CELLS 2020

# Cell-to-Cell Metabolic Cross-Talk in Physiology and Pathology

17 DECEMBER 2020 – 17 JANUARY 2021 | ONLINE

## EXTRACELLULAR VESICLES (EVs) DERIVED BY HUMAN ENDOTHELIAL PROGENITOR CELLS (EPCs) PROTECT HUMAN RENAL GLOMERULAR ENDOTHELIAL CELLS AND PODOCYTES FROM TUMOR NECROSIS FACTOR-α INJURY

cells MDPI

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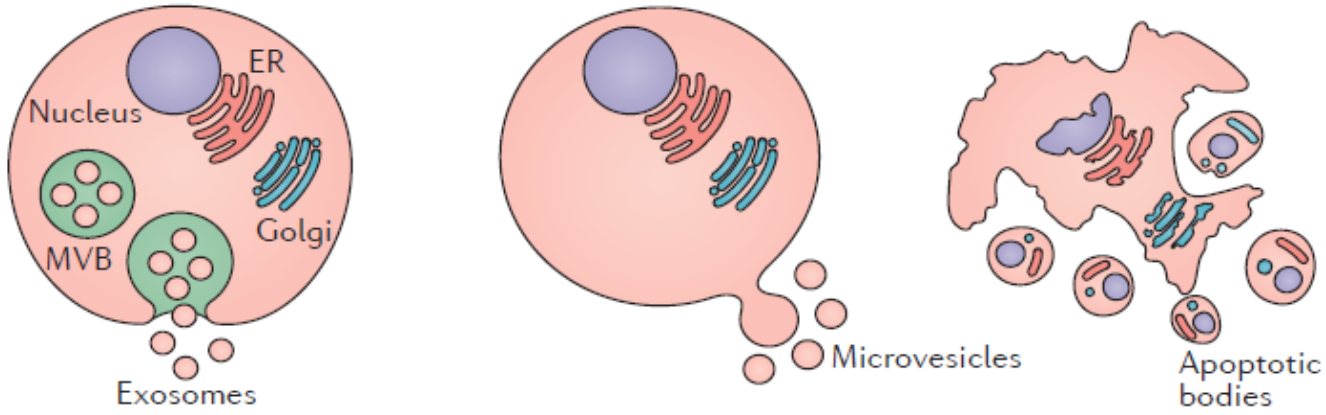
# Extracellular Vesicles (EVs)

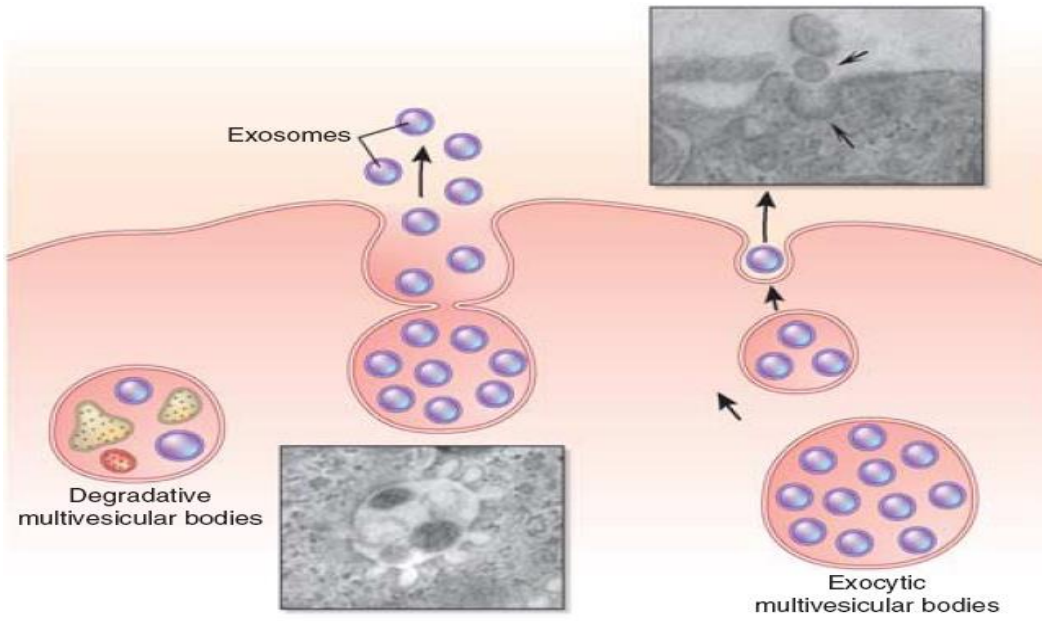
| | Exosomes | Microvesicles | Apoptotic bodies |
|---|---|---|---|
| **Formation** | Endosomal pathway, internal budding, exocytosis | Budding off the plasma membrane | Cell fragmentation/ blebbing |
| **Size** | 30–100 nm | 100–1,000 nm | 1–5 μm |
| **Content** | Proteins, lipids, mRNA, miRNA and cytosol | Proteins, lipids, mRNA, miRNA and cytosol | Proteins, lipids, DNA, rRNA, organelles and cytosol |




*Extracellular vesicles in renal disease*
*D Karpman, A Ståhl & I Arvidsson*
*Nature Reviews Nephrology (2017)*

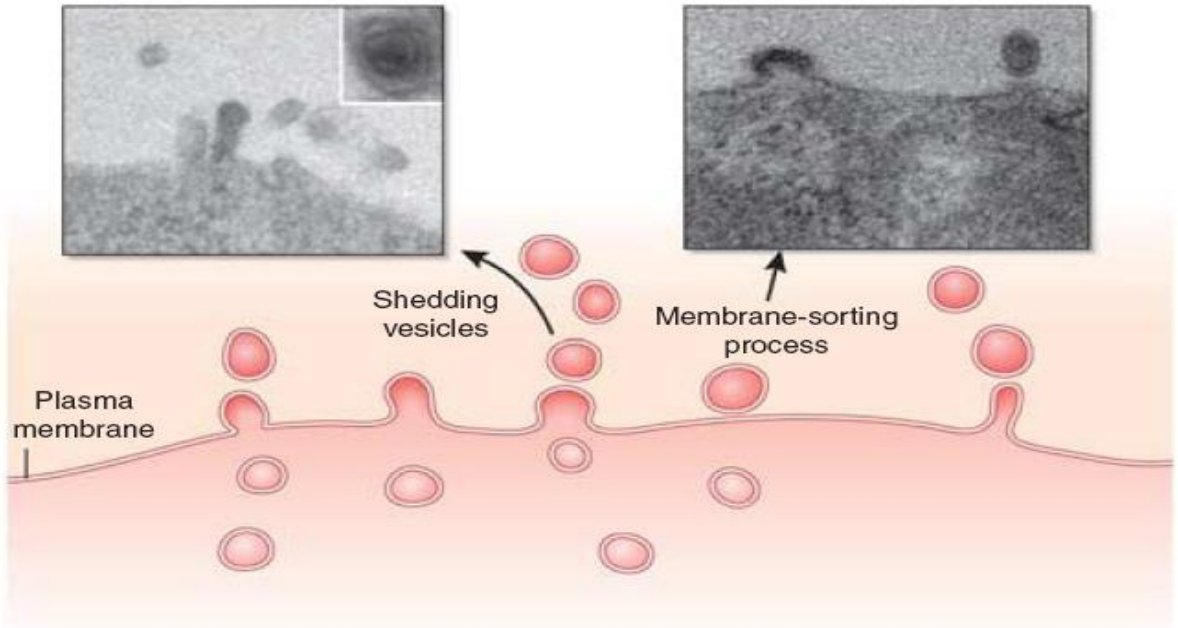
# Extracellular Vesicles (EVs)– Formation and Release

## Exosomes




Exosomes are released by exocytosis through a mechanism dependent on cytoskeleton activation and under the regulation of p53 protein.

## Microvesicles

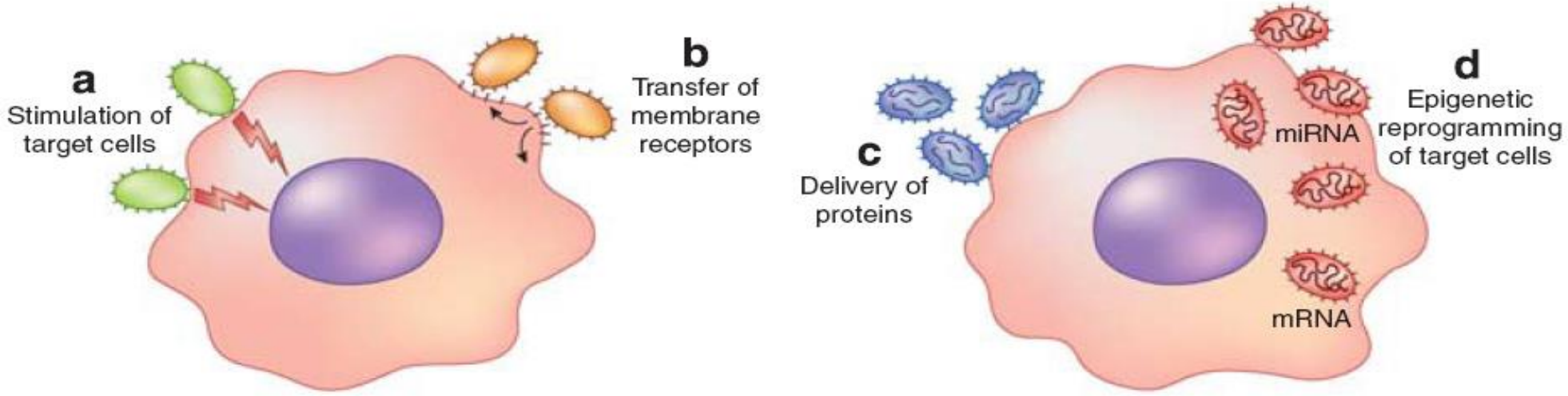



Microvesicles takes place from the budding of small cytoplasmic protrusions followed by their detachment from the cell surface dependent on calcium influx, calpain and cytoskeleton reorganization.

*Exosomes/microvesicles as a mechanism of cell-to-cell communication.*
*Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L. Kidney Int. 2010*

# Extracellular Vesicles (EVs)– Uptake and Biological Activity

EVs may mediate a cell-to cell horizontal transfer of biological material after uptake by Pinocytisis, Membrane Fusion, Endocytosis, or Receptor-mediated Endocytosis




*Exosomes/microvesicles as a mechanism of cell-to-cell communication.*
*Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L. Kidney Int. 2010*

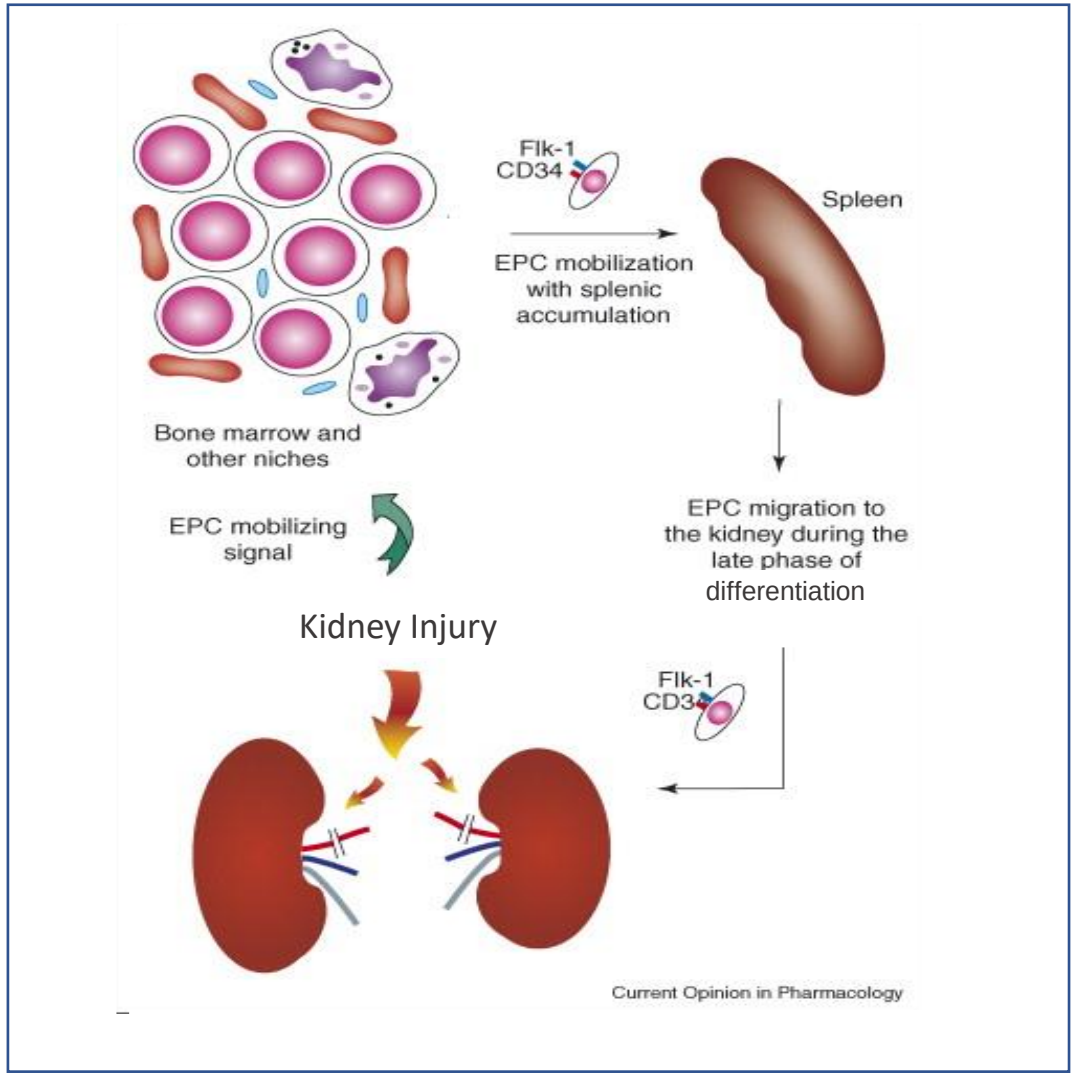
# Endothelial Progenitor Cells (EPCs)

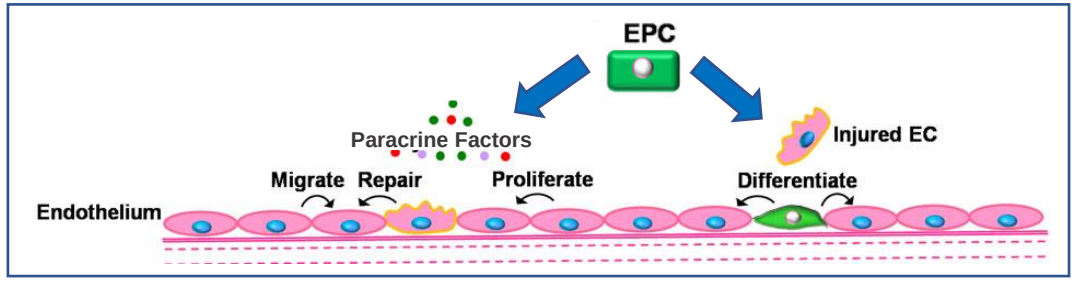
EPCs are adult stem cells derived from the bone marrow that circulate in the peripheral blood

EPCs play an important role in the regulation of vascular homeostasis and participate in the regeneration of injured endothelium and of different organs

EPC-induced endothelial cell repair is mainly ascribed to the release of paracrine factors:

-Growth Factors (VEGF, HGF, etc.)
-Extracellular vesicles (EVs)

