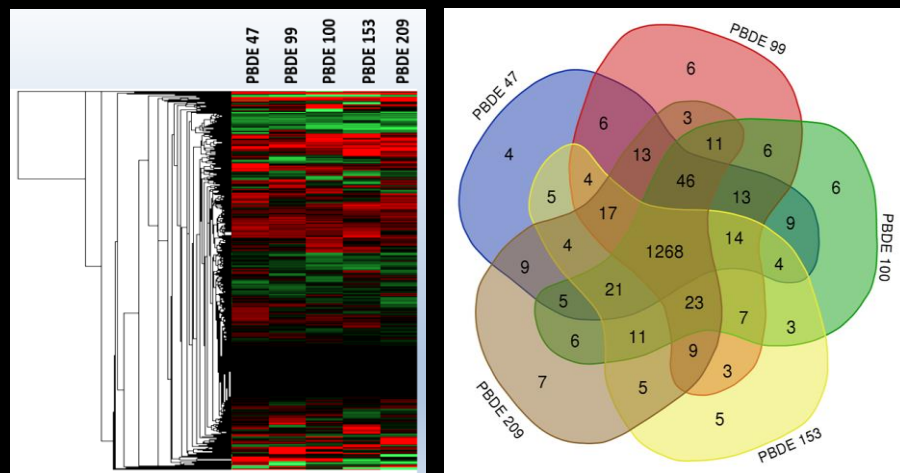
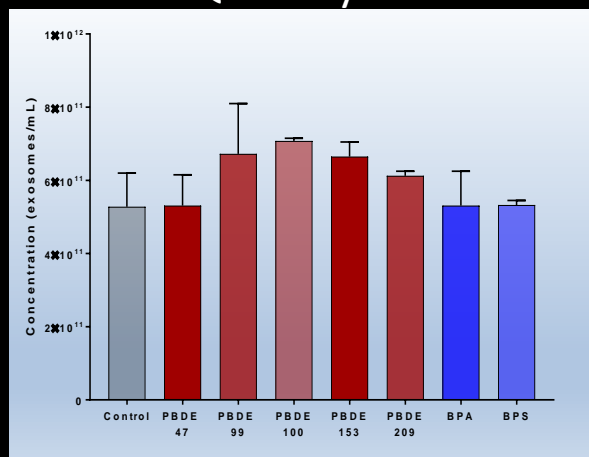
Morphology



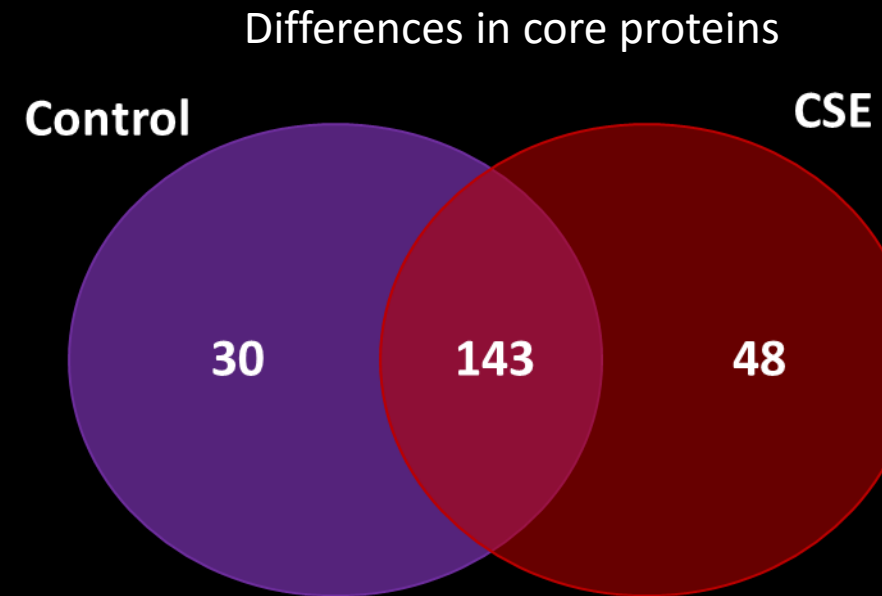
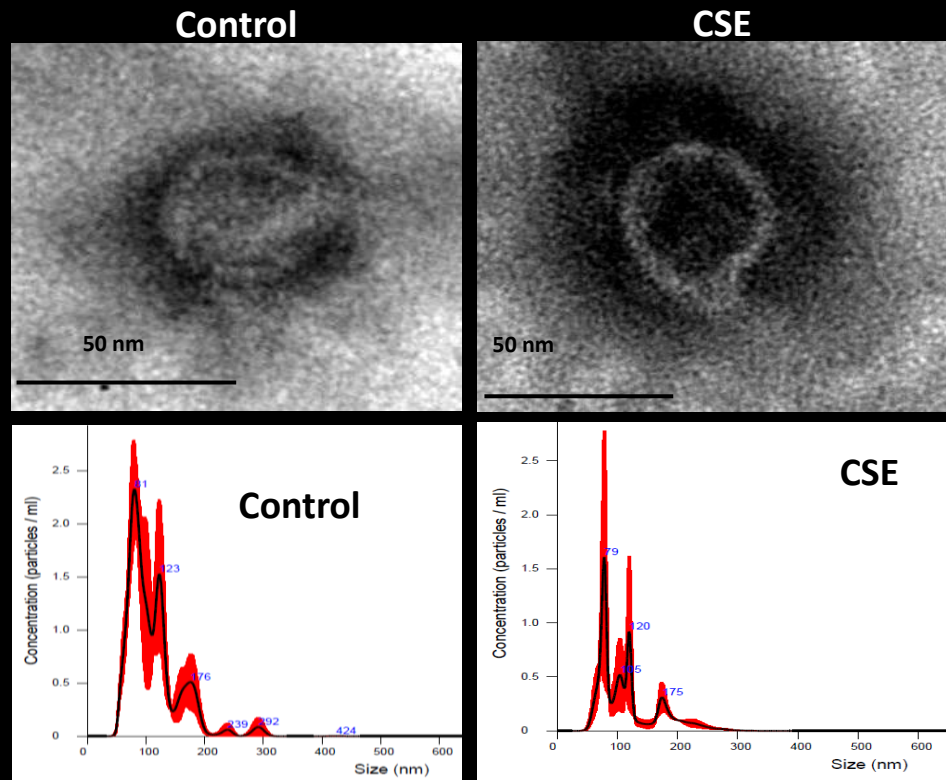
HMGB1 is increased in PBDE treated cell derived exosomes



Quantity

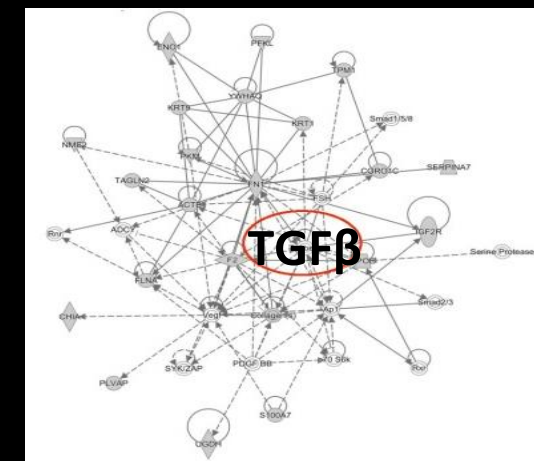
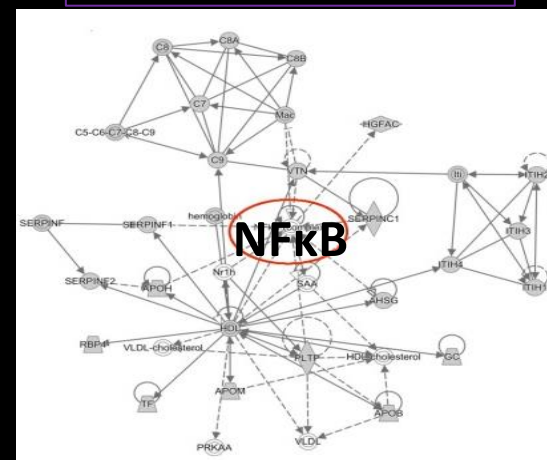
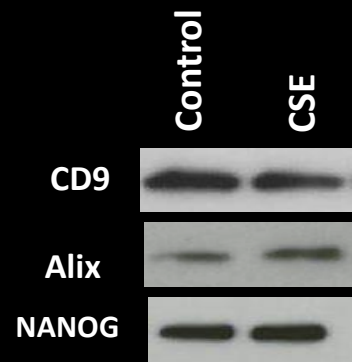