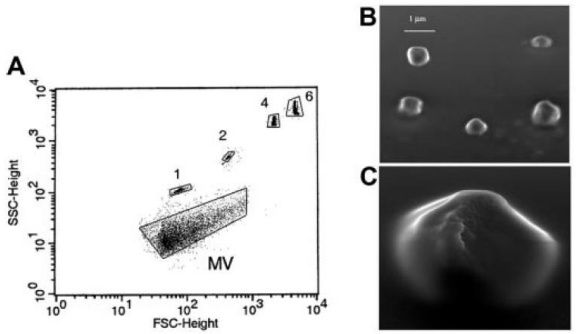


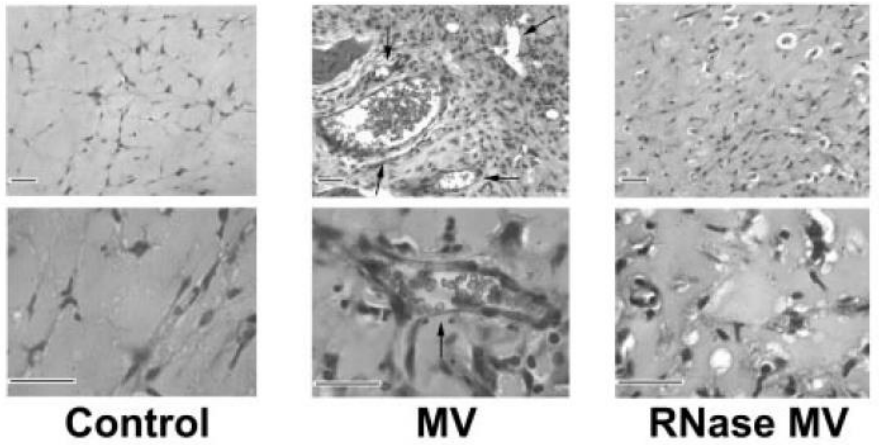



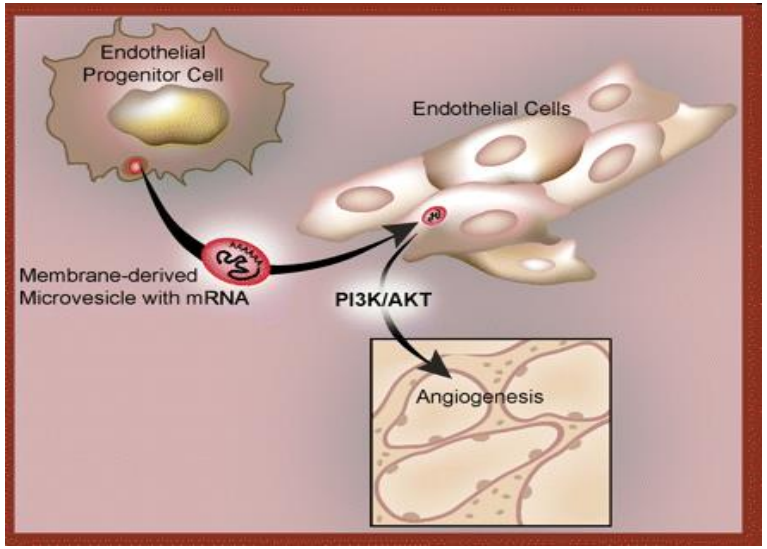
BLOOD, 1 OCTOBER 2007 • VOLUME 110, NUMBER 7

# Endothelial progenitor cell–derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA

Maria Chiara Deregibus,[1] Vincenzo Cantaluppi,[1] Raffaele Calogero,[2] Marco Lo Iacono,[2] Ciro Tetta,[3] Luigi Biancone,[1] Stefania Bruno,[1] Benedetta Bussolati,[1] and Giovanni Camussi[1]



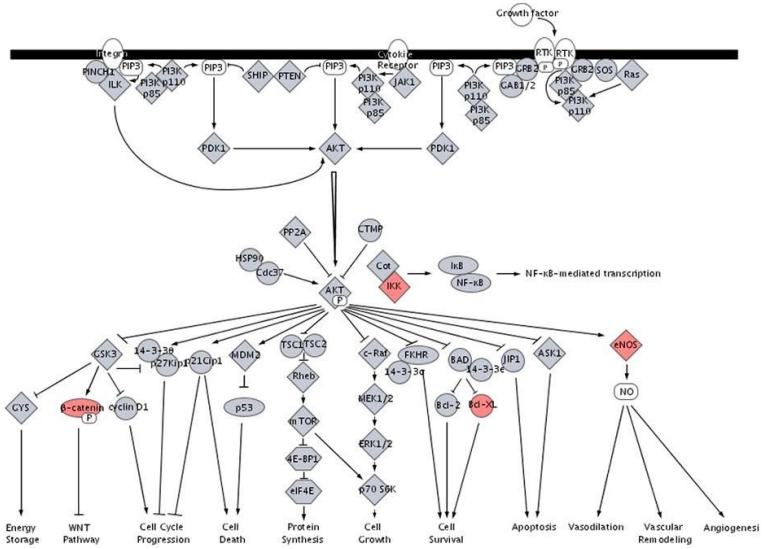




Bcl-XL

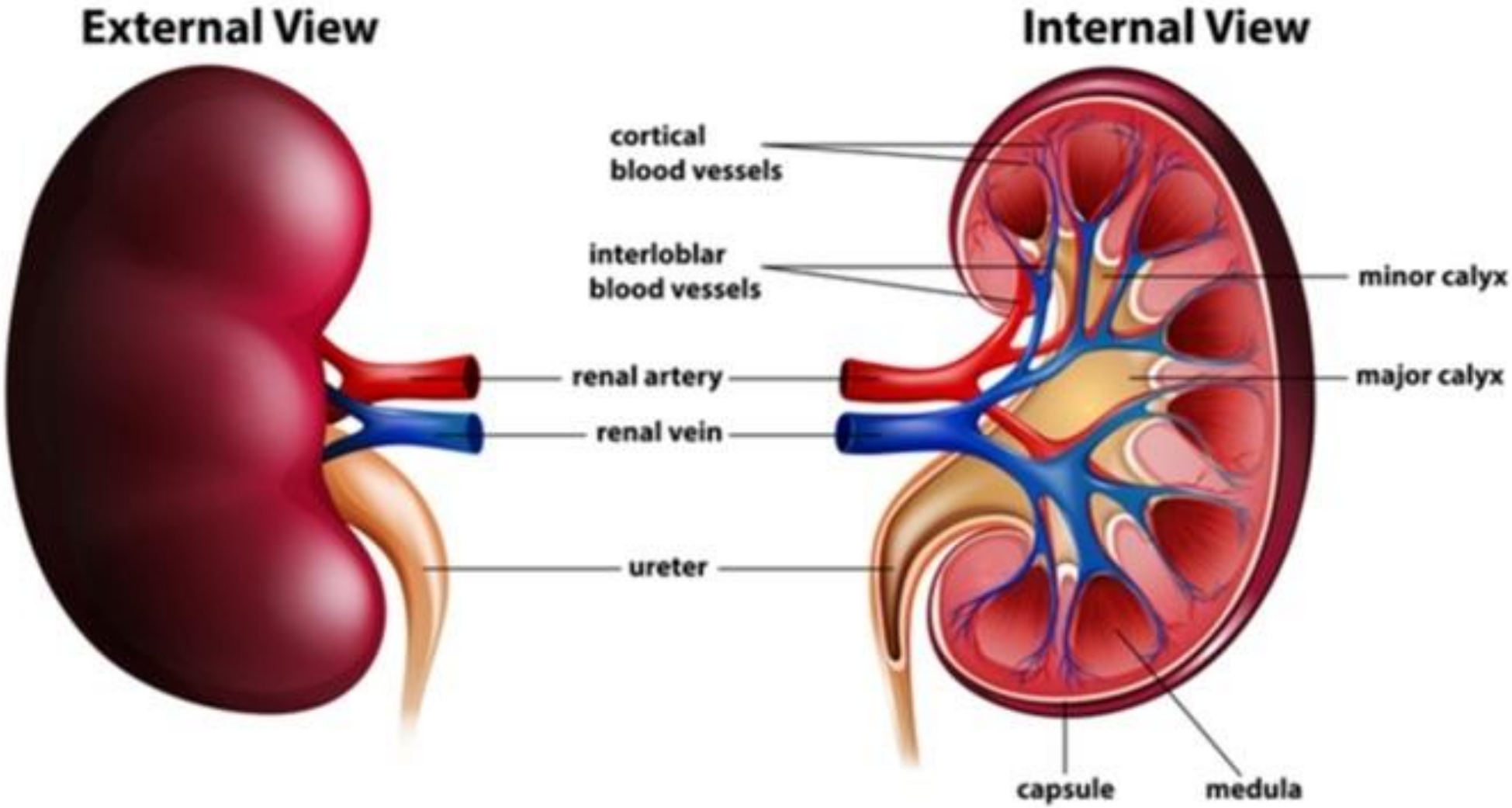
eNOS

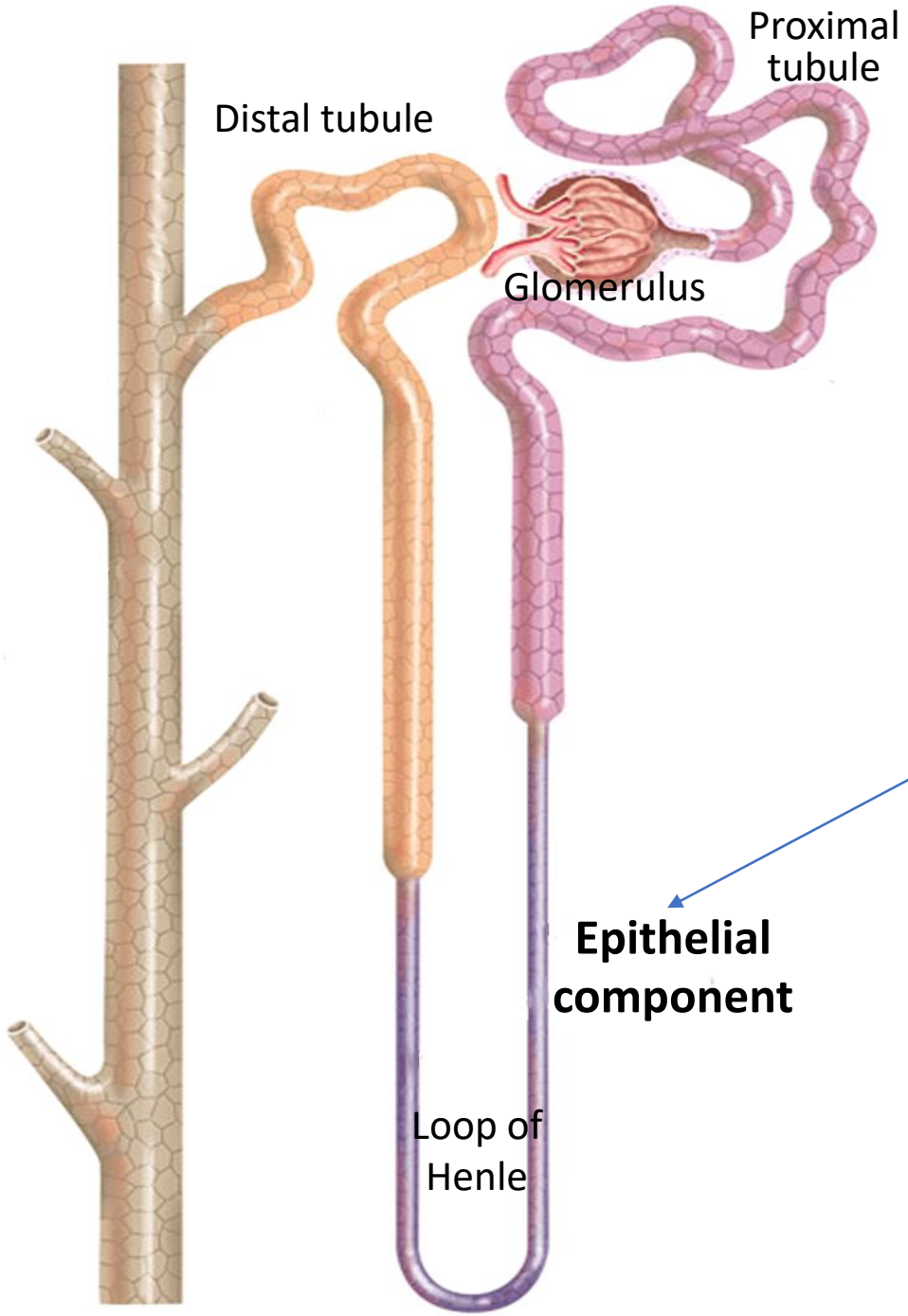
B-catenin

P-Akt/Akt



# Kidney

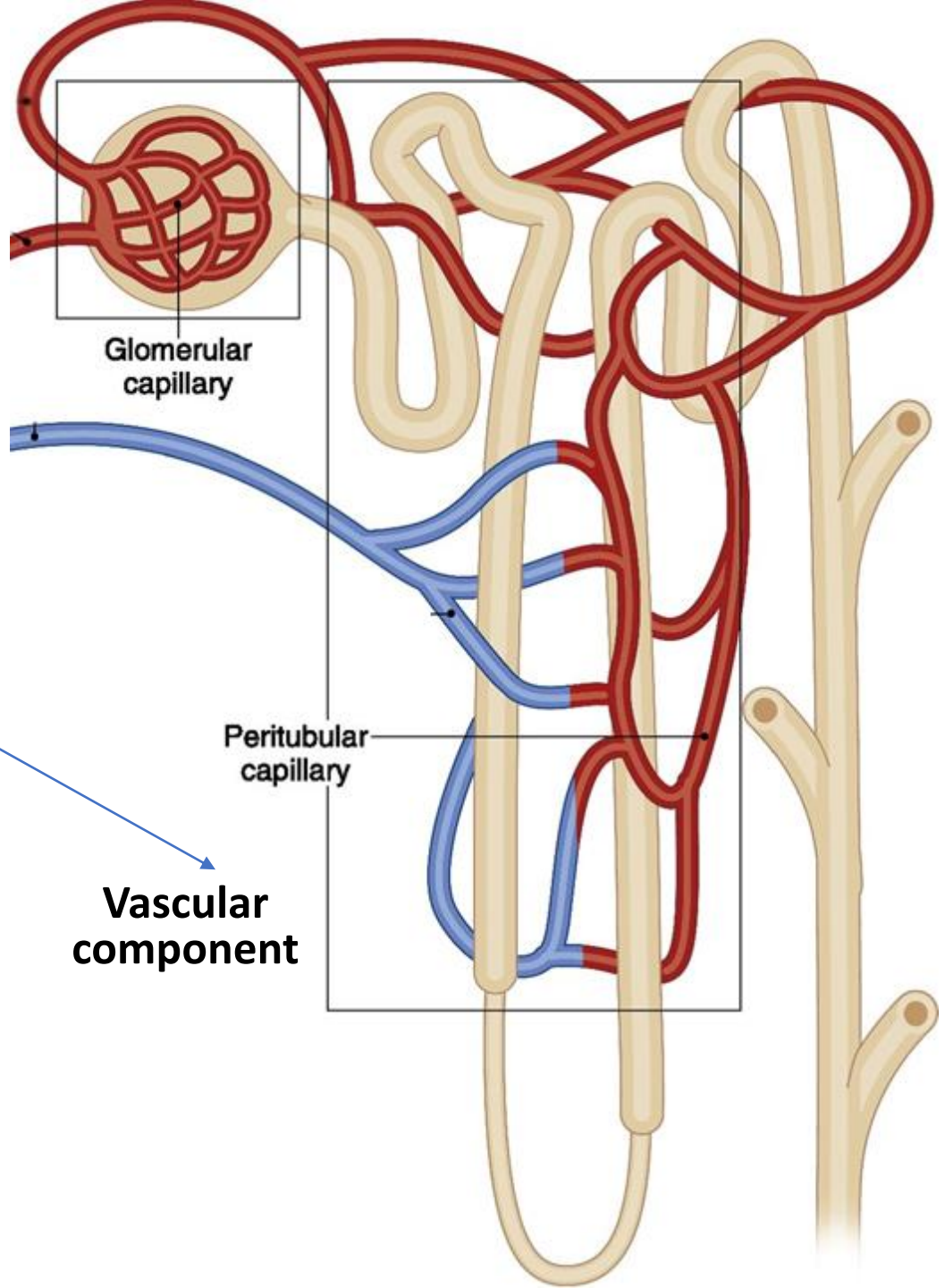

External View
Internal View
cortical blood vessels
interloblar blood vessels
minor calyx
major calyx
renal artery
renal vein
ureter
capsule
medula