# Exosomal Characteristics from Cigarette Smoke Extract Treated Fetal Cells



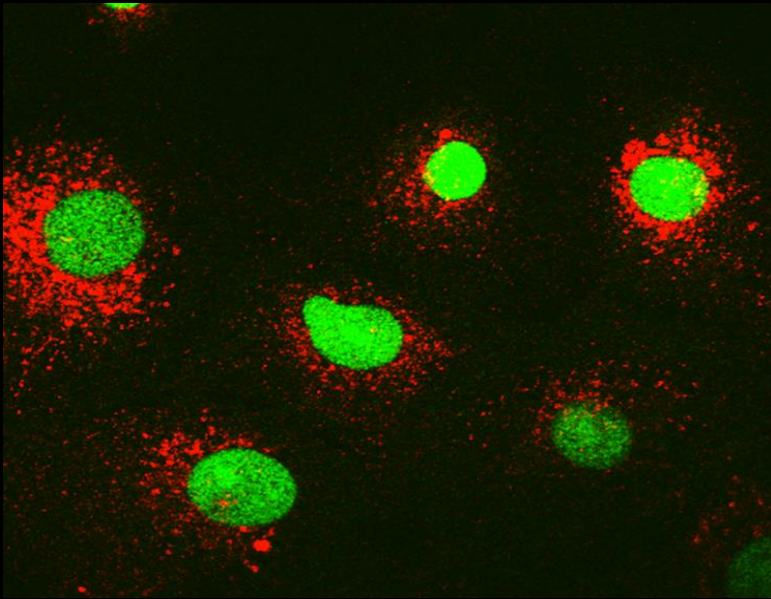
Control Exosomes

CSE Exosomes

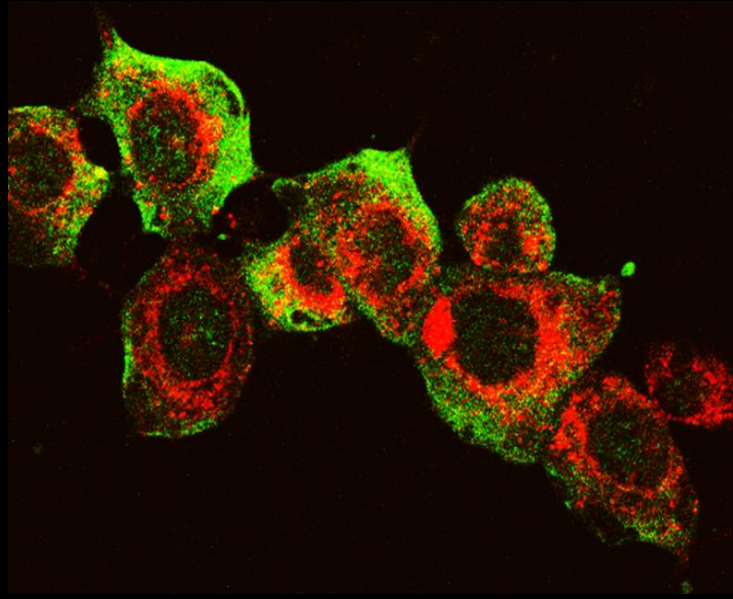


# HMGB1 is Released by Oxidative Stress and Packaged by Exosomes

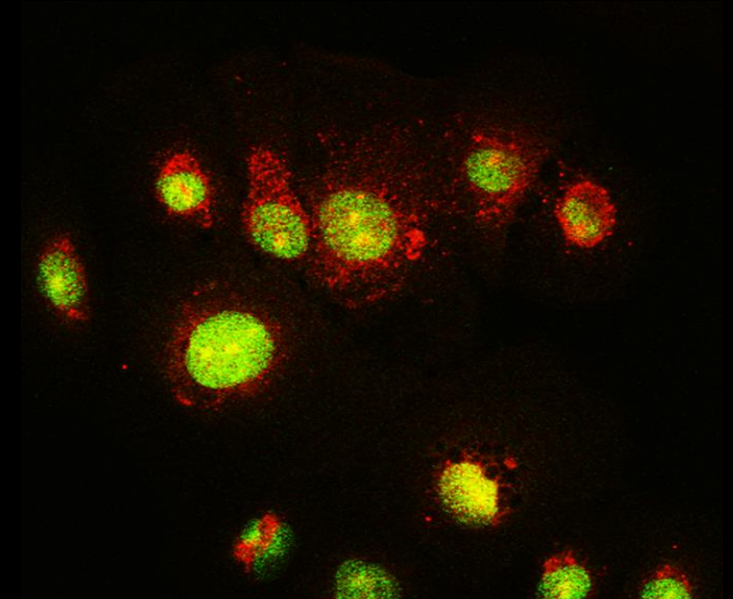
Untreated Amnion cells



Amnion cells treated with cigarette smoke



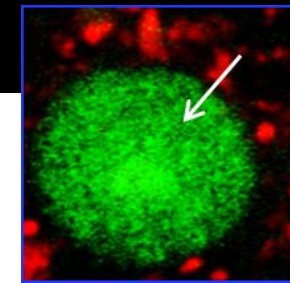
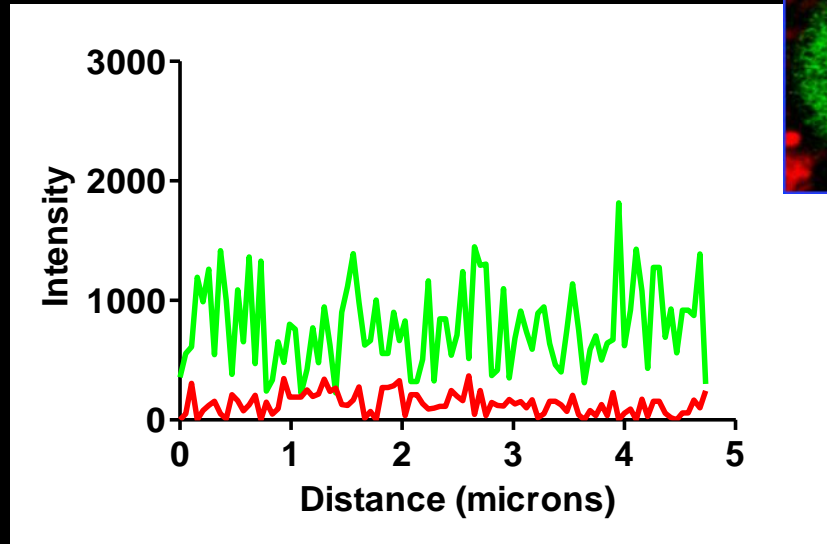
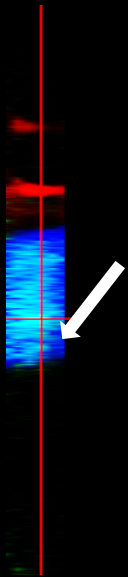
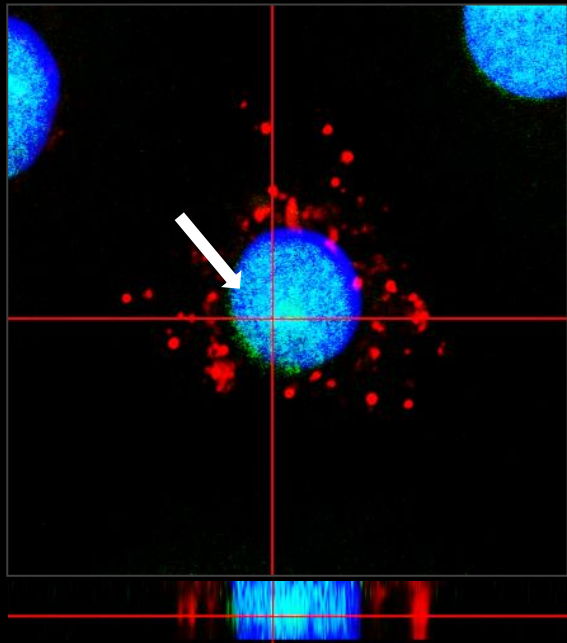
Amnion cells with cigarette smoke + N-Acetyl cysteine



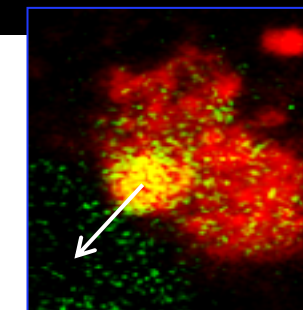
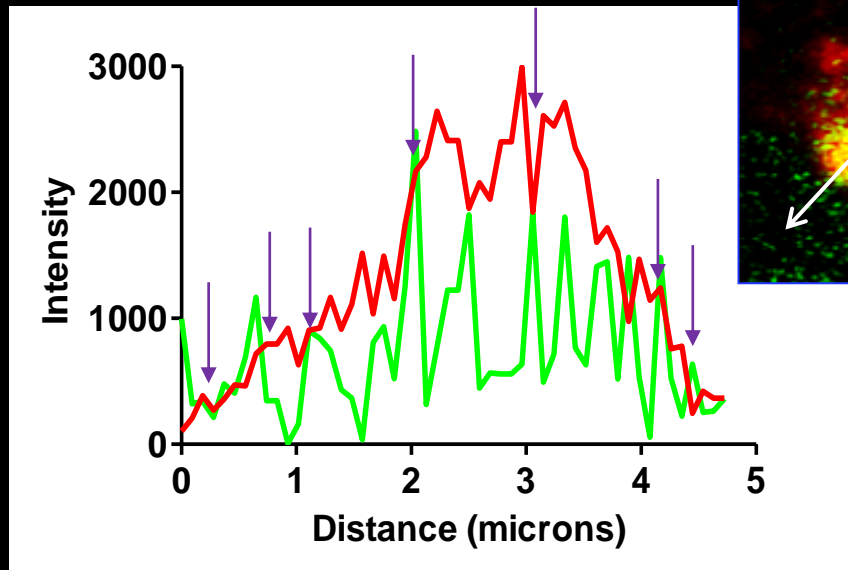
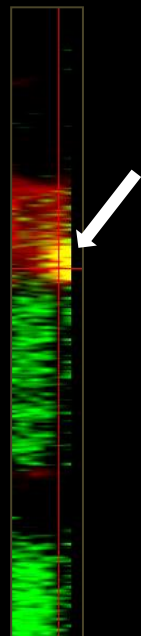
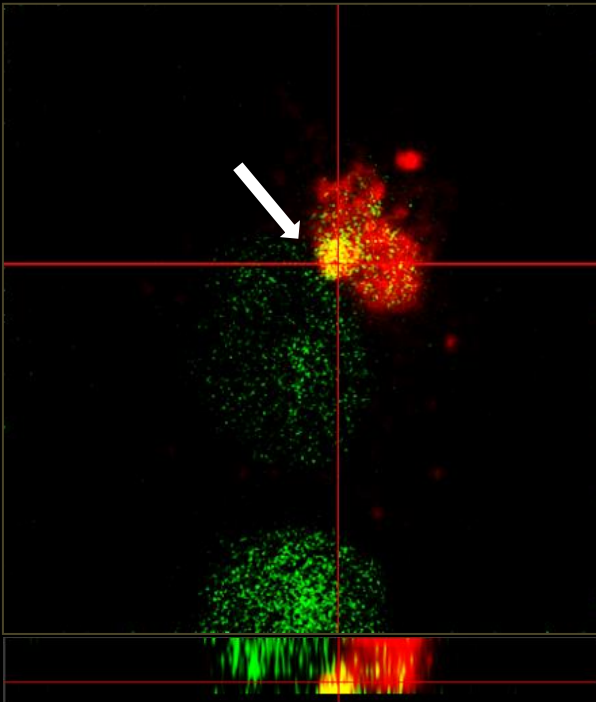
• CD9 Exosome marker