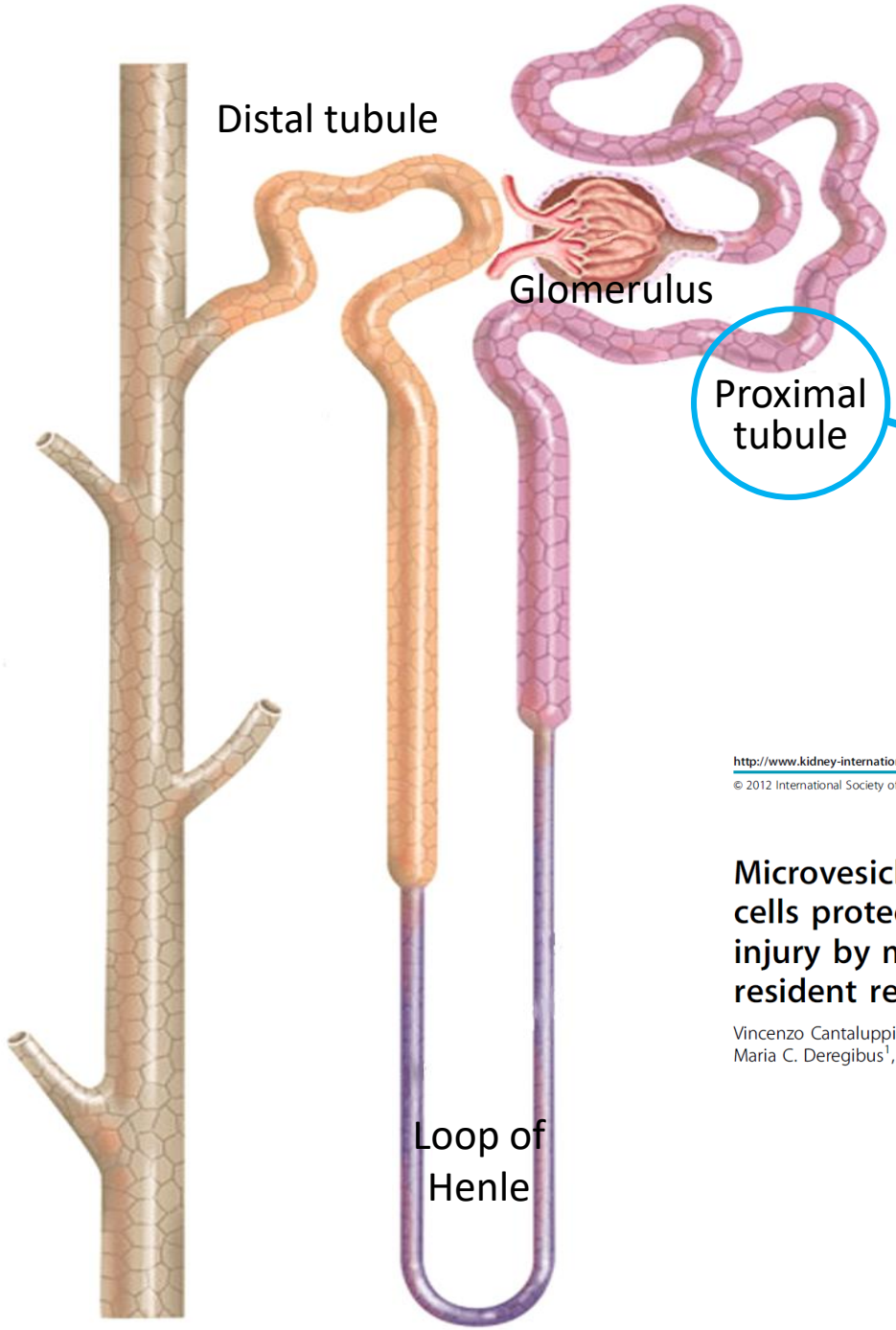



# Nephron

**Epithelial component**

**Vascular component**





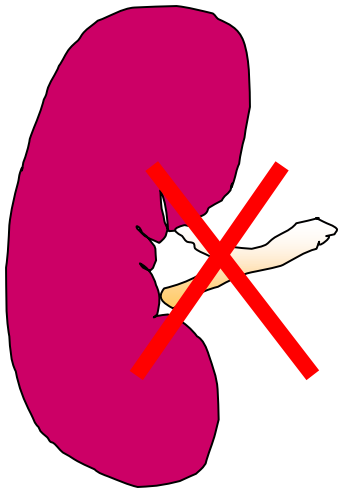

**Analysis of biological effect of EPC-derived EVs on Proximal Tubular Cells and Peritubular Endothelial Cells**

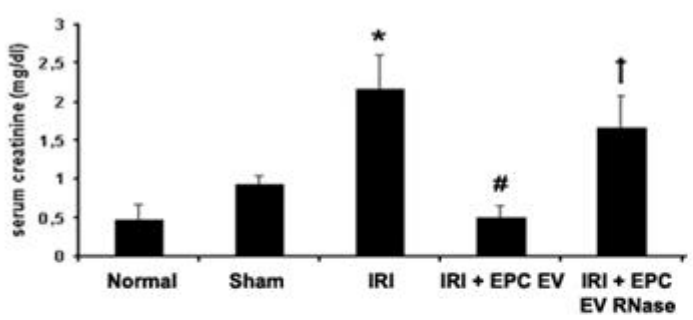
http://www.kidney-international.org original article

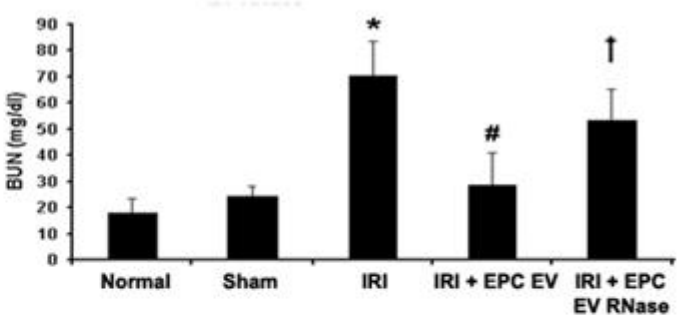

**Microvesicles derived from endothelial progenitor cells protect the kidney from ischemia–reperfusion injury by microRNA-dependent reprogramming of resident renal cells**

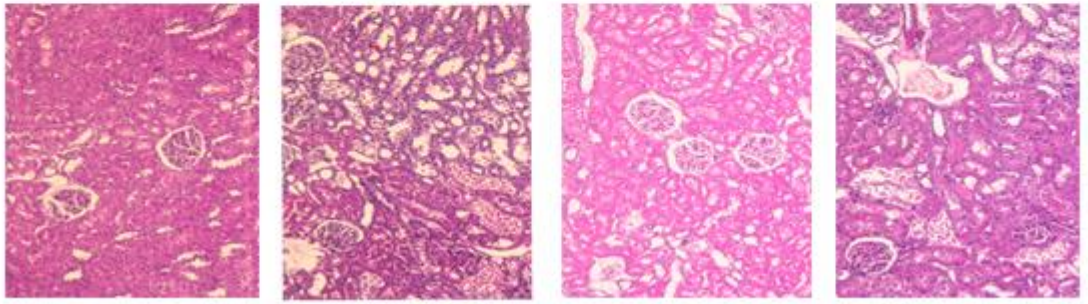
Vincenzo Cantaluppi[1], Stefano Gatti[2], Davide Medica[1], Federico Figliolini[1], Stefania Bruno[1], Maria C. Deregibus[1], Andrea Sordi[2], Luigi Biancone[1], Ciro Tetta[3,4] and Giovanni Camussi[1]

# EPC-derived EVs exerted functional and morphologic protection from renal Ischemia Reperfusion Injury



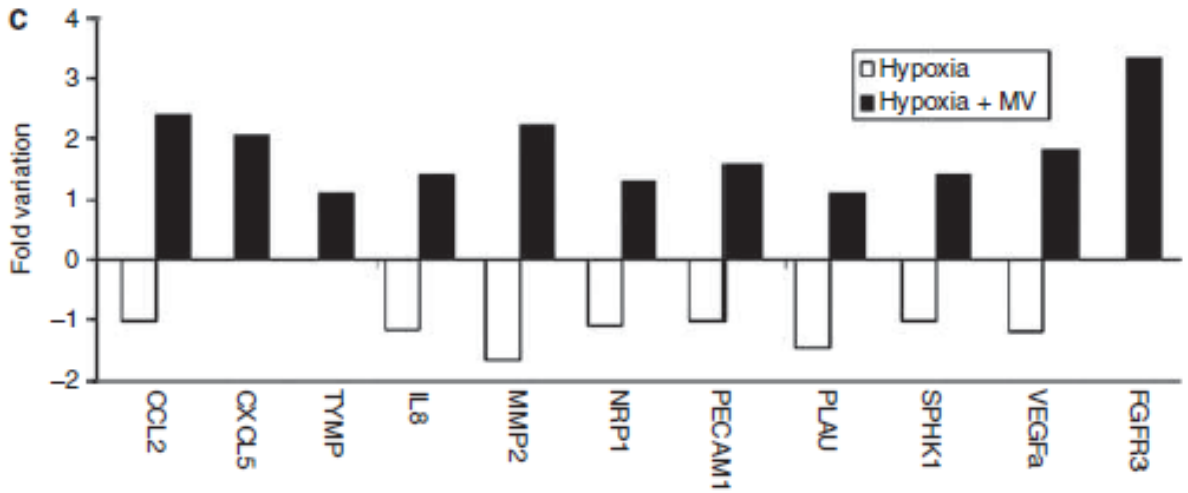


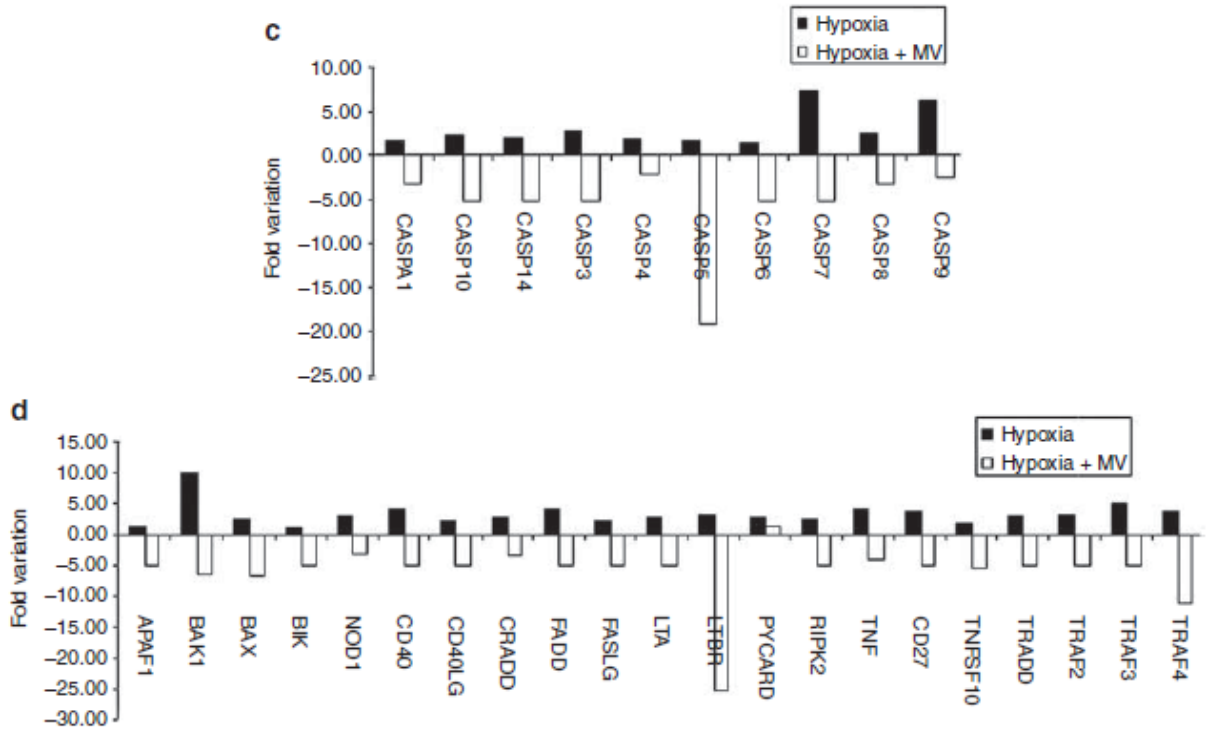




| | Normal | Sham | IRI | IRI + EPC EV | IRI + EPC EV Rnase |
|---|---|---|---|---|---|
| Casts (n/HPF) | 0 | 0 | 2.6 ± 1.2 | 0.48 ± 0.21 | 2.93 ± 0.84 |
| Tubular Apoptosis (n/HPF) | 1.6 ± 0.3 | 1.8 ± 0.6 | 17.6 ± 2.9 | 3.4 ± 1.3 | 14.8 ± 2.7 |
| Infiltrating Granulocytes | 2.2 ± 0.8 | 2.9 ± 1.2 | 29.6 ± 3.3 | 9.6 ± 2.2 | 23.8 ± 4.6 |
| Infiltrating Monocytes | 6.7 ± 1.1 | 6.5 ± 1.5 | 44.2 ± 0.6 | 12.3 ± 4.4 | 28.2 ± 3.8 |

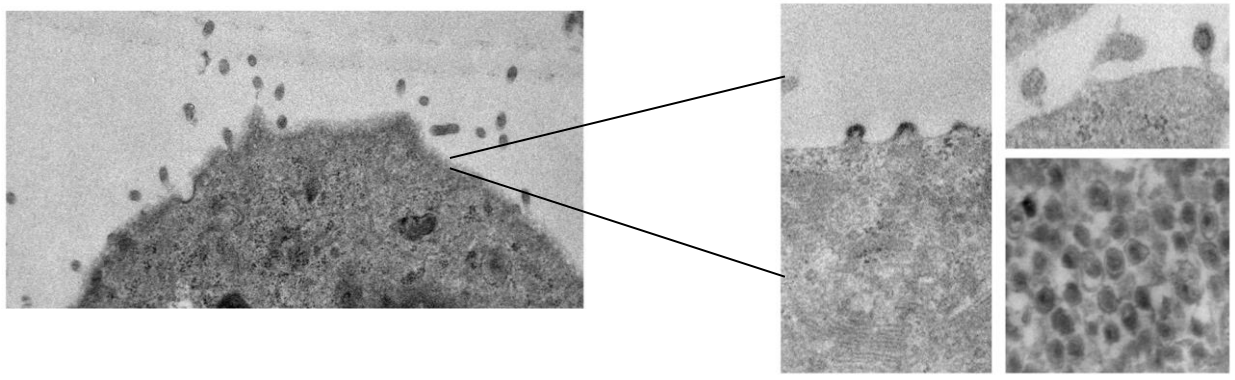
## EPC-derived EVs stimulate Angiogenesis on Peritubular Endothelial Cells in Hypoxia

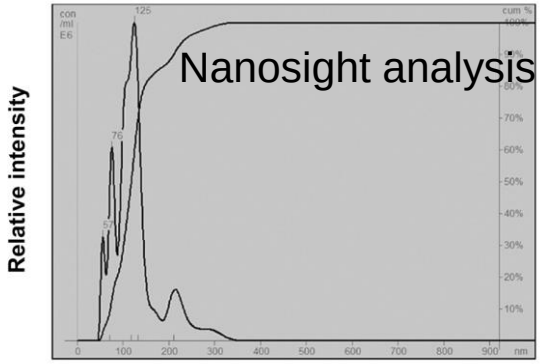



## EPC-derived EVs increase Resistance to Apoptosis on Tubular Epithelial Cells in Hypoxia



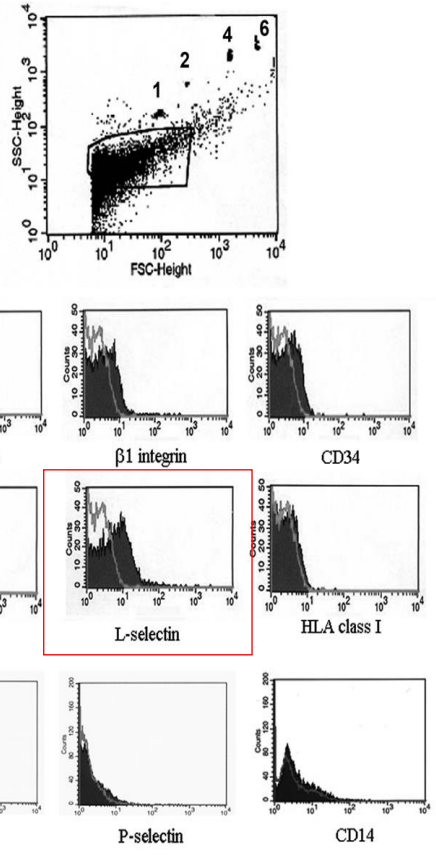
# Characterization of EPC-derived EVs

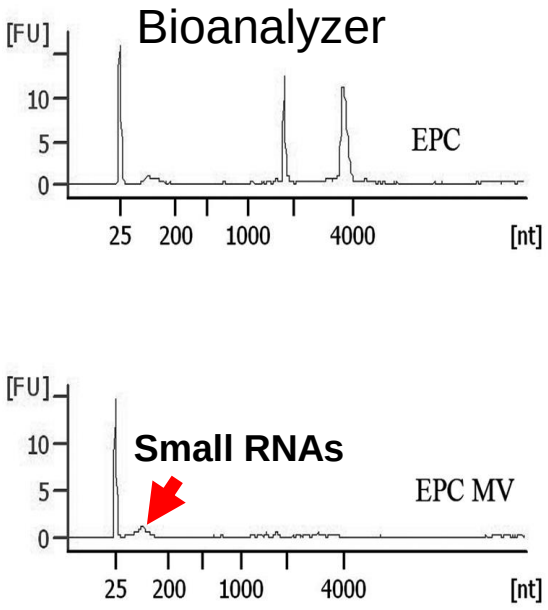





**Average size: 160 nm**

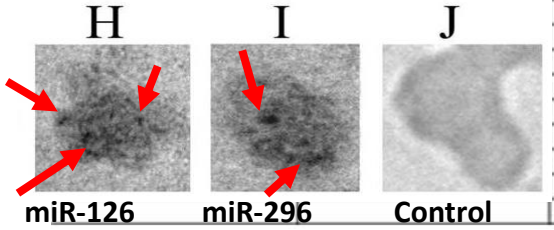
FACS

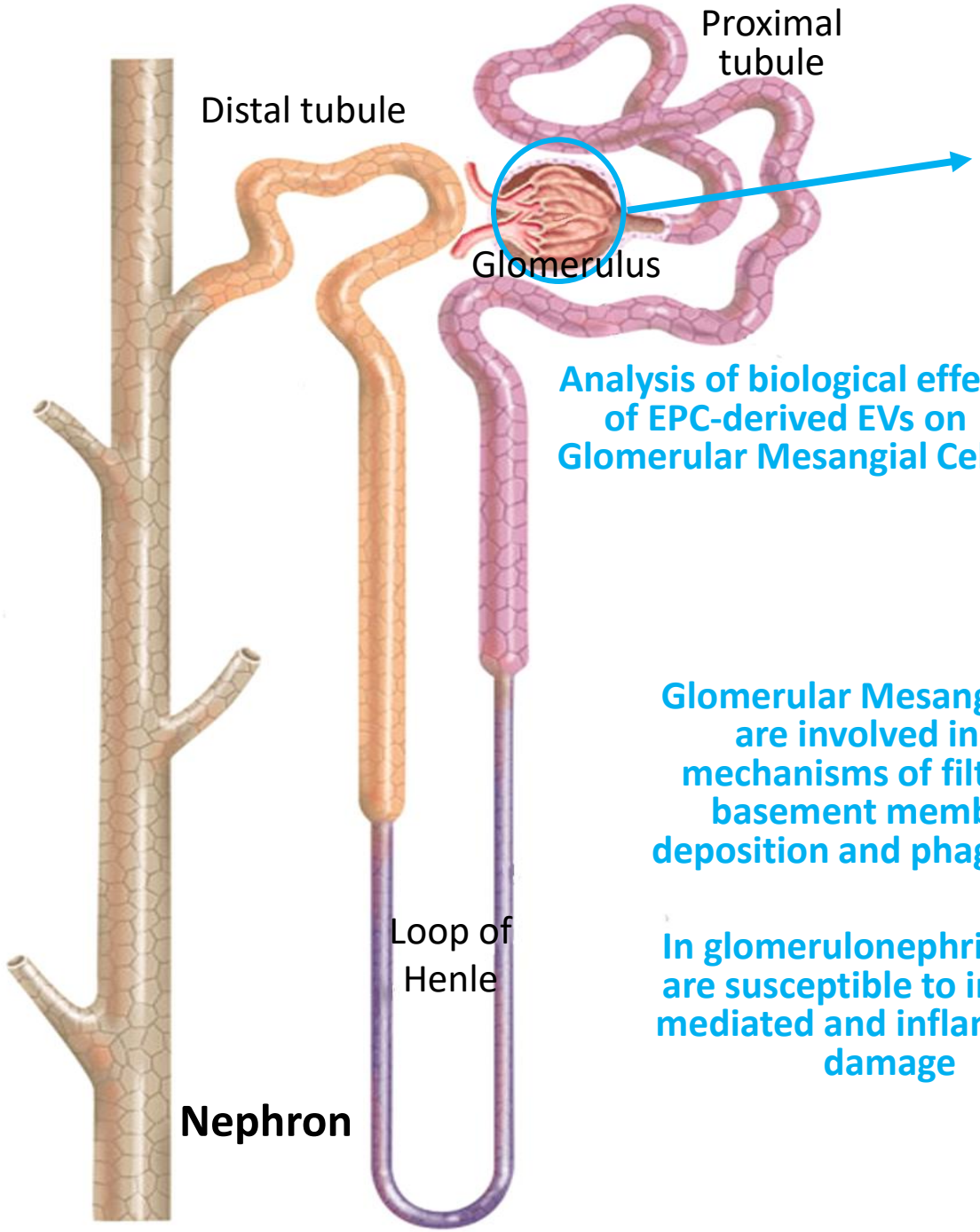



**160 microRNAs**

| ANGIOGENESIS | PROLIFERATION | APOPTOSIS INHIBITION | FIBROGENESIS INHIBITION |
|---|---|---|---|
| let-7a | miR-106b | let-7c | miR-192 |
| let-7b | miR-125a | miR-17-5p | miR-200a |
| let-7c | miR-125b | miR-103 | miR-200b |
| let-7d | miR-130a | miR-125a | let-7b |
| let-7e | miR-15a | miR-125b | let-7c |
| let-7f | miR-181b | miR-145 | let-7d |
| let-7g | miR-181c | miR-155 | let-7e |
| miR-126 | miR-181d | miR-181b | |
| miR-130 | miR-223 | miR-181c | |
| miR-19b | miR-23a | miR-181d | |
| miR-210 | miR-24 | miR-191 | |
| miR-296 | miR-26a | miR-21 | |
| miR-378 | miR-26b | miR-210 | |
| miR-92 | miR-27a | miR-23a | |
| | miR-31 | miR-23b | |
| | miR-484 | miR-24 | |
| | | miR-26a | |
| | | miR-26b | |
| | | miR-27a | |
| | | miR-27b | |
| | | miR-29a | |
| | | miR-30a | |
| | | miR-30b | |
| | | miR-30c | |
| | | miR-30d | |
| | | miR-30e | |
| | | miR-378 | |





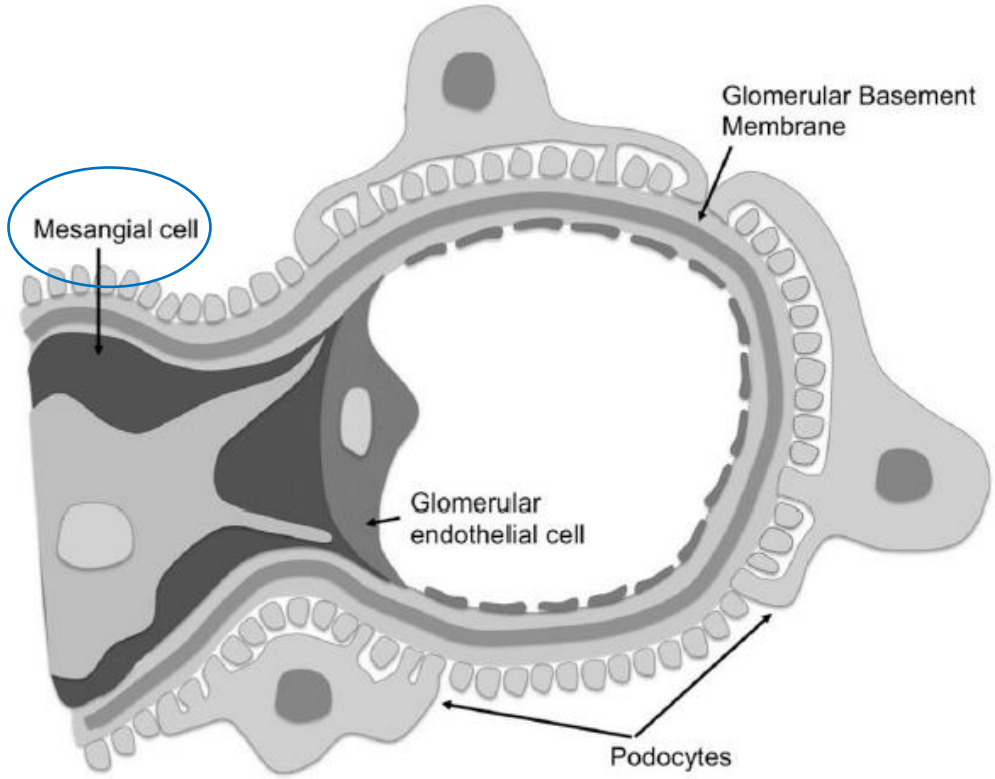

**Analysis of biological effect of EPC-derived EVs on Glomerular Mesangial Cells**

**Glomerular Mesangial Cells are involved in the mechanisms of filtration, basement membrane deposition and phagocytosis**

**In glomerulonephritis, they are susceptible to immune-mediated and inflammatory damage**


Endothelial progenitor cell-derived extracellular vesicles protect from complement-mediated mesangial injury in experimental anti-Thy1.1 glomerulonephritis

Vincenzo Cantaluppi[1], Davide Medica[1], Claudio Mannari[2], Giulia Stiaccini[2], Federico Figliolini[1], Sergio Dellepiane[1], Alessandro Domenico Quercia[1], Massimiliano Migliori[3], Vincenzo Panichi[3], Luca Giovannini[2], Stefania Bruno[1], Ciro Tetta[4], Luigi Biancone[1] and Giovanni Camussi[1]



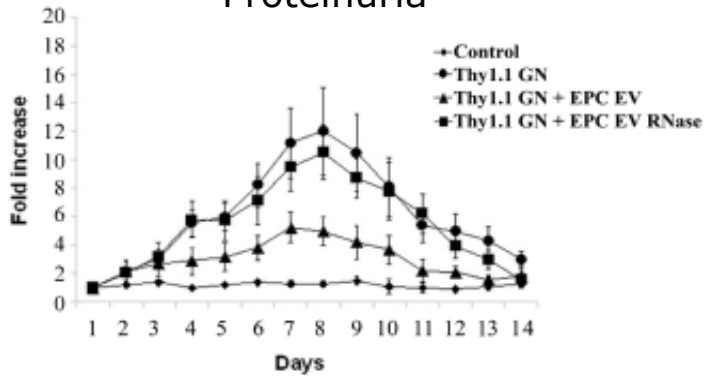

*Crosstalk in Glomerular Injury and Repair*
*Henrik Dimke, Yoshiro Maezawa, and Susan E. Quaggin 4*
*Curr Opin Nephrol Hypertens. 2015 May ; 24(3): 231–238.*

# EPC-derived EVs protect glomerular mesangium in experimental anti-Thy1.1 glomerulonephritis through inhibition complement cascade

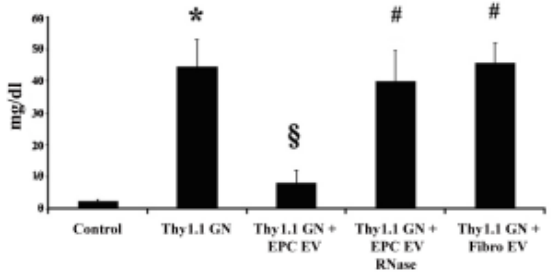
Endothelial progenitor cell-derived extracellular vesicles protect from complement-mediated mesangial injury in experimental anti-Thy1.1 glomerulonephritis

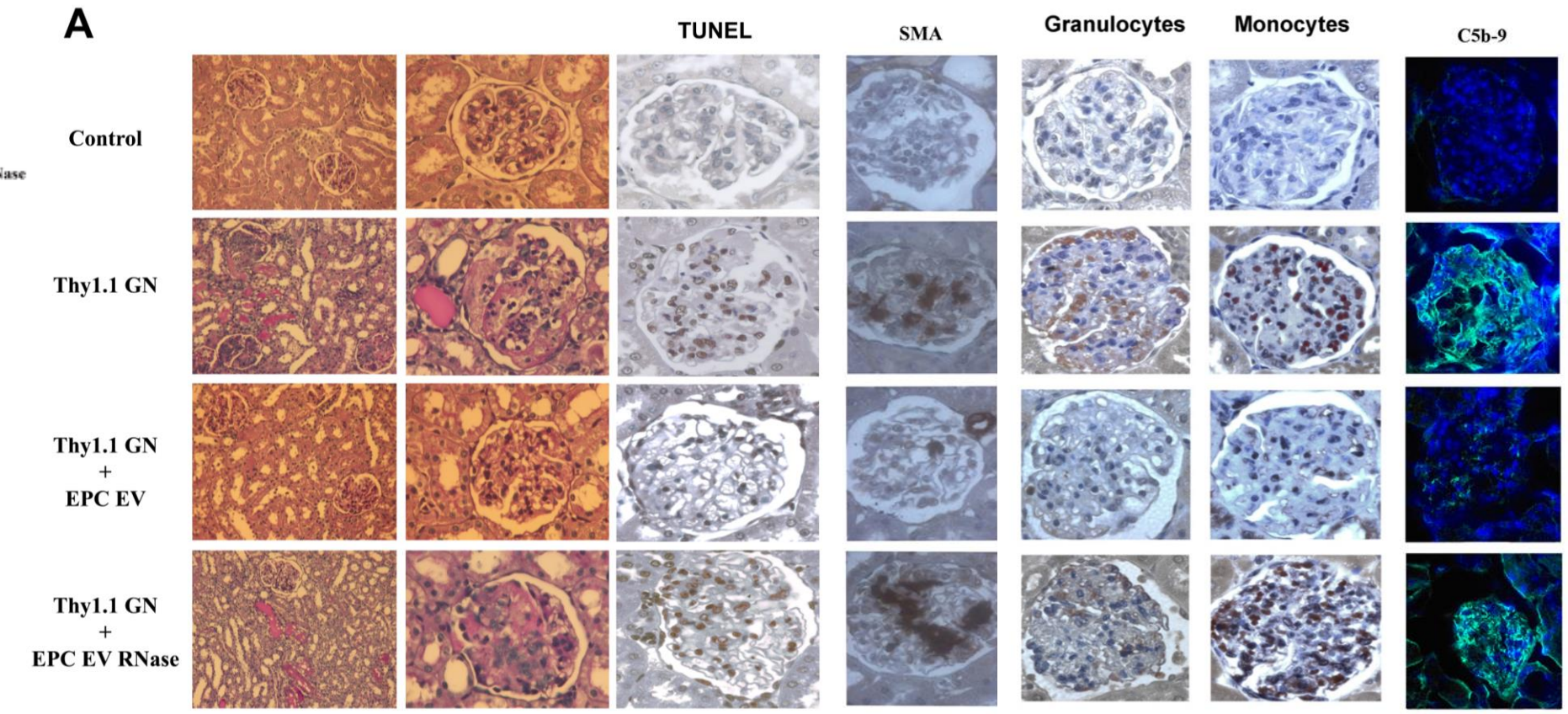
Vincenzo Cantaluppi[1], Davide Medica[1], Claudio Mannari[2], Giulia Stiaccini[2], Federico Figliolini[1], Sergio Dellepiane[1], Alessandro Domenico Quercia[1], Massimiliano Migliori[3], Vincenzo Panichi[3], Luca Giovannini[2], Stefania Bruno[1], Ciro Tetta[4], Luigi Biancone[1] and Giovanni Camussi[1]