• HMGB1

# Co-localization

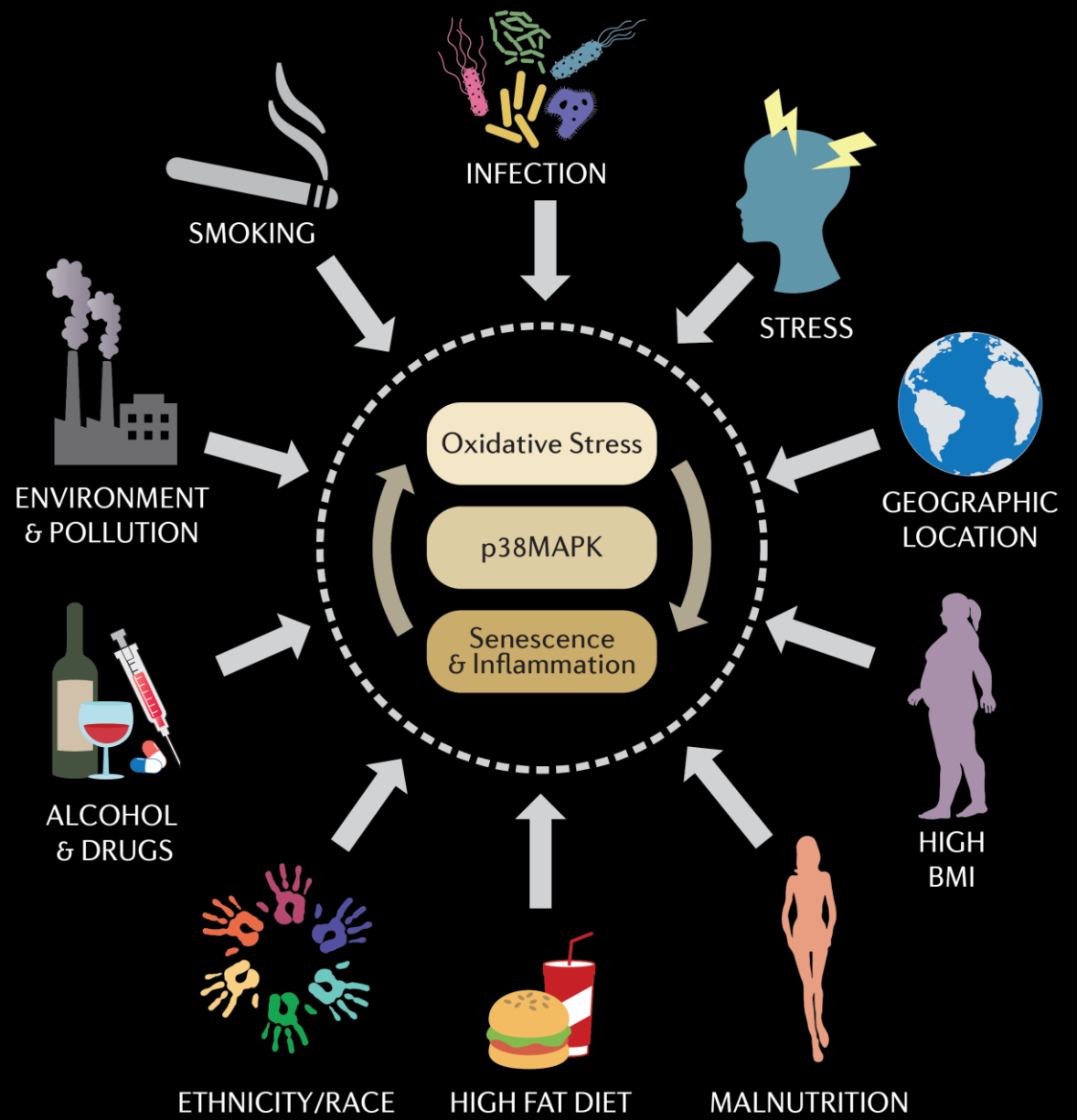
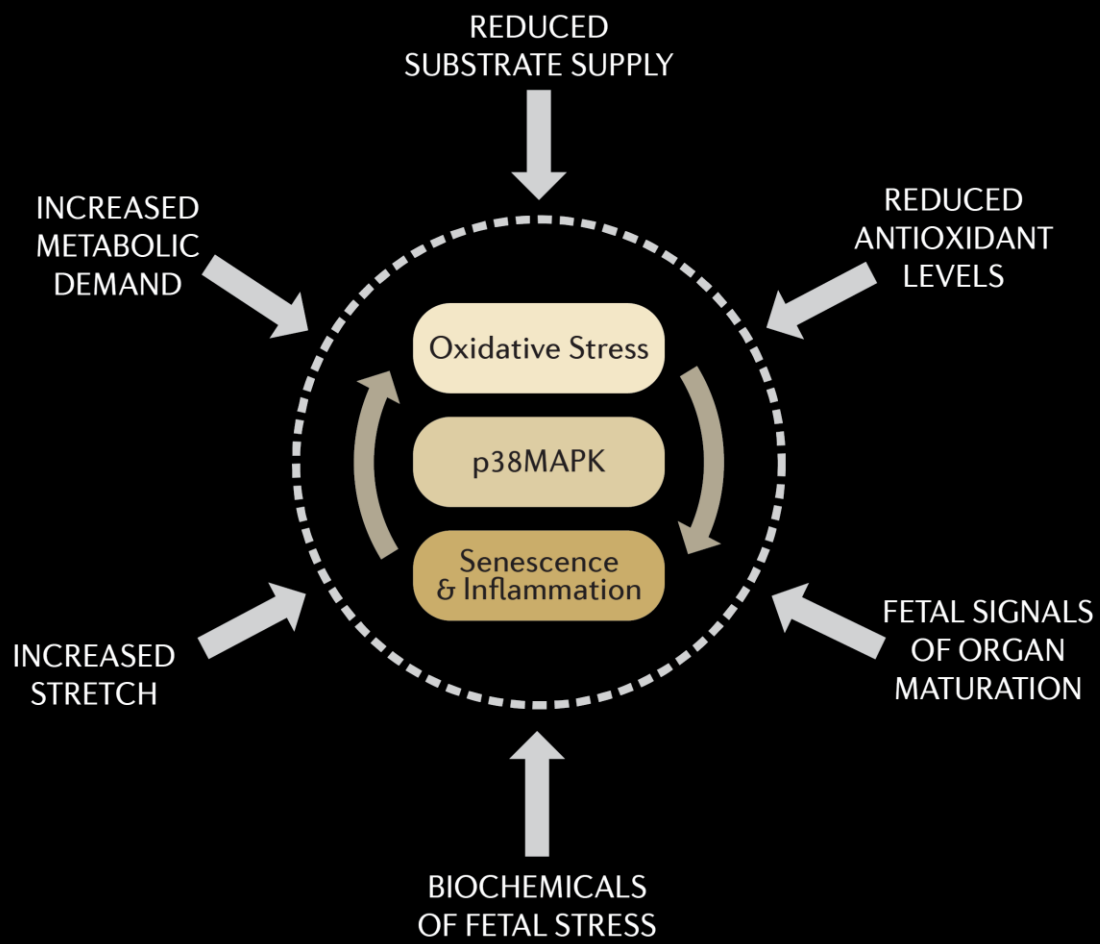


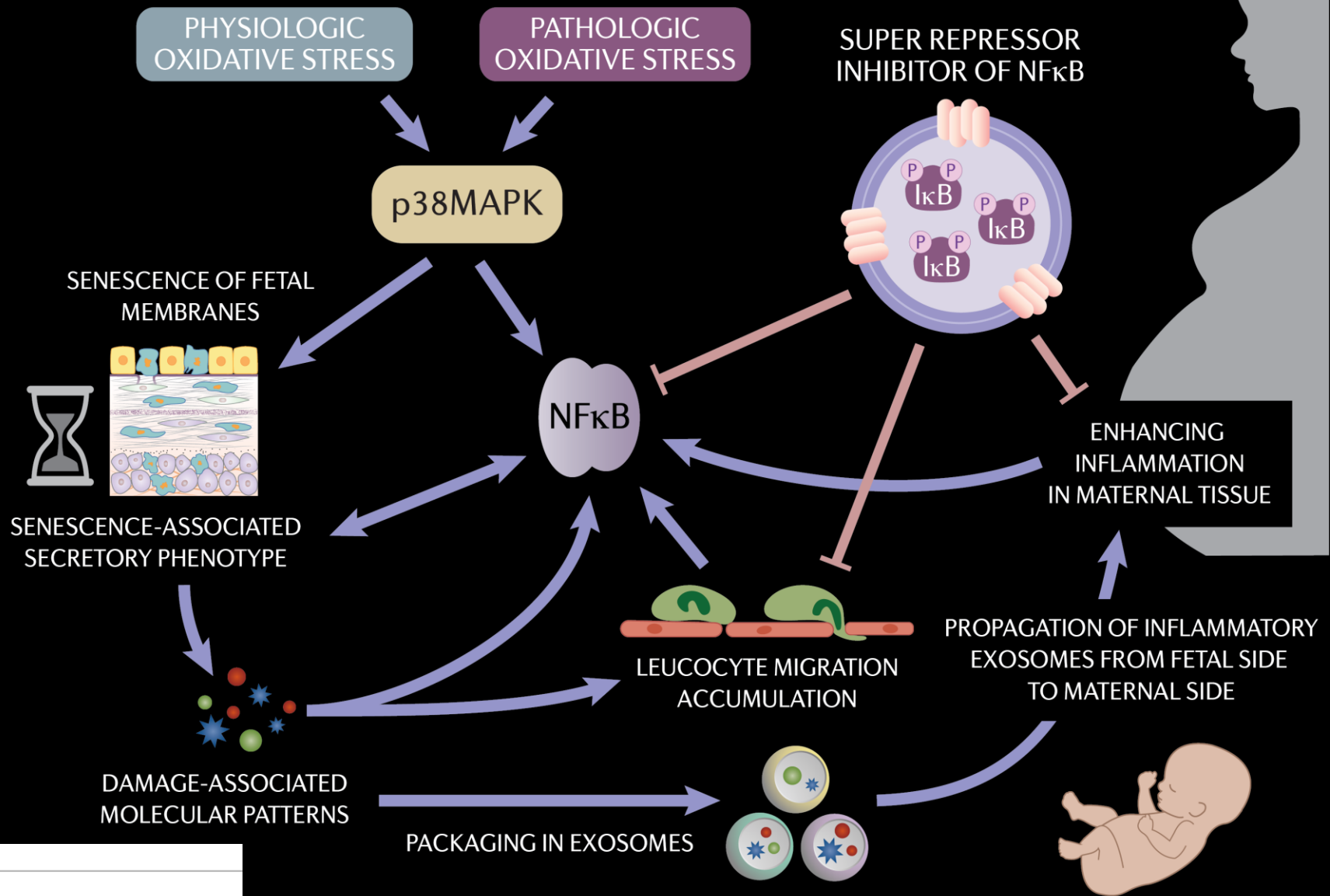
Control



CSE  
Oxidative stress







**Exosomal delivery of NF-κB inhibitor delays LPS-induced preterm birth and modulates fetal immune cell profile in mouse models**

Samantha Sheller-Miller<sup>1</sup>, Enkhtuya Radnaa<sup>1</sup>, Jae-Kwang Yoo<sup>2</sup>, Kyungsun Choi<sup>2,3</sup>, Youngeun Kim<sup>2</sup>, Yu Na Kim<sup>2</sup>, Eunsoo Kim<sup>2</sup>, Lauren Richardson<sup>1</sup>, Chulhee Choi<sup>2,3</sup>, Ramkumar Menon<sup>1\*</sup>