Proteinuria

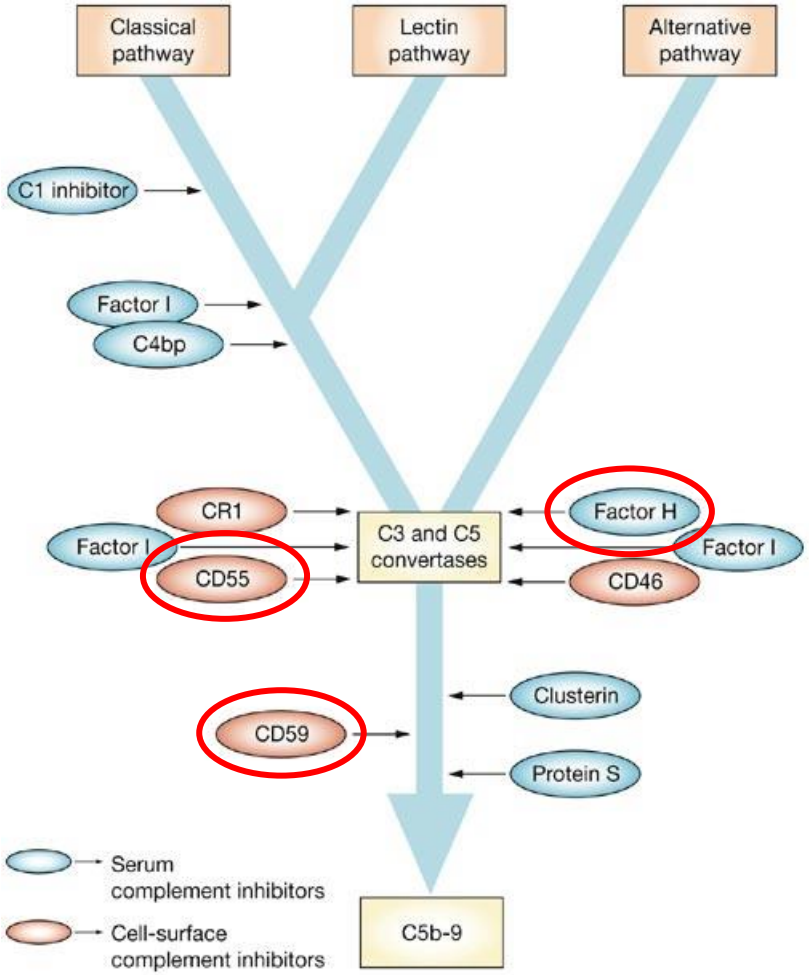


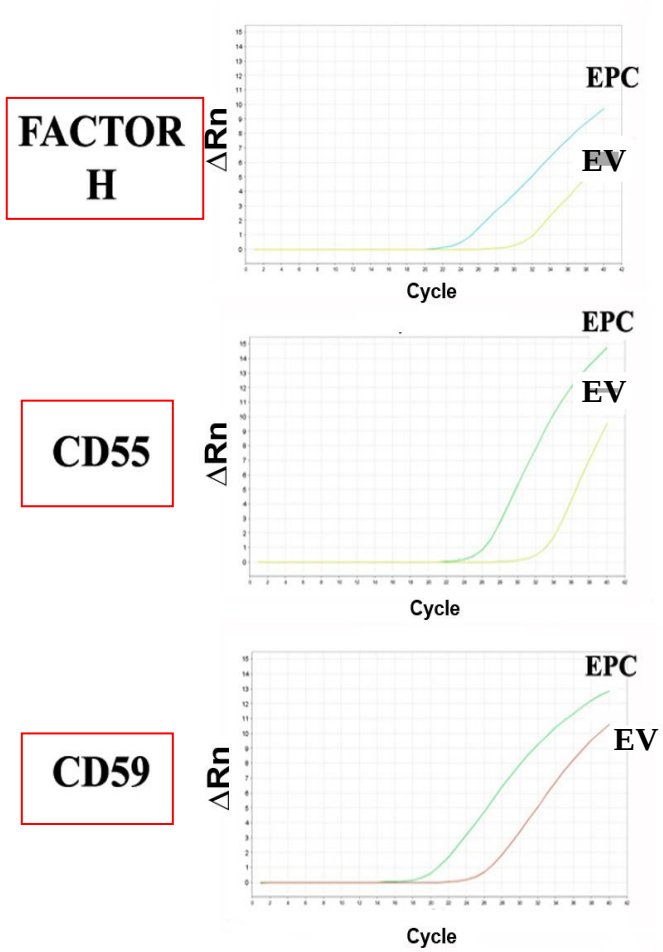

BUN

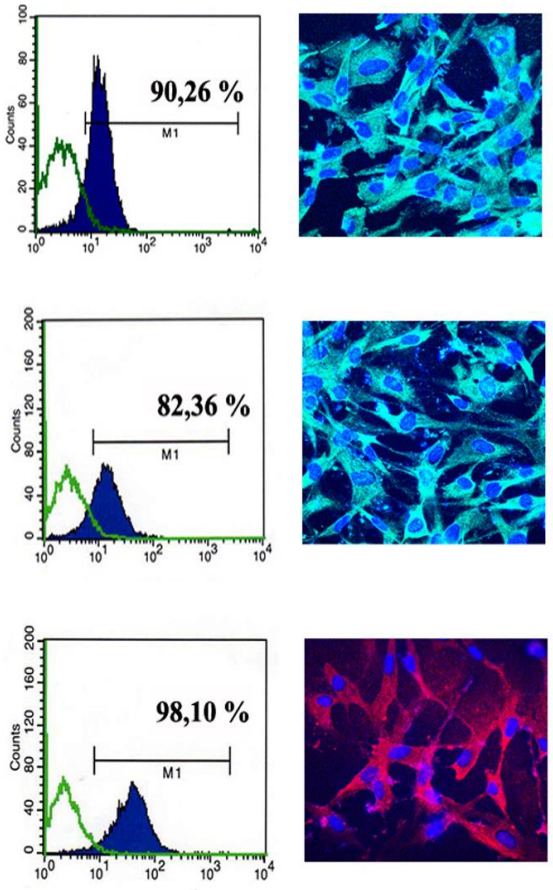


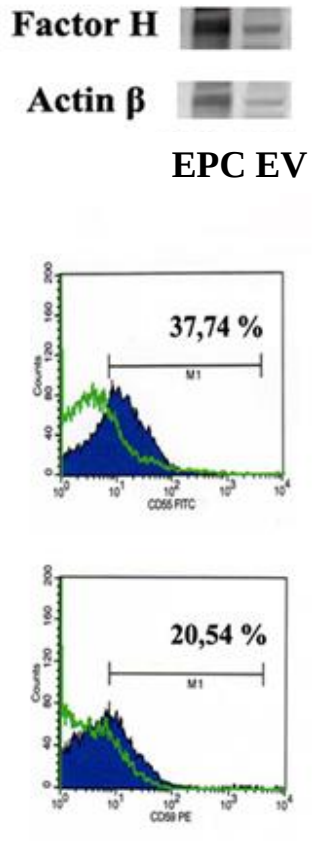
# Expression of complement inhibitors by EPCs and EPC-derived EVs





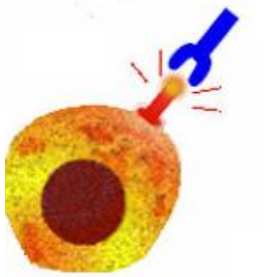




EPCs

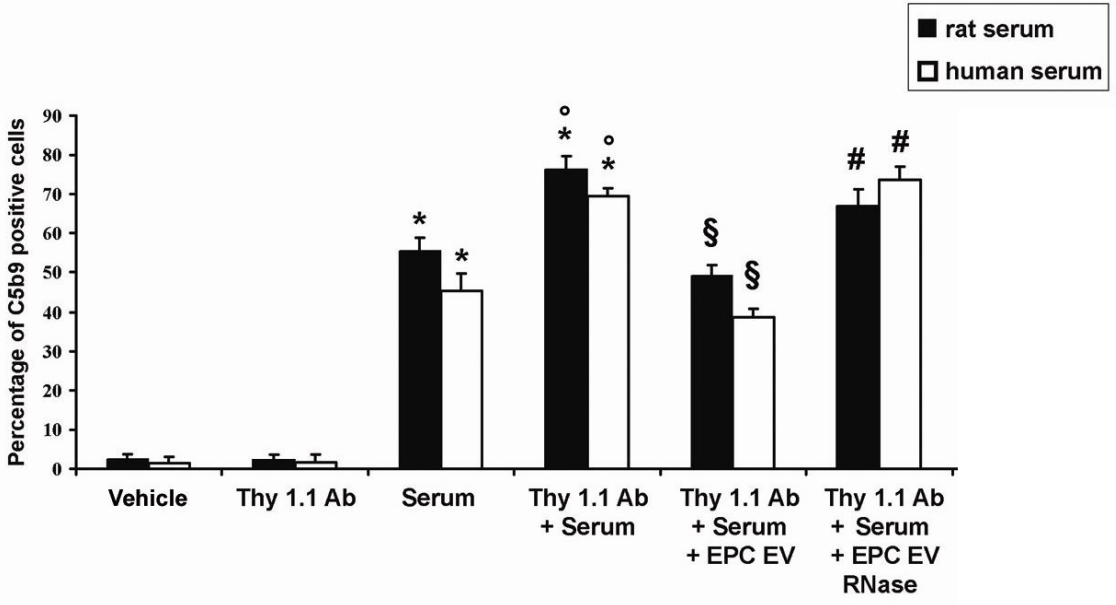
EVs

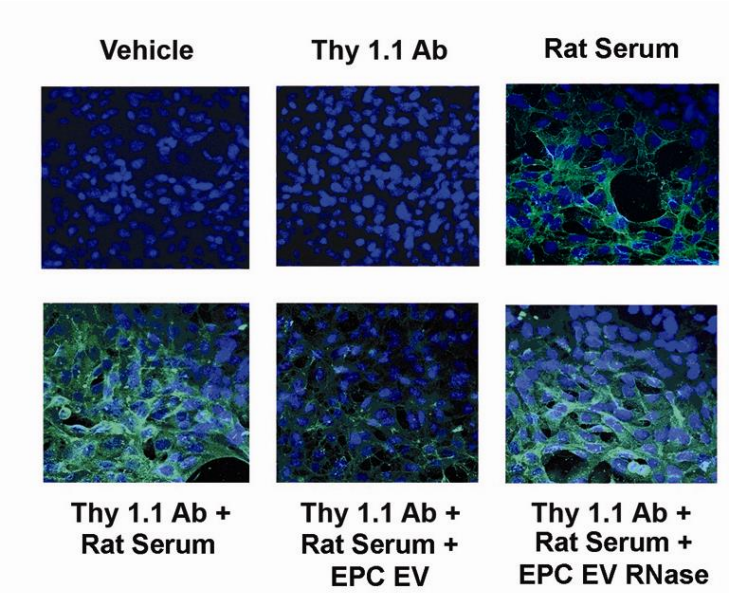
# EPC-derived EVs significantly reduced C5b-9 deposition in Rat Mesangial Cells *in vitro*

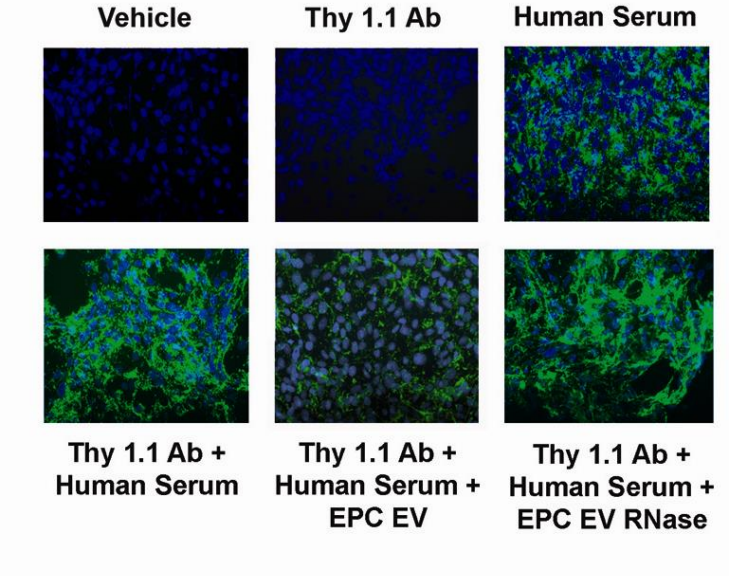
Ab Thy1.1

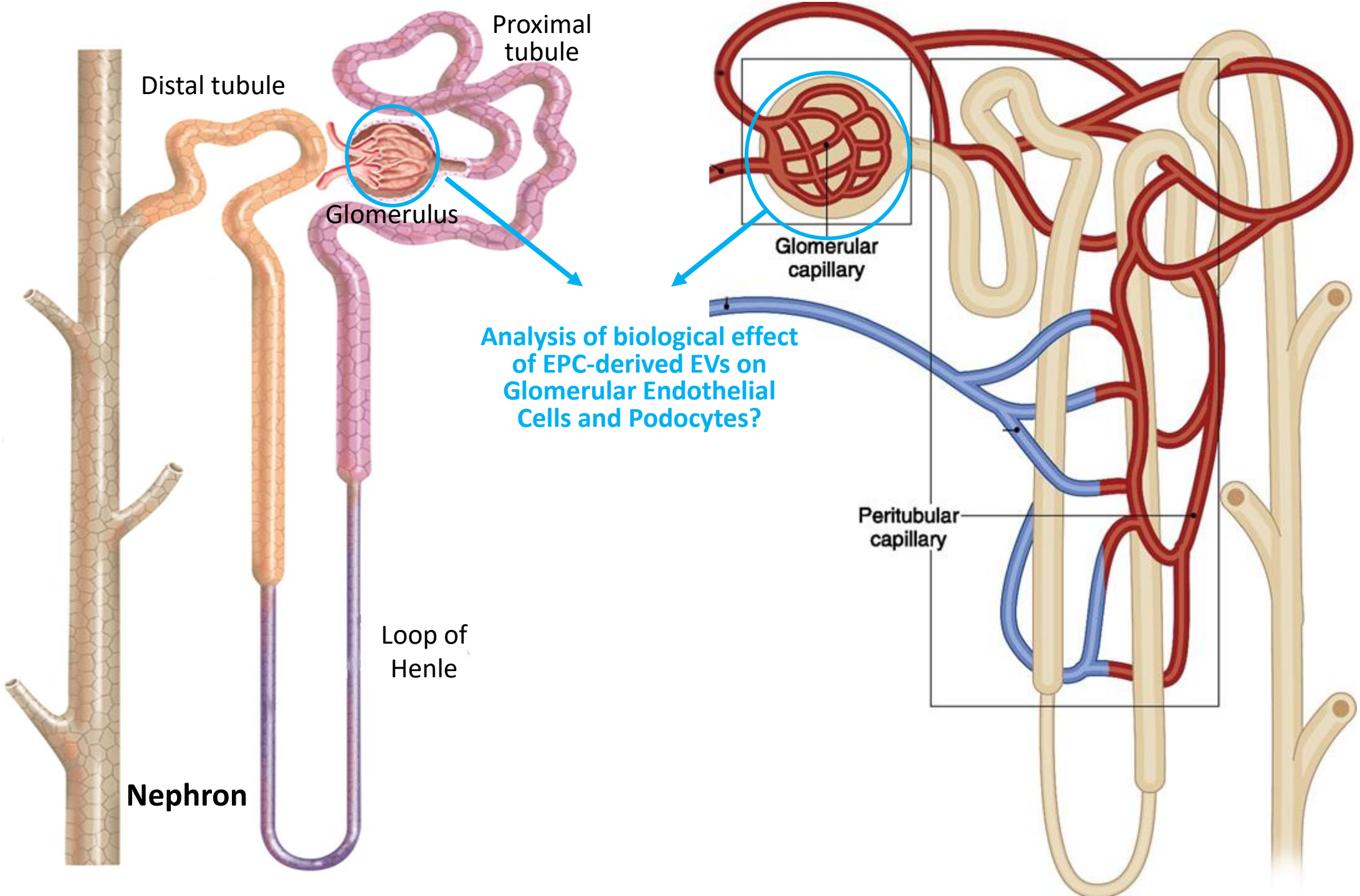


Rat Mesangial Cell



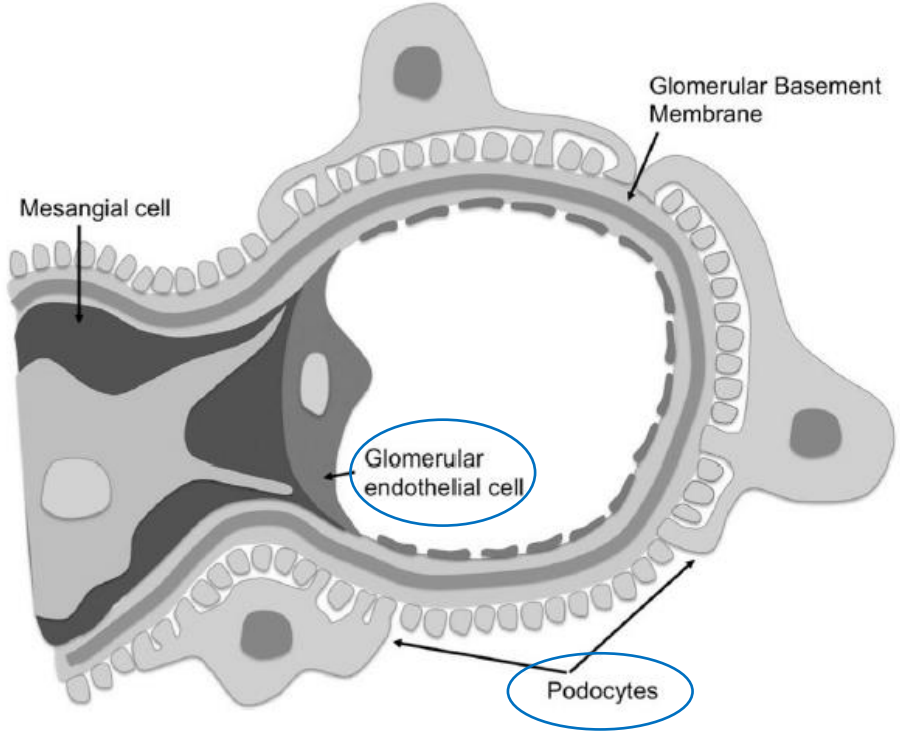






**Analysis of biological effect of EPC-derived EVs on Glomerular Endothelial Cells and Podocytes?**

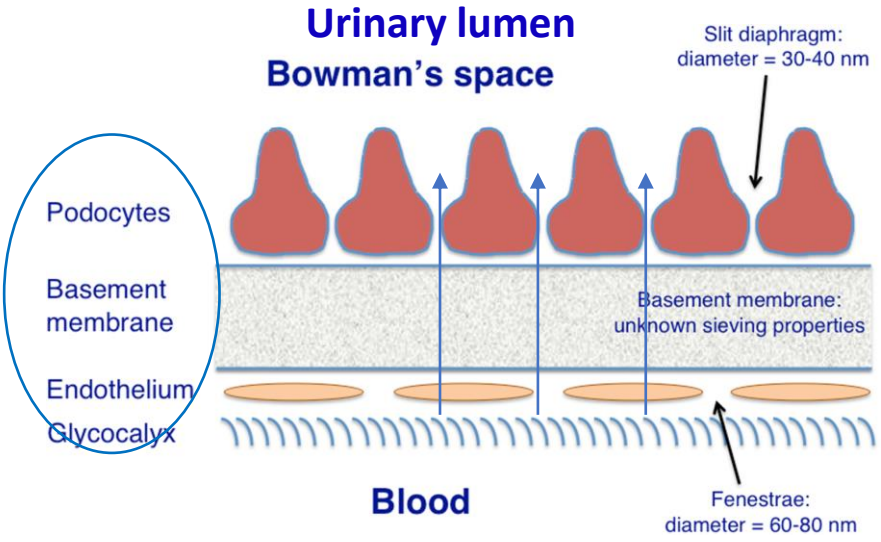
**Glomerular Endothelial Cells (GECs), Basement membrane and Podocytes are part of the Glomerular Filtration Barrier (GFB)**

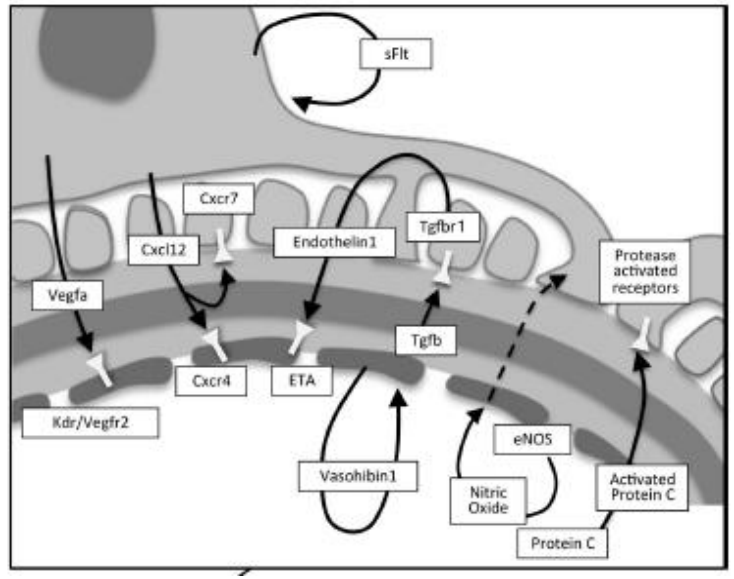
**Filtration of blood in pre urine in Bowman's space**



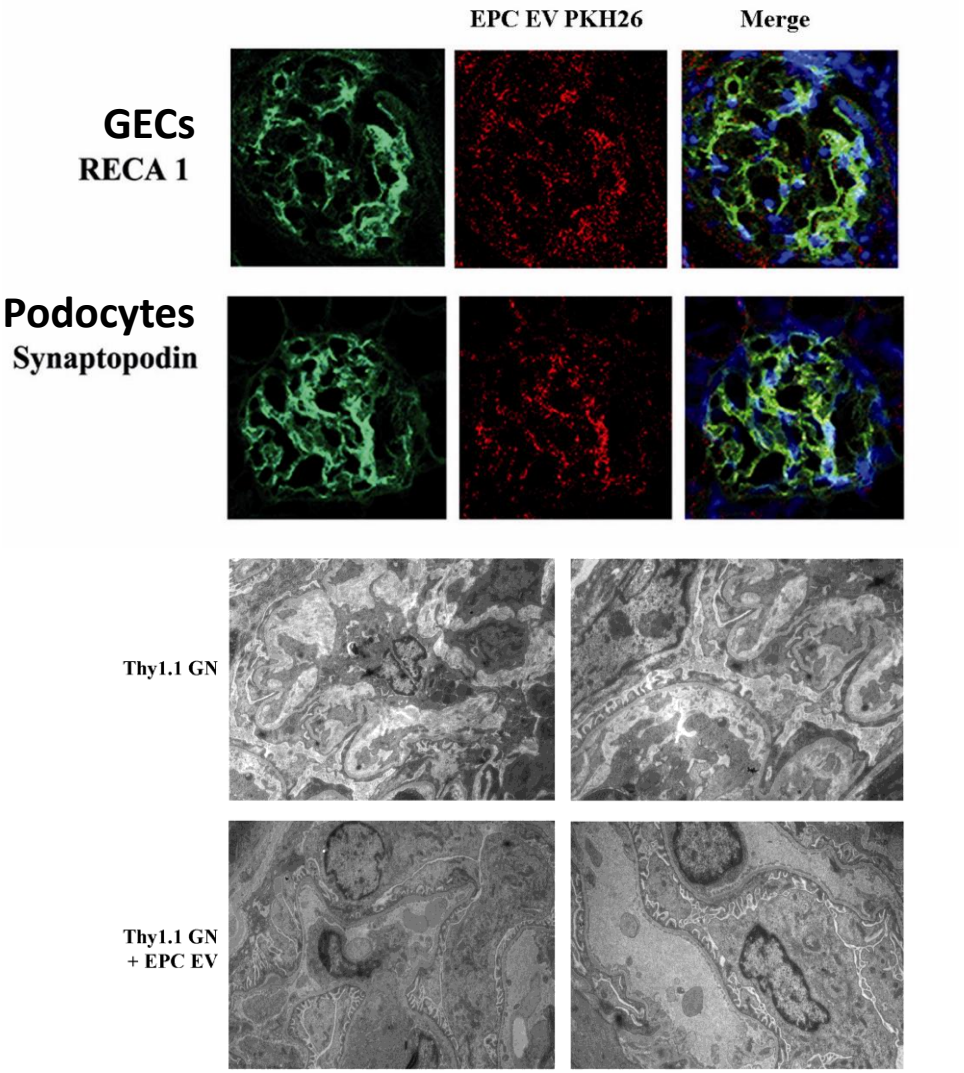

**Complex cross-talk of growth factors between Glomerular Endothelial Cells (GECs) and Podocytes to maintain GFB integrity**

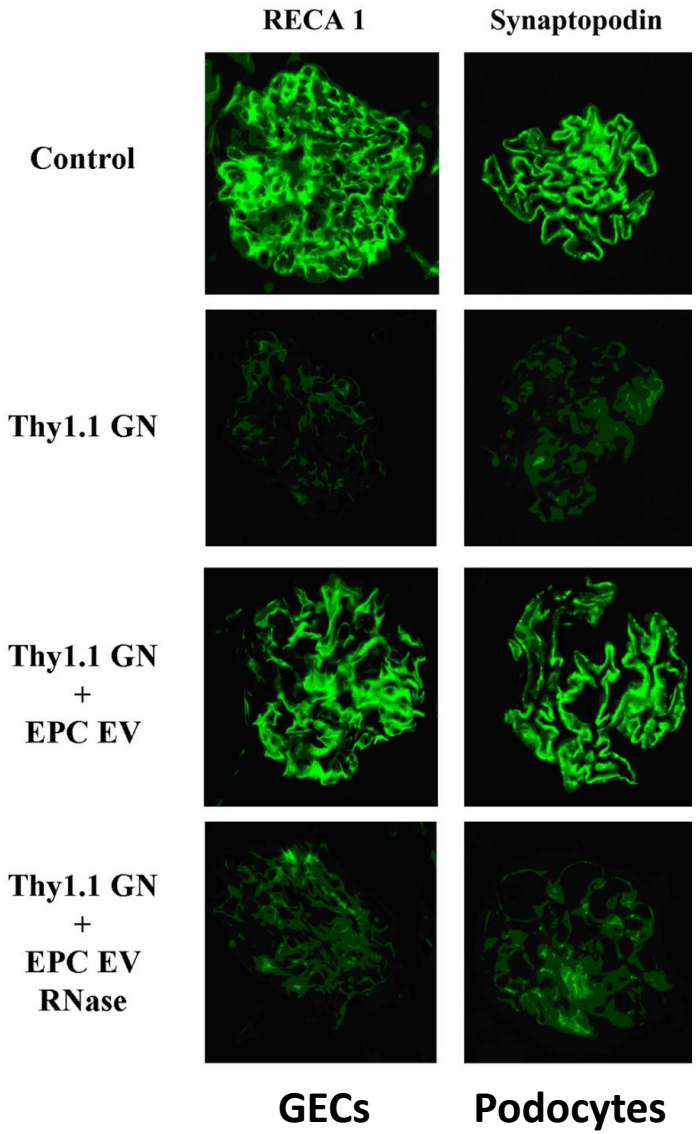
**Angiogenesis,**
**Proliferation**
**Apoptosis,**
**Differentiation**
**Basement membrane production**



# EPC-derived EVs protect in Glomerular Endothelium and Podocytes in experimental anti-Thy1.1 glomerulonephritis



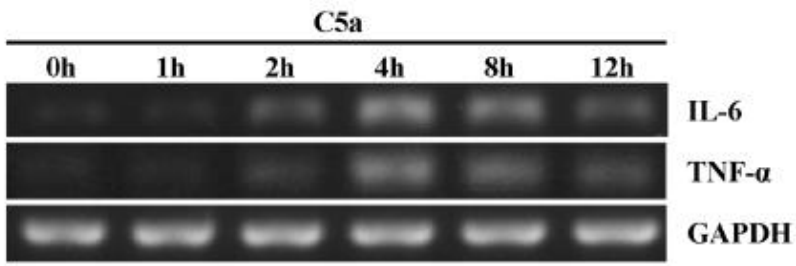
PLOS ONE

RESEARCH ARTICLE

# C5a Induces the Synthesis of IL-6 and TNF-α in Rat Glomerular Mesangial Cells through MAPK Signaling Pathways

Mingde Ji[1,2], Yanlai Lu[1], Chenhui Zhao[3], Wenxing Gao[4], Fengxia He[1], Jing Zhang[1], Dan Zhao[1], Wen Qiu[1]*, Yingwei Wang[1]