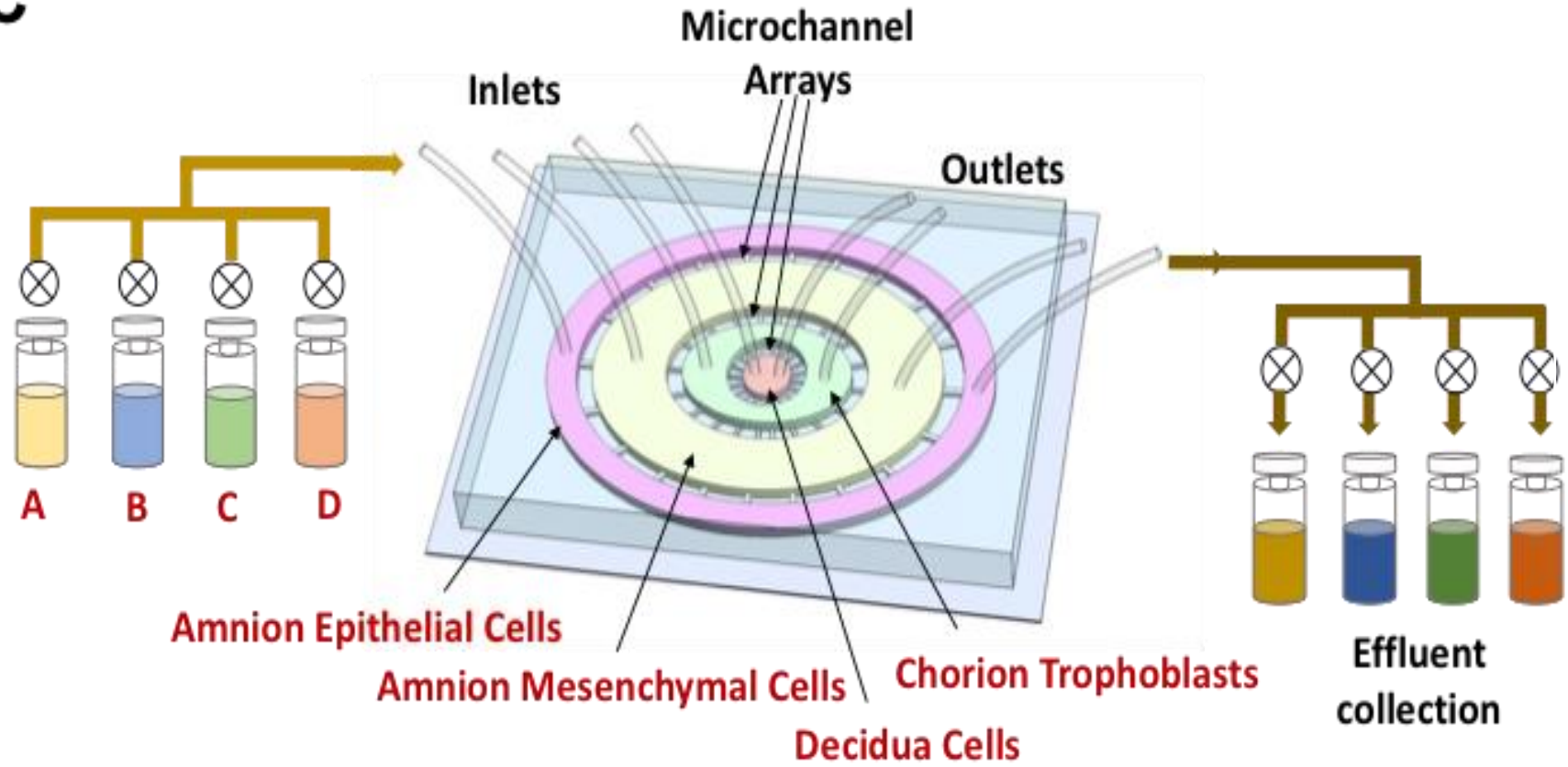
# How to test feto-maternal communication?

Organ-On-a-Chip – Maintain intercellular interactions

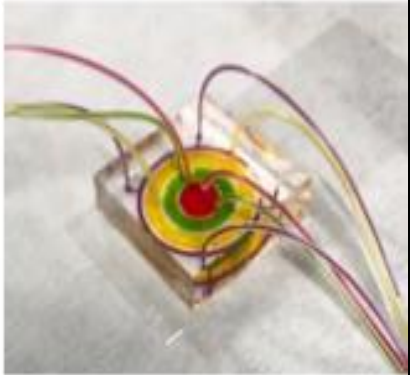
# Fetal-Maternal Interface-On-Chip

Fetal Membrane Organ on a Chip (FMI-OOC)

C



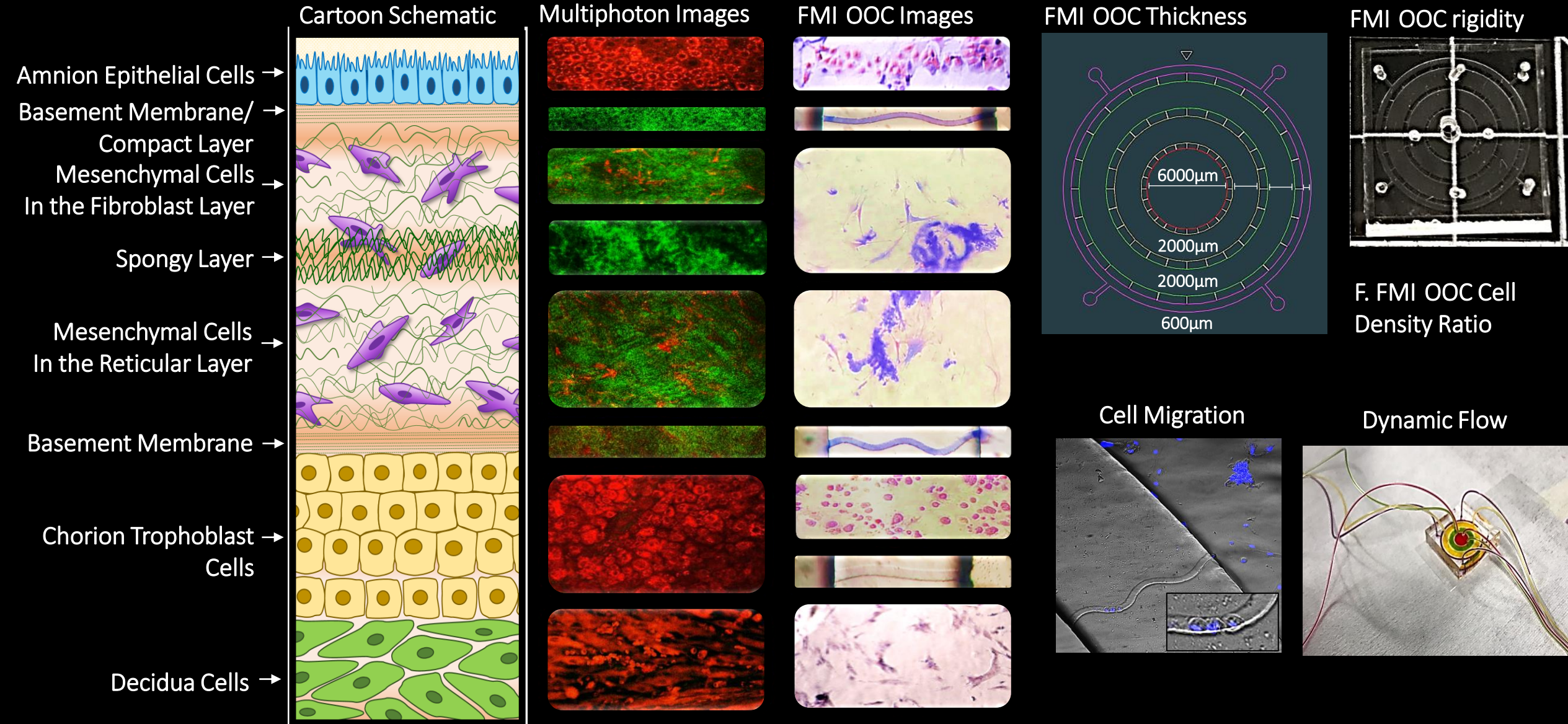
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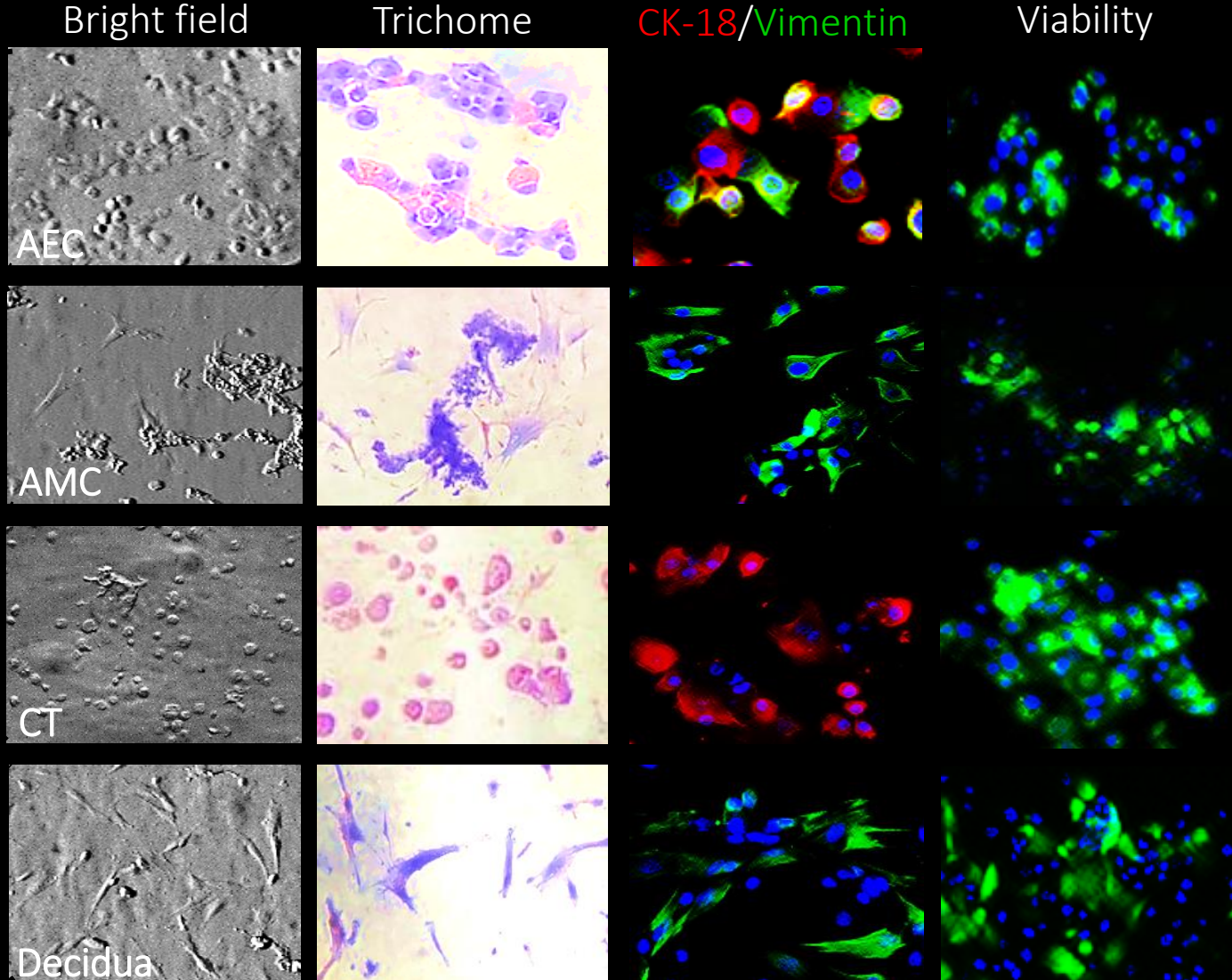
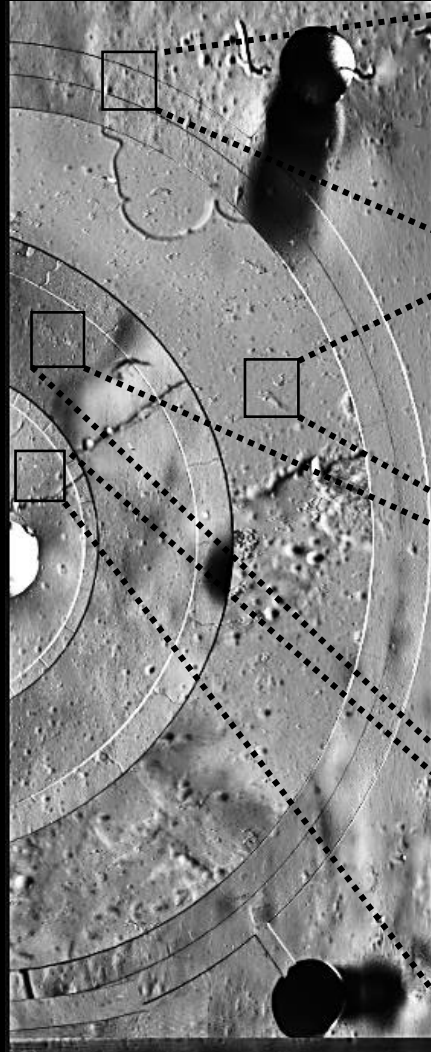
E



# Fetal Maternal Interface-On-Chip



# Fetal Maternal Interface-On-Chip



# Fetal Maternal Interface-On-Chip

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**Journal of Hazardous Materials**

journal homepage: [www.elsevier.com/locate/jhazmat](http://www.elsevier.com/locate/jhazmat)

**Molecular mechanisms of environmental toxin cadmium at the feto-maternal interface investigated using an organ-on-chip (FMi-OOC) model**

Sungjin Kim<sup>a,1</sup>, Lauren Richardson<sup>a,b,1</sup>, Enkhtuya Radnaa<sup>b</sup>, Zunwei Chen<sup>c</sup>, Ivan Rusyn<sup>c</sup>, Ramkumar Menon<sup>b,\*</sup>, Arum Han<sup>a,\*</sup>

<sup>a</sup> Department of Electrical and Computer Engineering, Texas A&M University, College Station, TX, USA  
<sup>b</sup> Department of Obstetrics & Gynecology, Division of Maternal-Fetal Medicine & Perinatal Research, The University of Texas Medical Branch at Galveston, 301 University Blvd., Galveston, TX 77555-1062, USA  
<sup>c</sup> Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

Impact of Cadmium toxicity during pregnancy and pathologic mechanism at the feto maternal interface

ROYAL SOCIETY OF CHEMISTRY

**Lab on a Chip**

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Cite this: DOI: 10.1039/d0lc01323d

**Extracellular vesicle mediated feto-maternal HMGB1 signaling induces preterm birth†**

Enkhtuya Radnaa, <sup>a</sup> Lauren S. Richardson, <sup>ab</sup> Samantha Sheller-Miller, <sup>a</sup> Tuvshintugs Baljinnyam, <sup>c</sup> Mariana de Castro Silva, <sup>a</sup> Ananth Kumar Kammala, <sup>a</sup> Rheanna Urrabaz-Garza, <sup>a</sup> Talar Kechichian, <sup>a</sup> Sungjin Kim, <sup>b</sup> Arum Han <sup>bd</sup> and Ramkumar Menon<sup>\*a</sup>

Feto-maternal exosome based signaling

ROYAL SOCIETY OF CHEMISTRY

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Cite this: *Lab Chip*, 2020, 20, 4486

**Modeling ascending infection with a feto-maternal interface organ-on-chip†**

Lauren S. Richardson, <sup>ab</sup> Sungjin Kim, <sup>b</sup> Arum Han <sup>bd</sup> and Ramkumar Menon <sup>da</sup>

Created an in vitro ascending model of infection. Physiologically validated in vivo using animal models

Received: 25 November 2020 | Revised: 3 February 2021 | Accepted: 5 February 2021

DOI: 10.1096/fj.202002590RRR

RESEARCH ARTICLE

THE FASEB JOURNAL

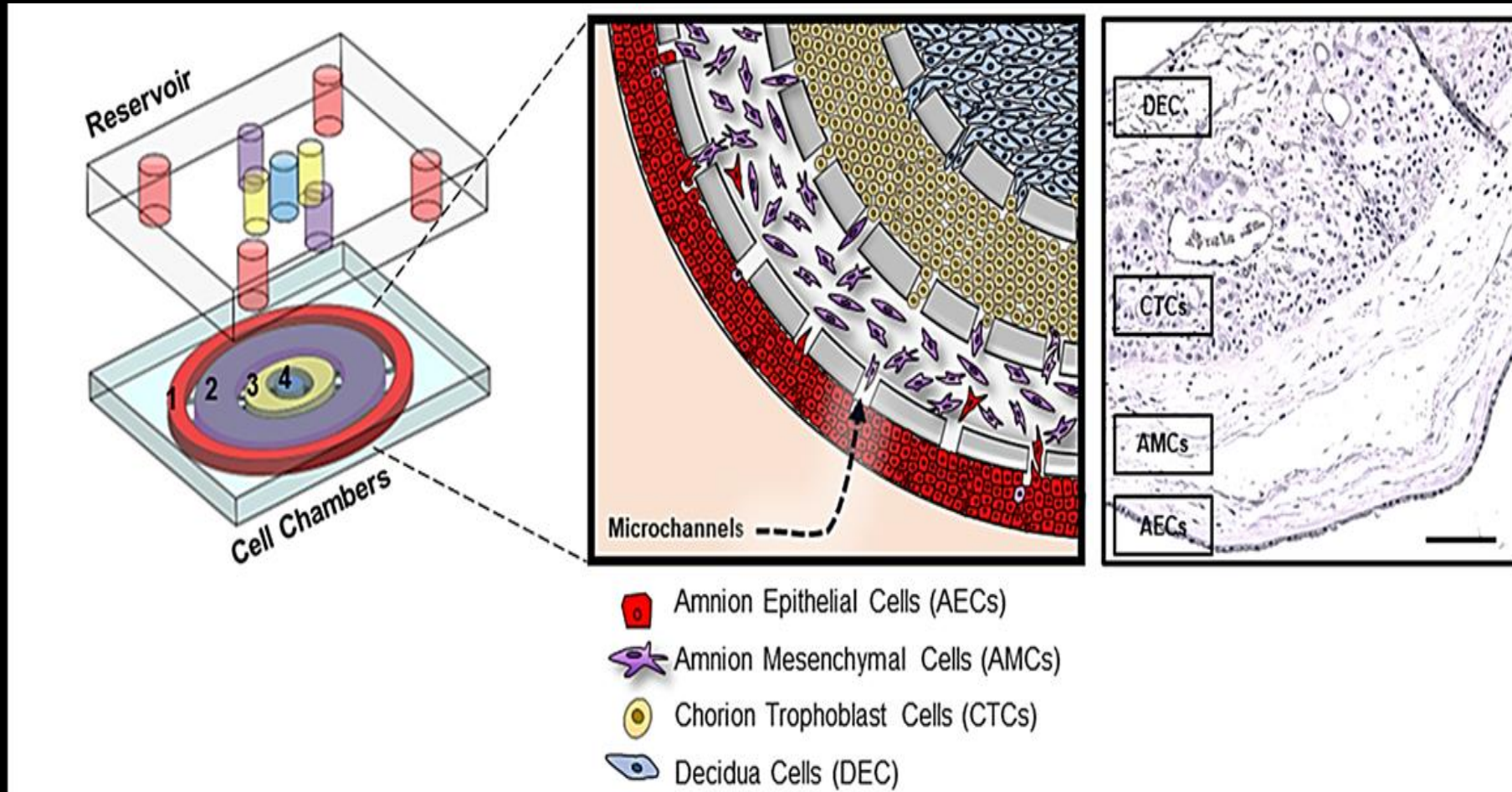
**Organ-on-chip of the cervical epithelial layer: A platform to study normal and pathological cellular remodeling of the cervix**

Ourlad Alzeus G. Tantengco<sup>1,2</sup> <sup>id</sup> | Lauren S. Richardson<sup>1,3</sup> <sup>id</sup> | Paul Mark B. Medina<sup>2</sup> <sup>id</sup> | Arum Han<sup>3</sup> <sup>id</sup> | Ramkumar Menon<sup>1</sup> <sup>id</sup>

Recreated cervical remodeling process in vitro

# Use of Microfluidic Devices to Test Propagation of Exosomal HMGB1

## Fetal Membrane Organ on a Chip (FMi-OOC)

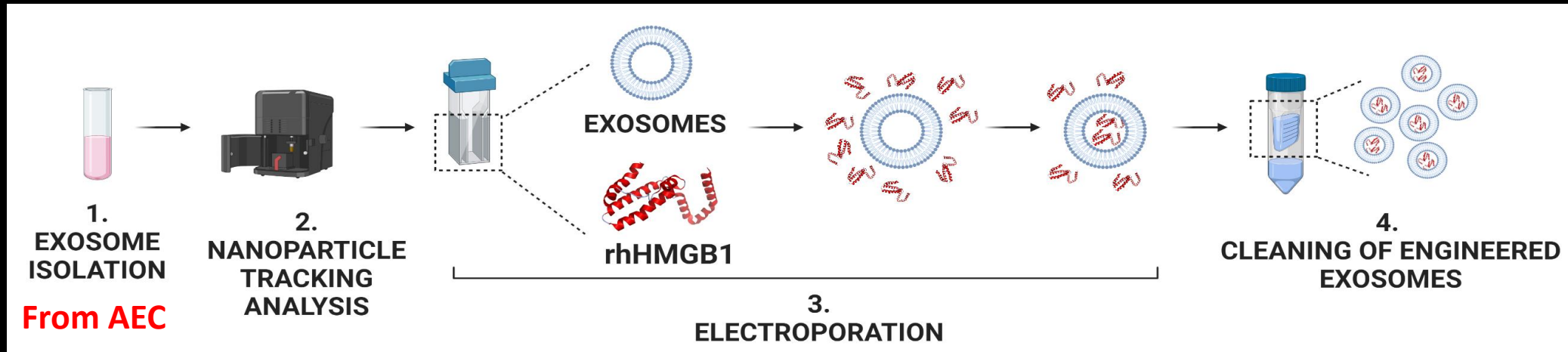




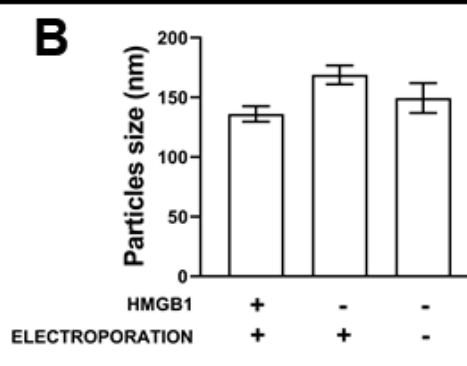
## Determining Functional Role of HMGB1 Encoded in Exosomes

- Engineering exosomes to contain HMGB1 – creating eHMGB1
  - Electroporation
- Validation of exosomal integrity after electroporation
- In vitro functional studies of eHMGB1 using Fetal Membrane-Decidua Organ on Chip (FMI- OOC)
- In vivo functional validation of eHMGB1 function
  - Mouse model
  - Determination of eHMGB1 propagation
  - Inflammation associated preterm birth