A

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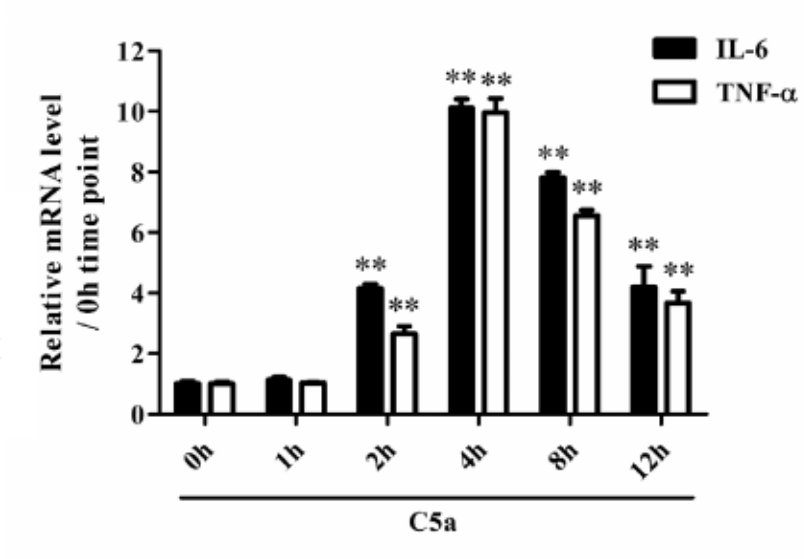
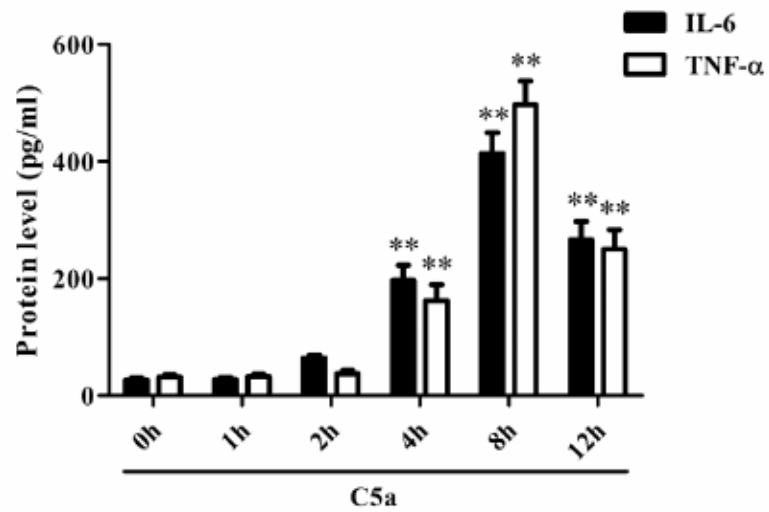
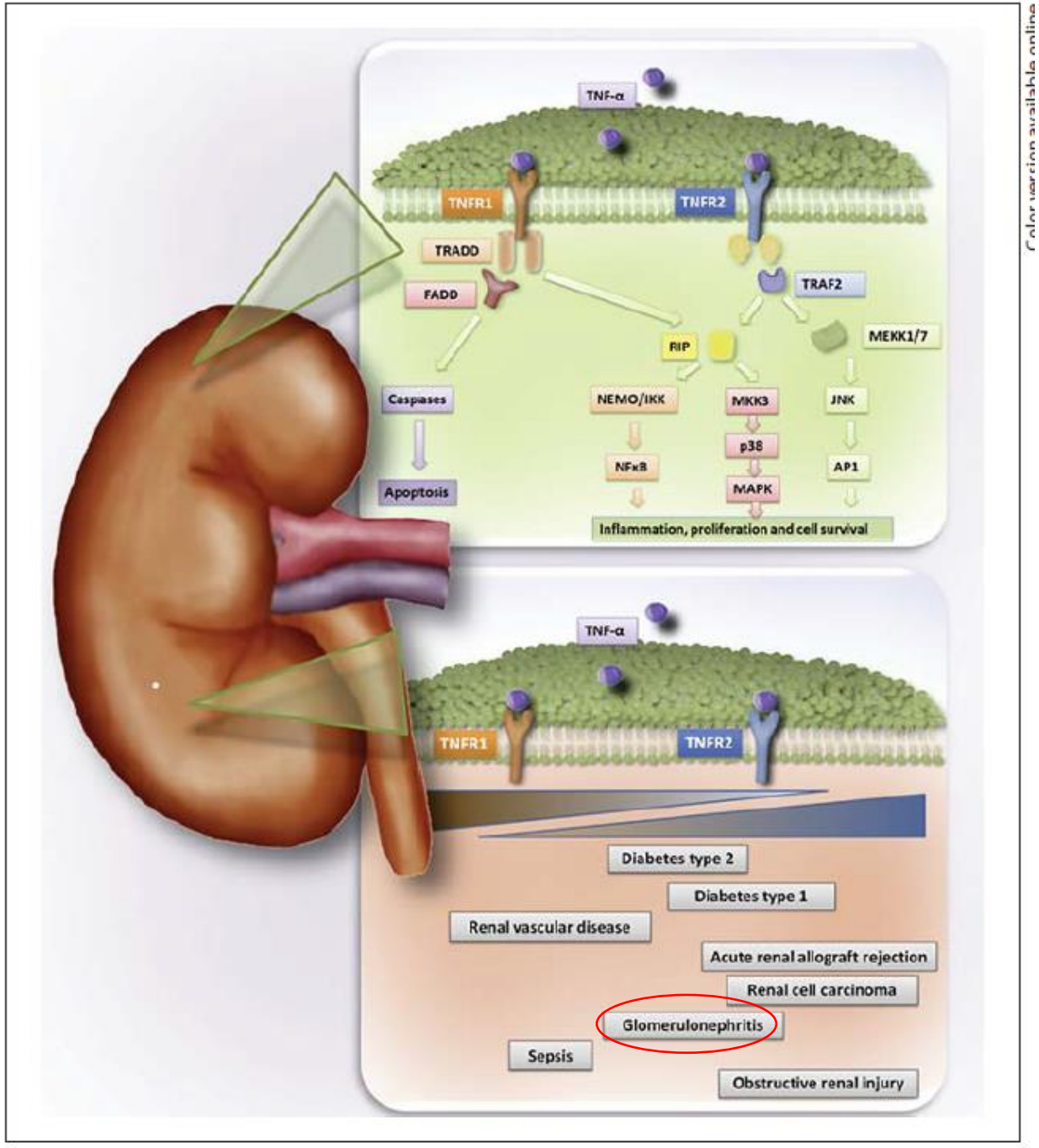



C



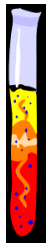
# Tumor Necrosis Factor-α (TNF-α) is a key mediator of inflammation in kidney diseases, particularly in glomerulonephritis

it acts on TNFR1 and TNFR2 receptors which are equally activated in glomerulonephritis in Podocytes and Glomerular Endothelial Cells




*Tumor Necrosis Factor Receptors: Biology and Therapeutic Potential in Kidney Diseases Marijn M. Speeckaert, Reinhart Speeckaert Margo Laute a Raymond Vanholder a Joris R. Delanghe Am J Nephrol 2012;36:261–270*

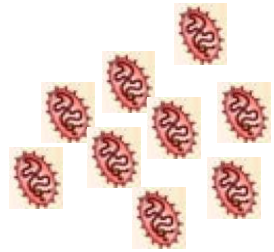
# Methods/1



Peripheral blood
of healthy volunteers

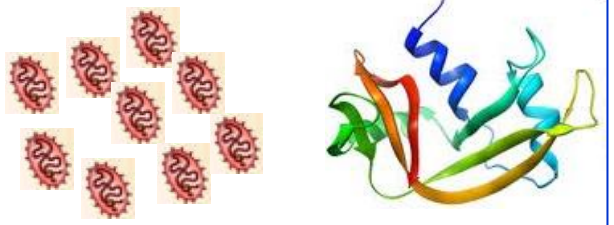
Isolation, characterization
and mantainance of
EPCs (CD133+/flk-1+)
in culture *in vitro*

Ultracentrifugation of culture
medium of EPCs
after 2-3 passages



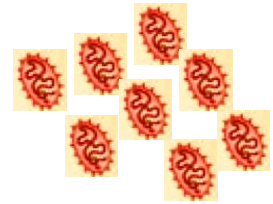
Isolation of EVs

# Methods/2

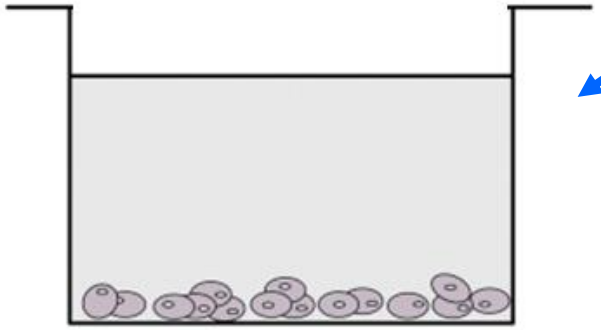


RNase pre-treatment of
EPC-derived EVs

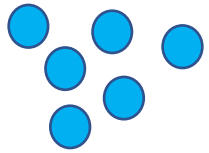
EPC-derived
EVs



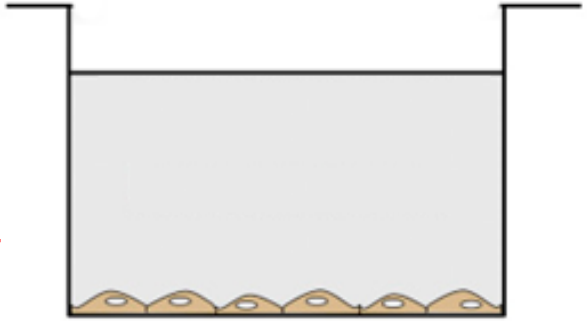
Intenalization on Cells
with red fluorescent
EPC-derived EVs stained
by PKH26 dye



Cytokines



TNF-α
IL-6
C5a



## Glomerular endothelial cells (GECs)

Angiogenesis

Leukocyte adhesion

Apoptosis

Oxidative Stress

## Podocytes

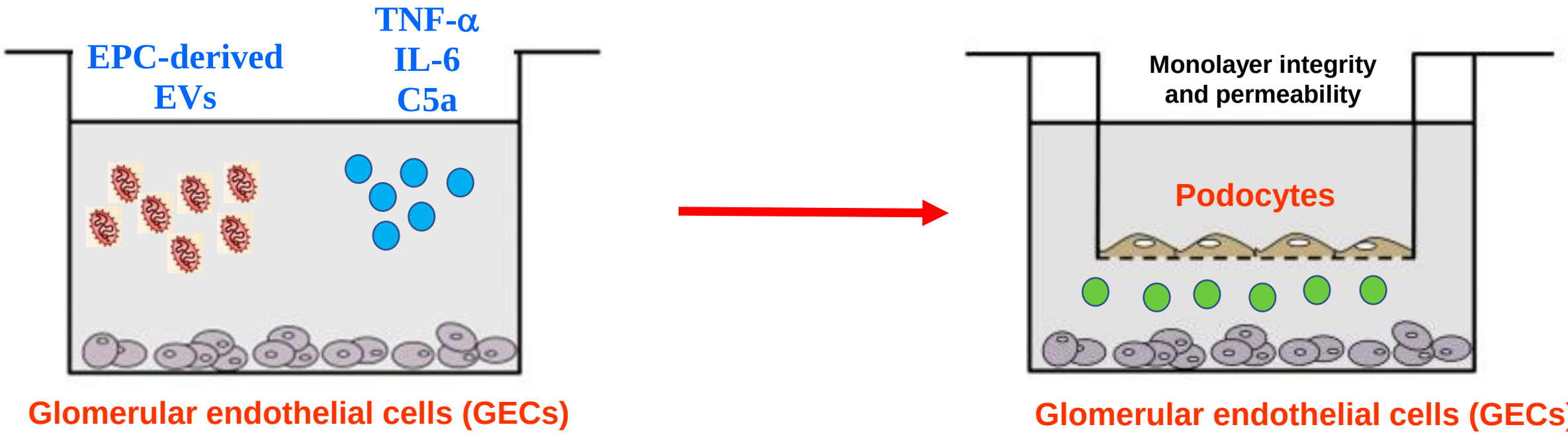
Viability

Apoptosis

Nephrin expression

# Methods/3

## Co-culture Model

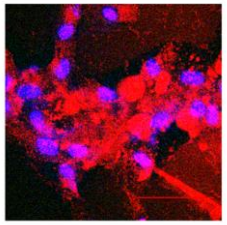


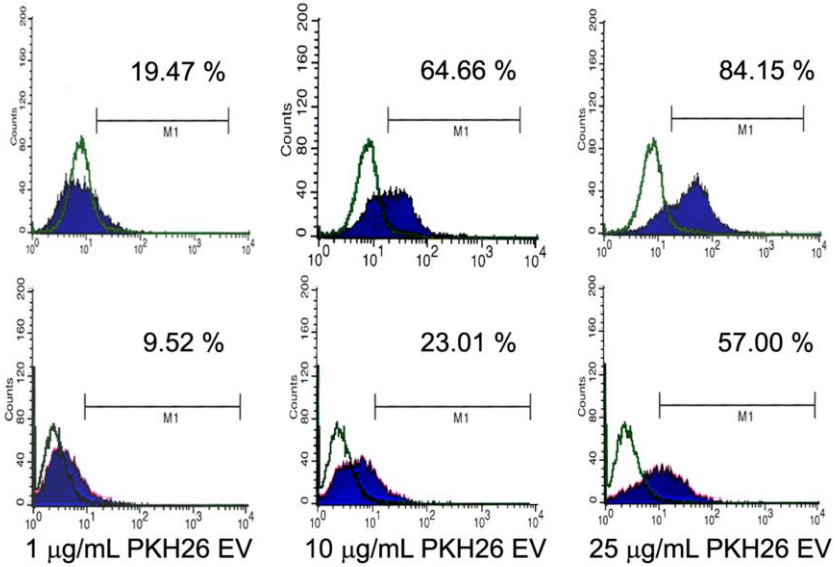
# Aims of the study

1) Description of the internalization mechanisms in Glomerular Endothelial Cells (GECs) and Podocytes
2) To evaluate the pro-angiogenic properties of EPC-derived EVs on GECs
3) To evaluate the protective effects of EPC-derived EVs in a inflammation model with TNF-$\alpha$ and other cytokines (CK) in GECs and Podocytes
4) Analysis of the effect of EPC-derived EVs in a co-culture model of GECs and Podocytes

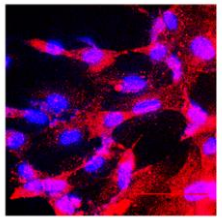
# Internalization of EPC-derived EVs in Glomerular Endothelial Cells (GECs) and Podocytes