# Engineering of Exosomes to load HMGB1 and its Characterization

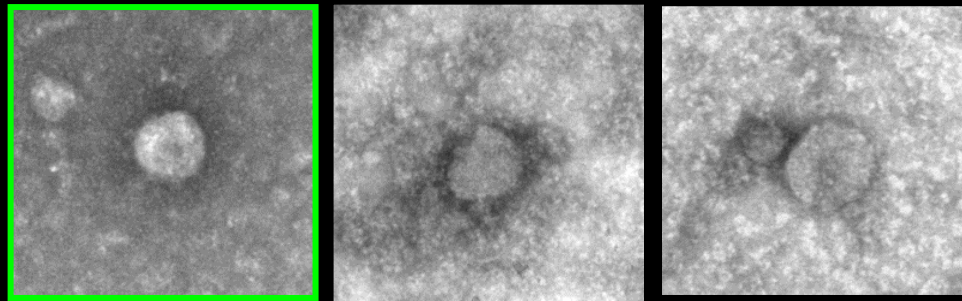


Particle size

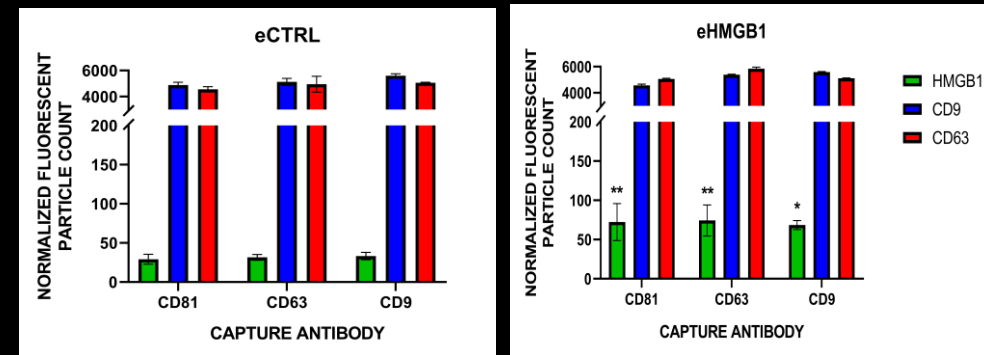


HMGB1	+	-	-
Electroporation	+	+	-

Morphology by TEM



Exosome markers

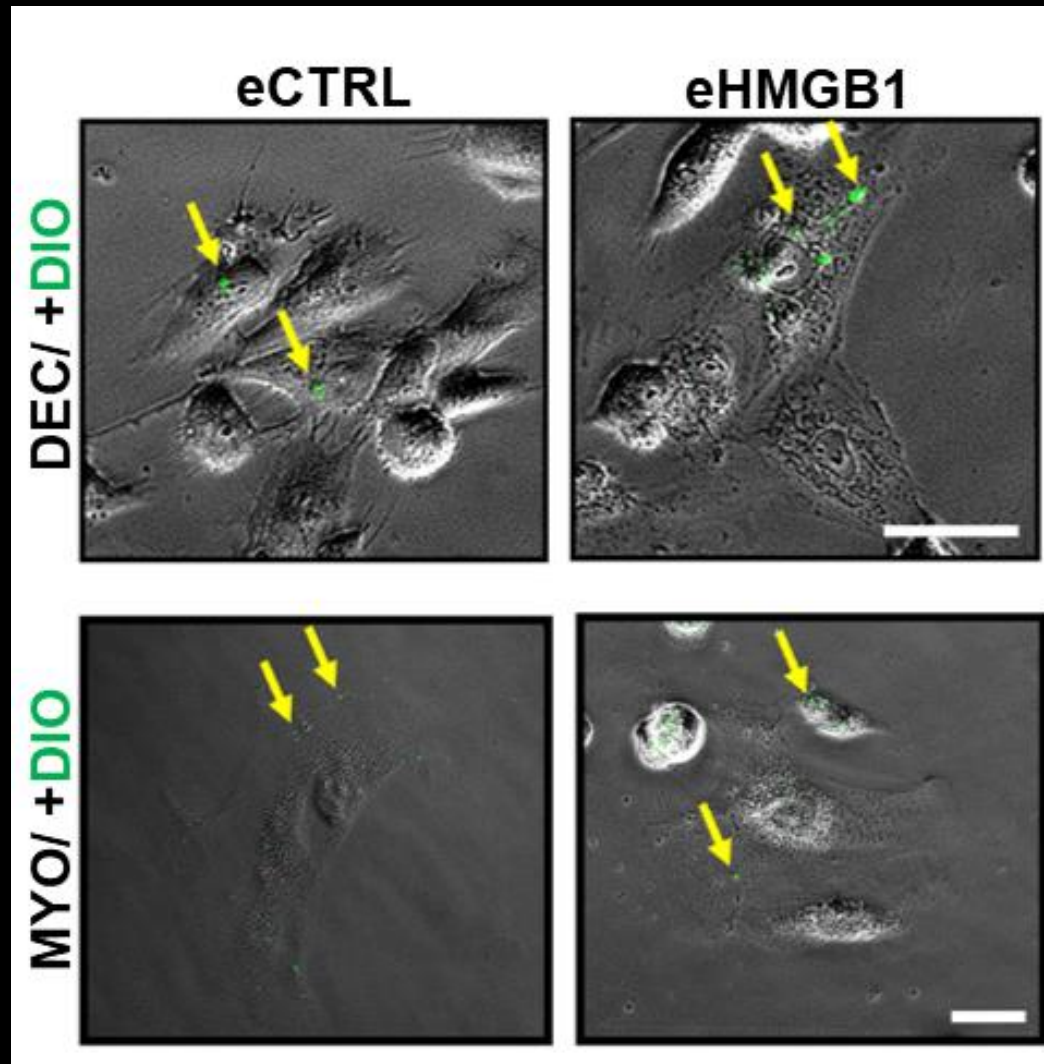


Exoview data

# eHMGB1 in Maternal Uterine Cells

- Exosomes containing 10ng HMGB1 was used for experiments

Endometrium/Decidua

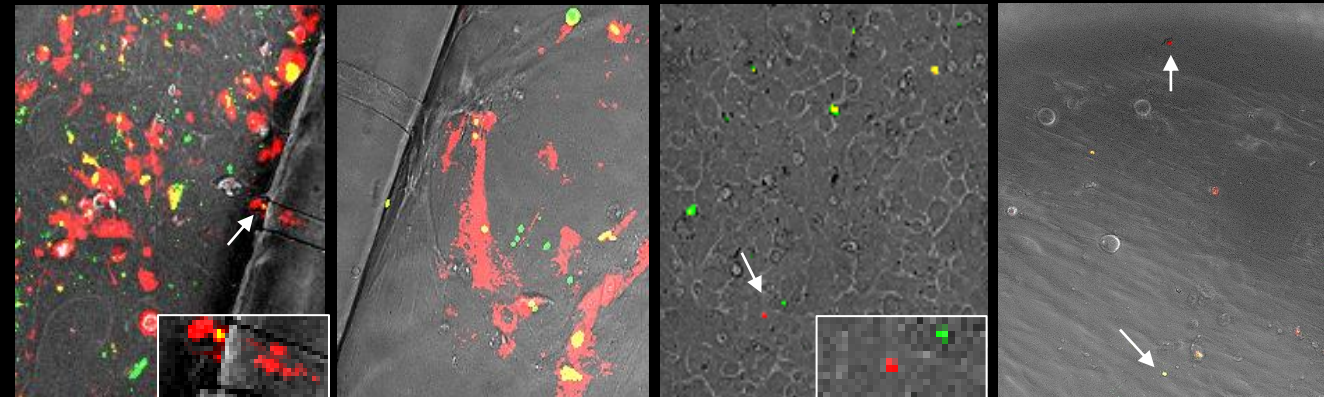
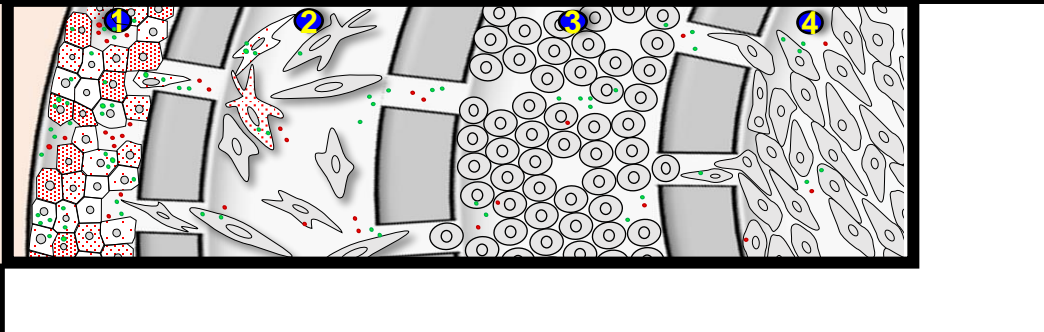
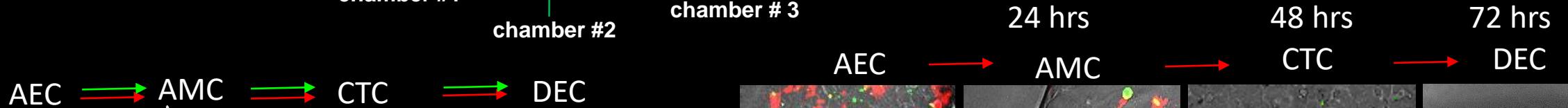
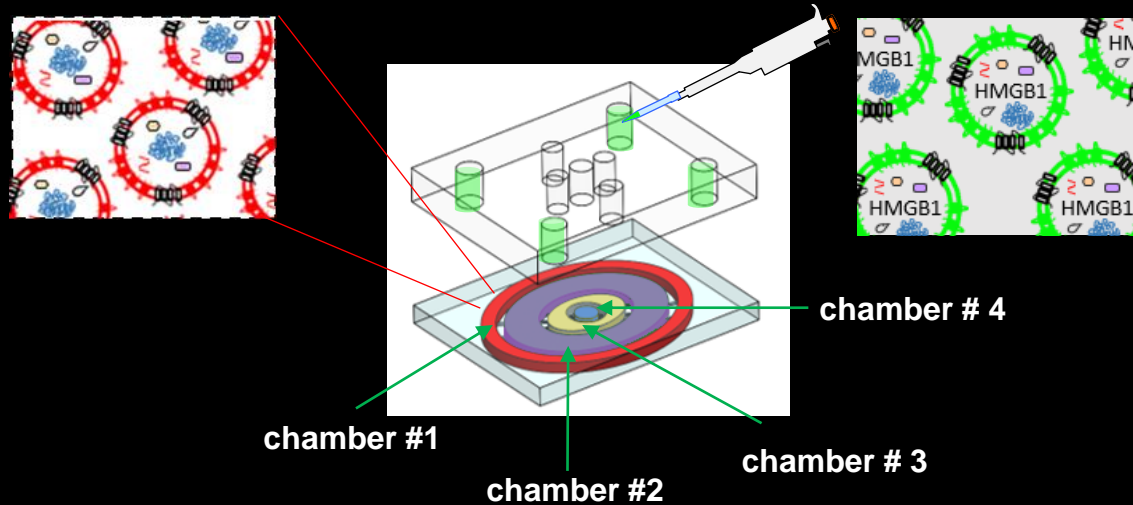


Myometrium

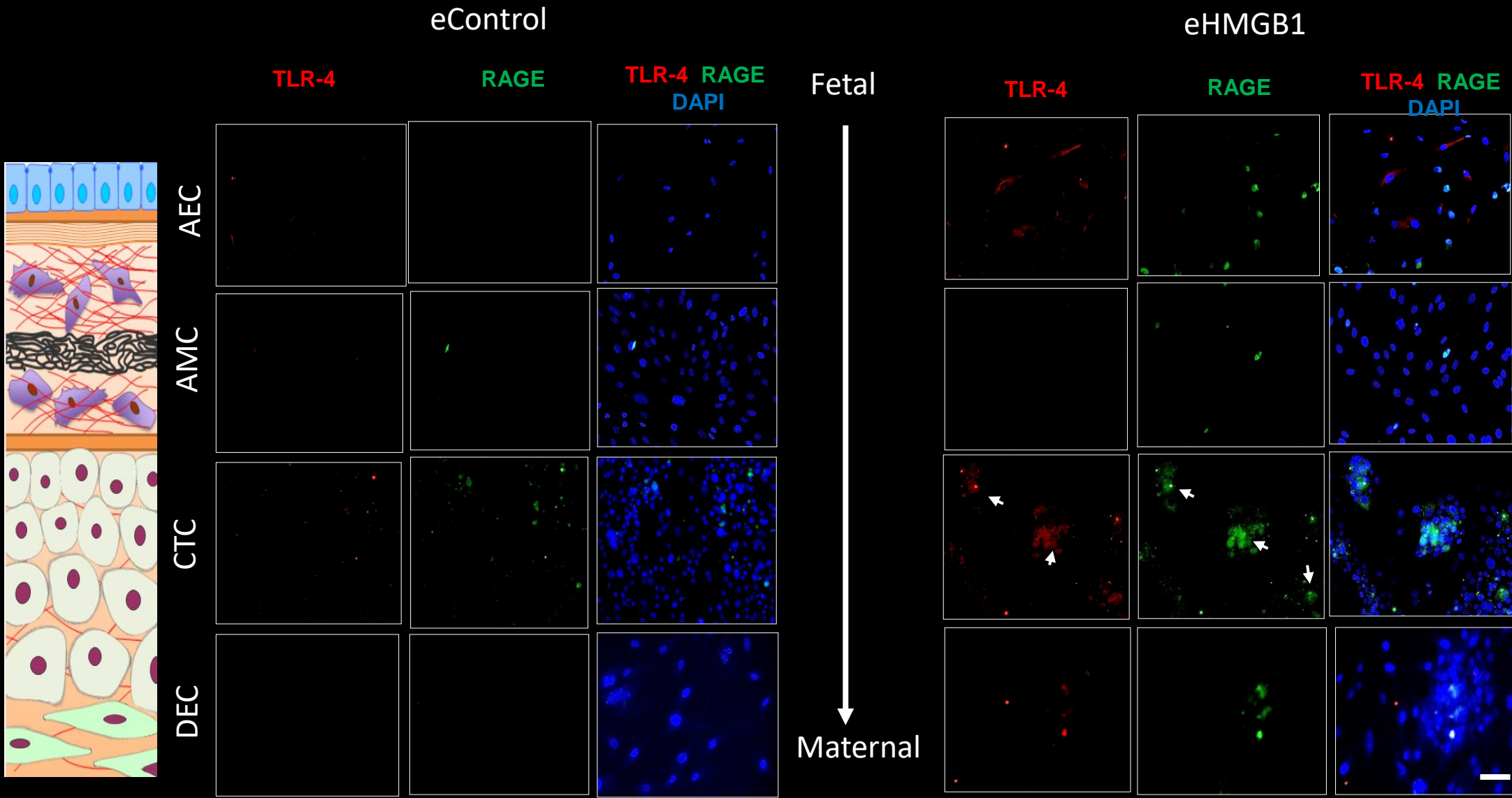
- Electroporation did not impact uptake of exosomes by recipient cells

# AEC-derived Exosomes Traffic Across the FMI-OOC

- Two types of exosomes derived from amnion epithelial cells (AECs) were tested
  1. Endogenous exosomes – Derived from **RFP Cells** → **red exosomes**
  2. Exogenous exosomes – Derived from AEC, electroporated to contain HMGB1 (eHMGB1) – **labelled with Green dye**



# eHMGB1 Trafficking Activates RAGE & TLR4 Expression Throughout the FMI-OOC

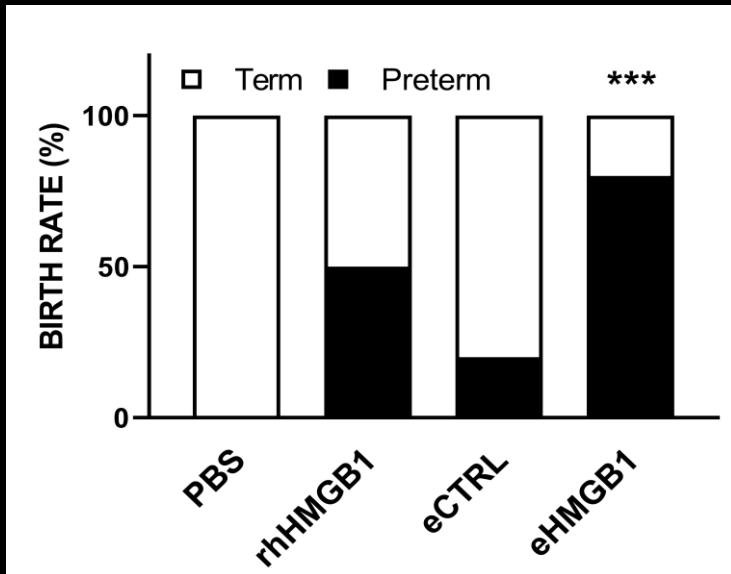
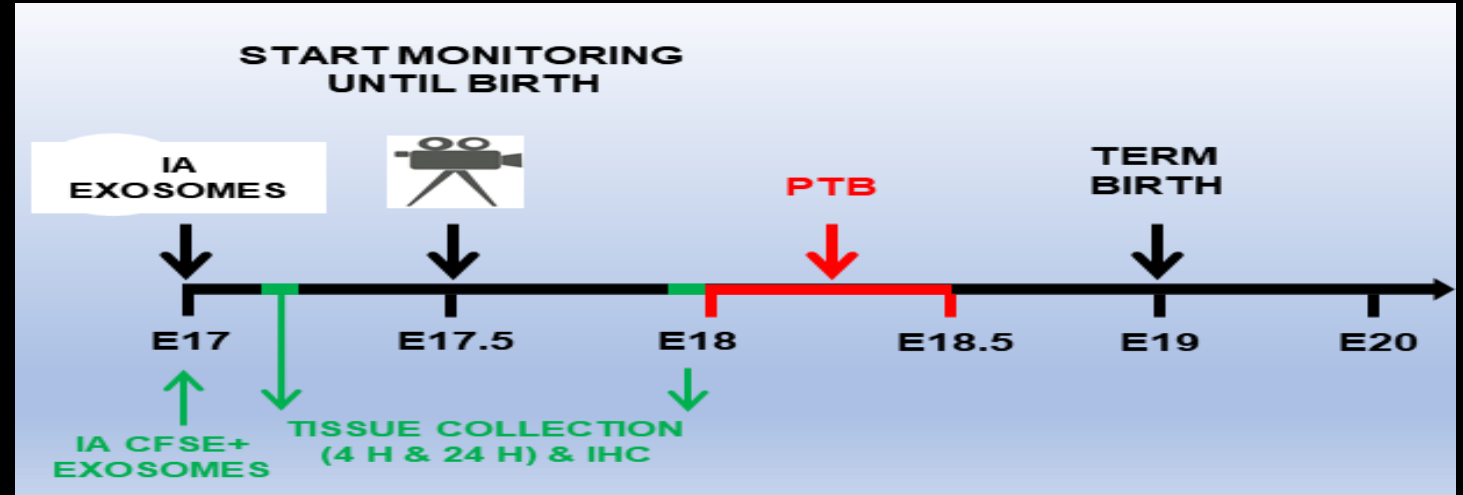
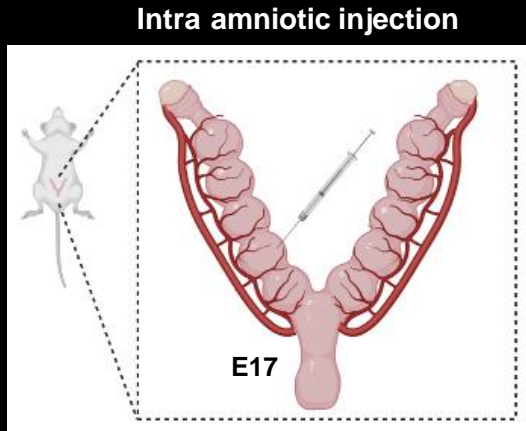


- eHMGB1 can traffic through fetal-maternal cells and increases its receptors
- eHMGB1 increases TNF- $\alpha$  in chorion (CTC) and decidua (DEC)

# eHMGB1 induces Preterm Birth

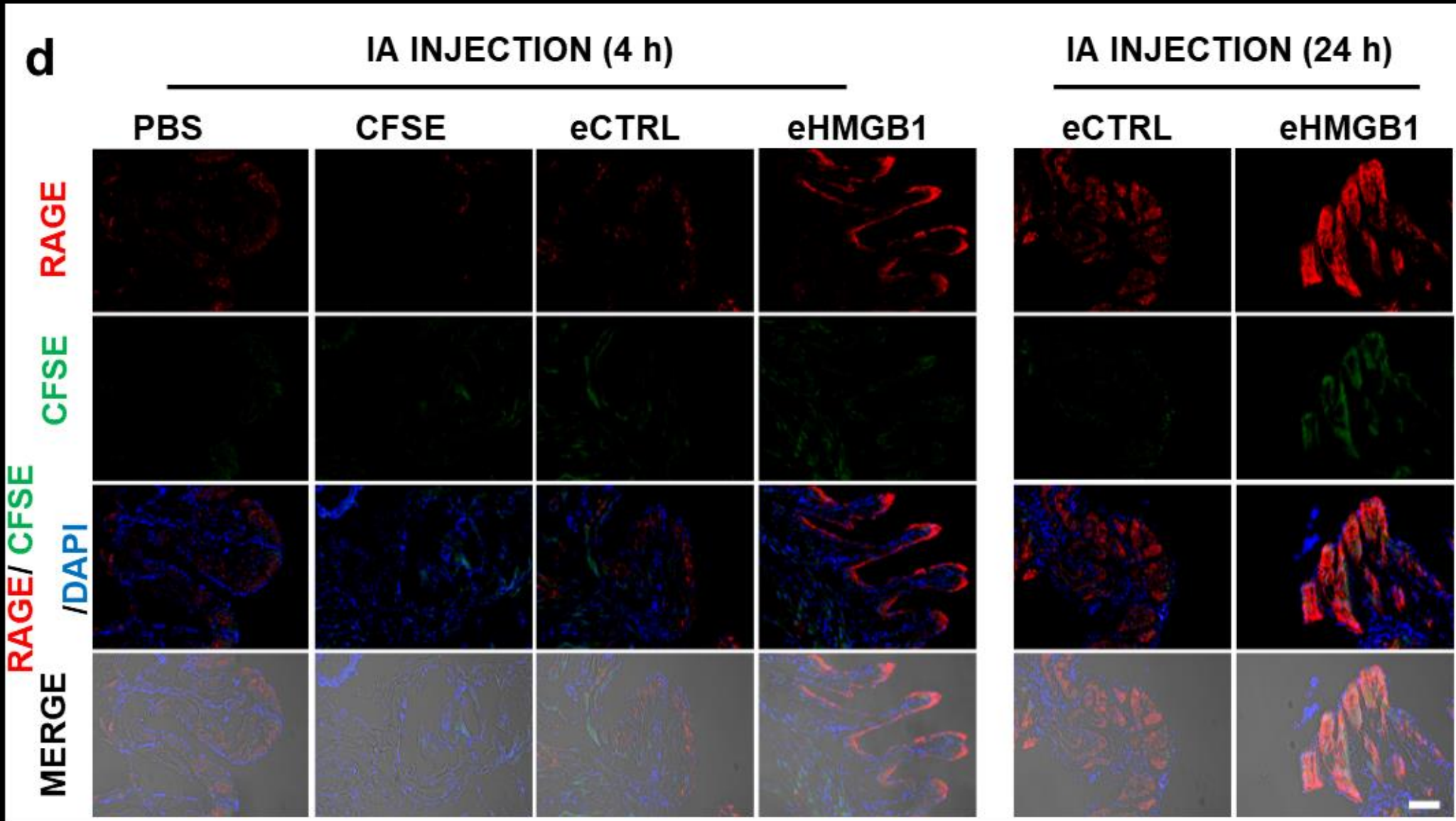
Physiologic validation of OOC data

10 ng HMGB1  
Amniotic sac



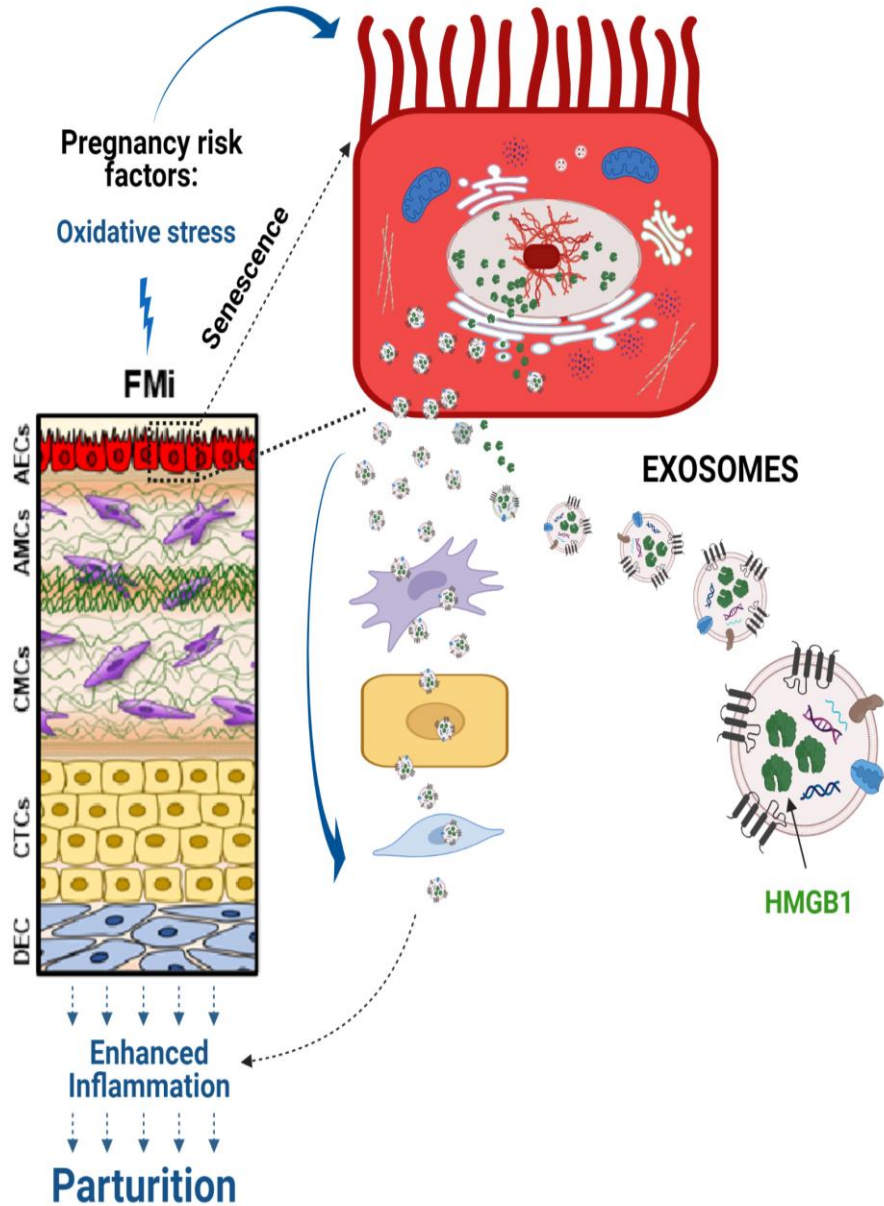
- Amniotic fluid cytokines are increased
- No change in maternal plasma cytokines
- Cervix and uterine cytokine levels are increased

# eHMGB1 from Amniotic Fluid to Uterus increases RAGE Expression

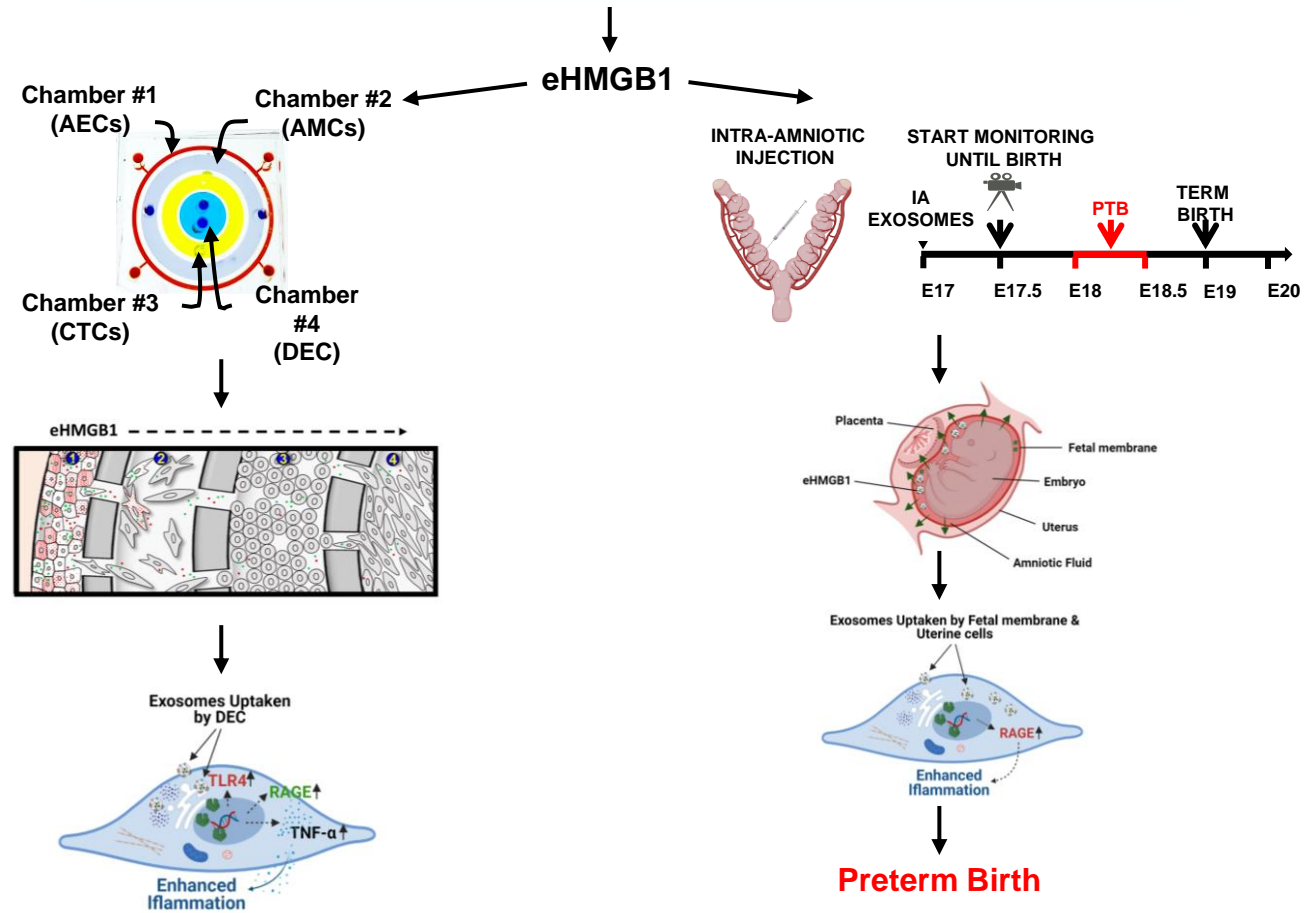
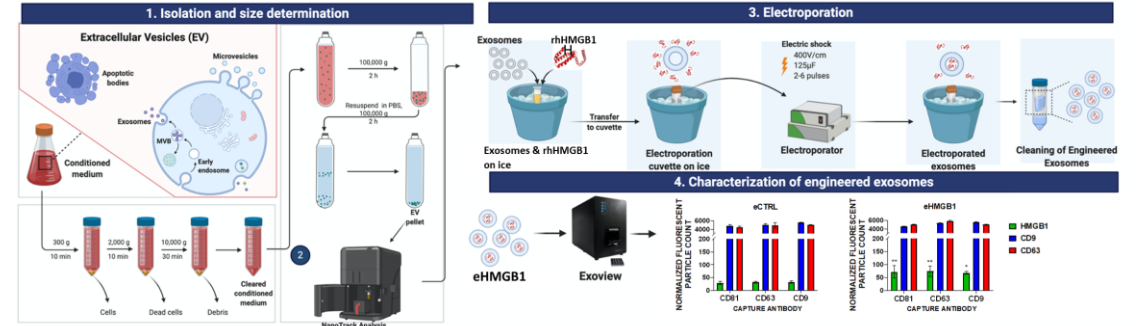


# Summary

## Theoretical concept



## Experimental approach and outcome





# Acknowledgements

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BILL &  
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GATES  
foundation



National Institute of  
Allergy and  
Infectious Diseases



March  
of Dimes  
*Saving babies, together*



amag  
PHARMACEUTICALS

HOLOGIC  
The Science of Sure



Health

Strategic funds

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Thank you!



**It's about saving babies!**