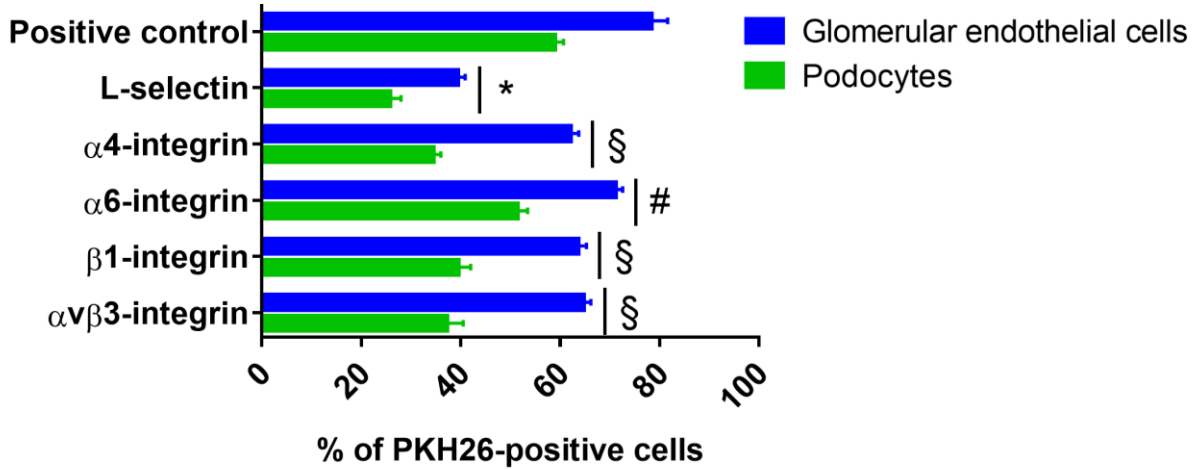
Glomerular Endothelial Cells






Podocytes

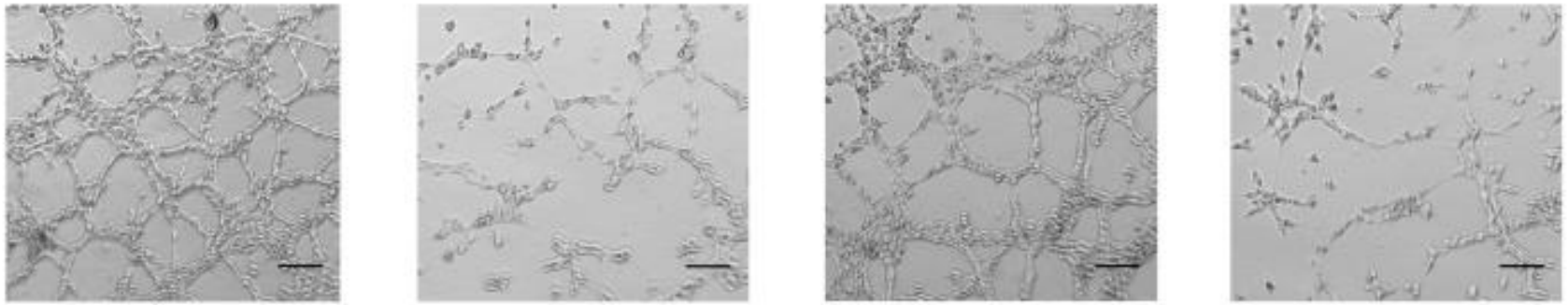




# EPC-derived EV effect on Angiogenesis of GECs

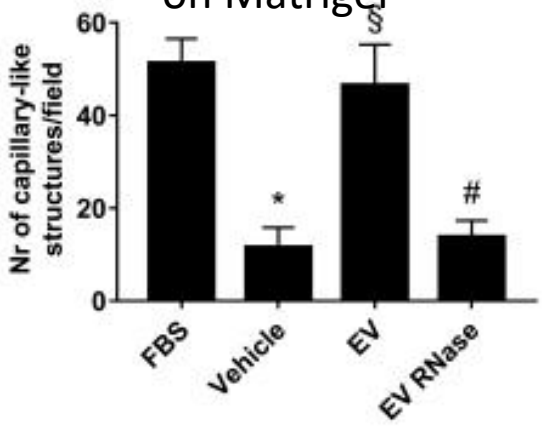
Angiogenesis on Matrigel

A



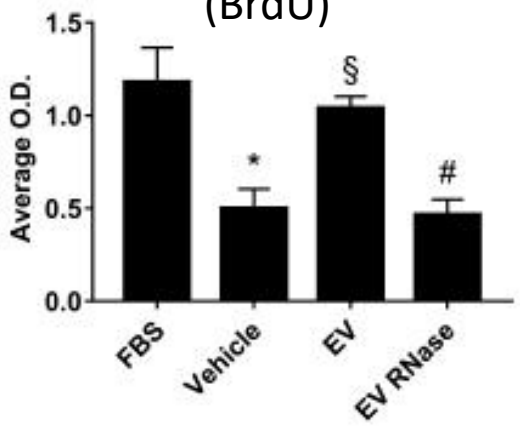

Count of Angiogenesis on Matrigel

B



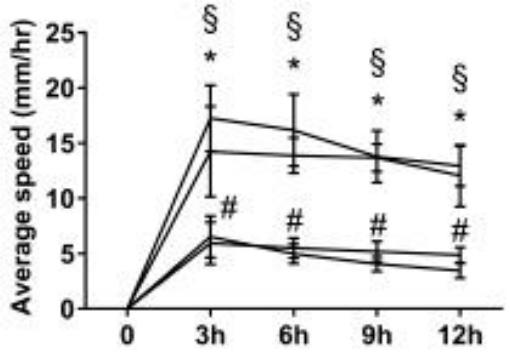

Proliferation assay (BrdU)

C

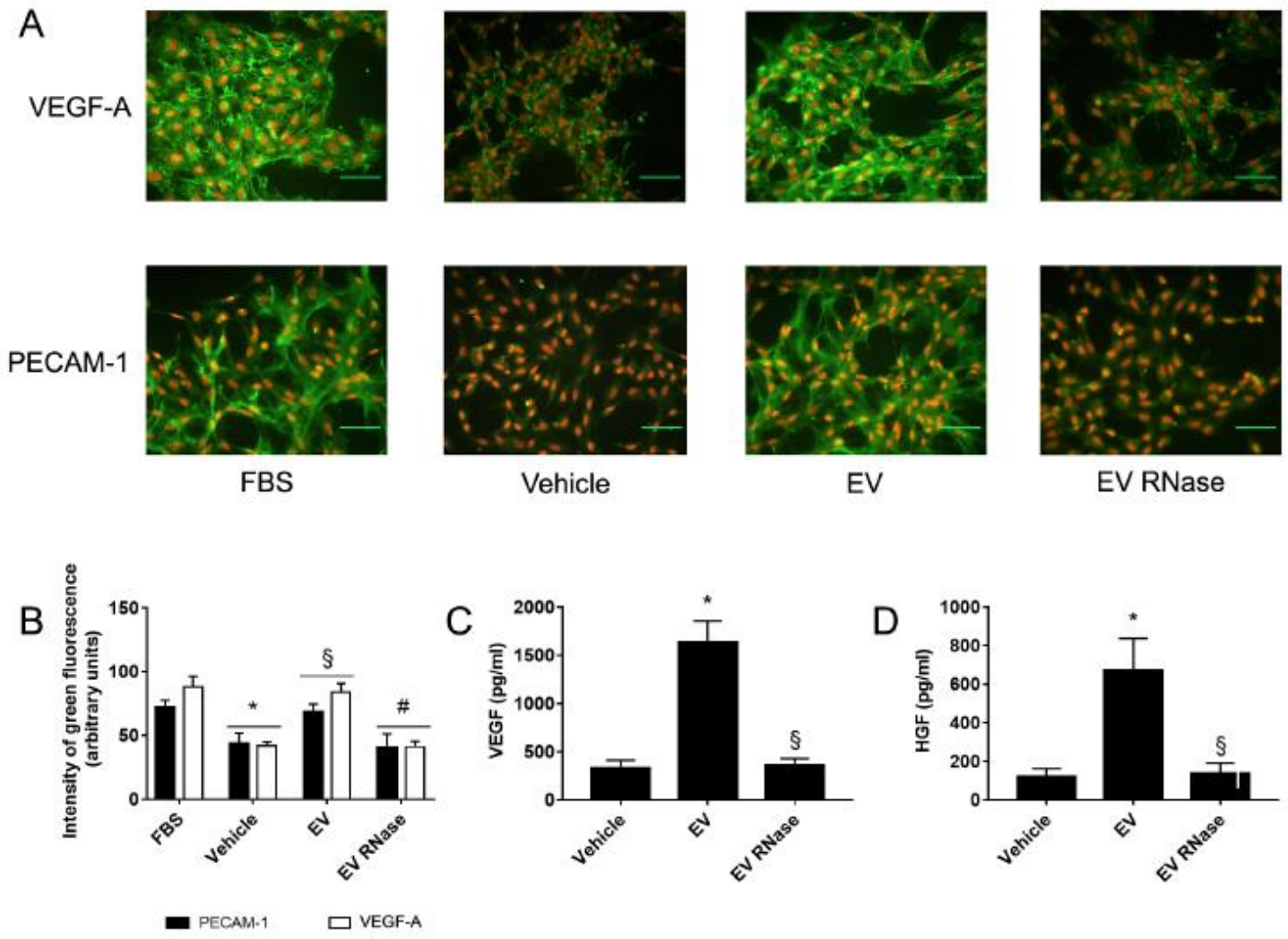


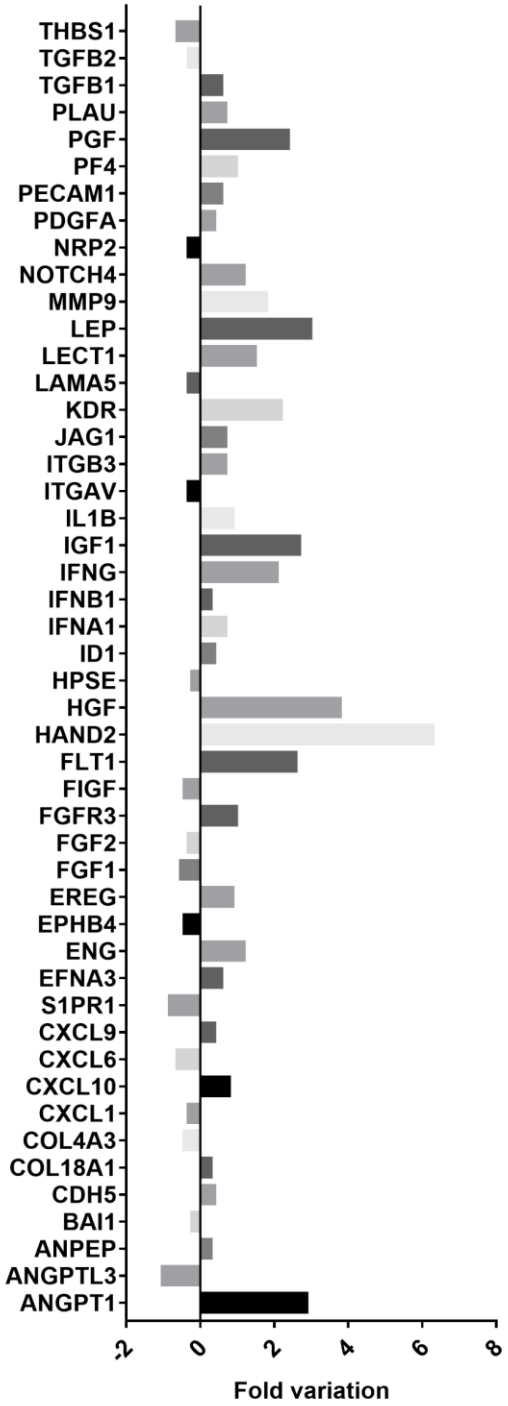

Migration assay

D



# EPC-derived EVs increase expression of proteins involved in GEC angiogenesis

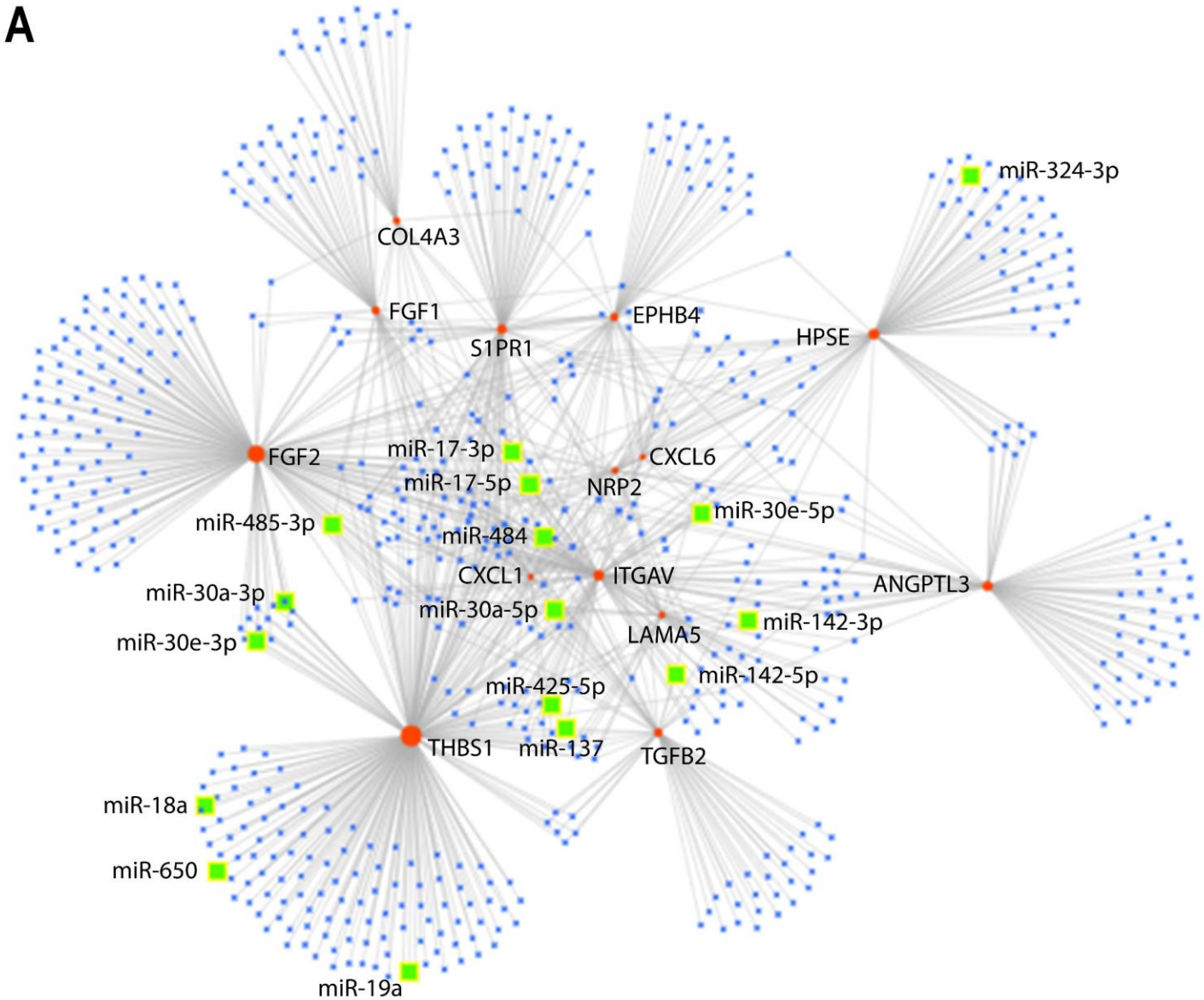





# EPC-derived EVs modulate gene expression of GECs

- Angiogenesis
- Anti-angiogenetic genes
- Growth factors for podocytes (cross-talk)
- Cell Migration
- Glomerular basement membrane

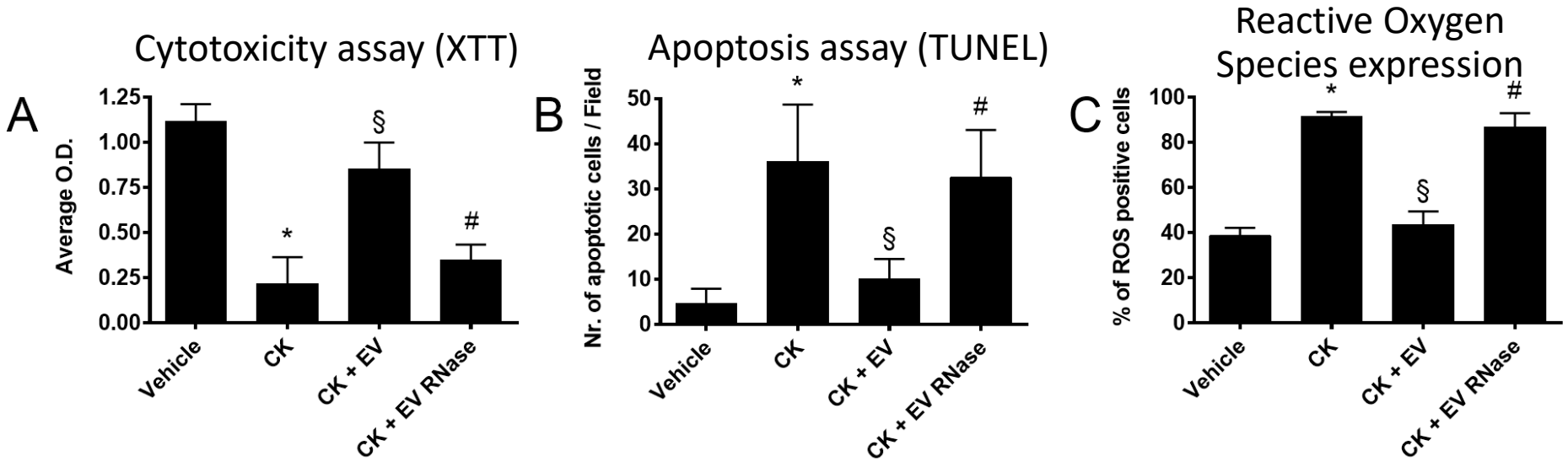
A

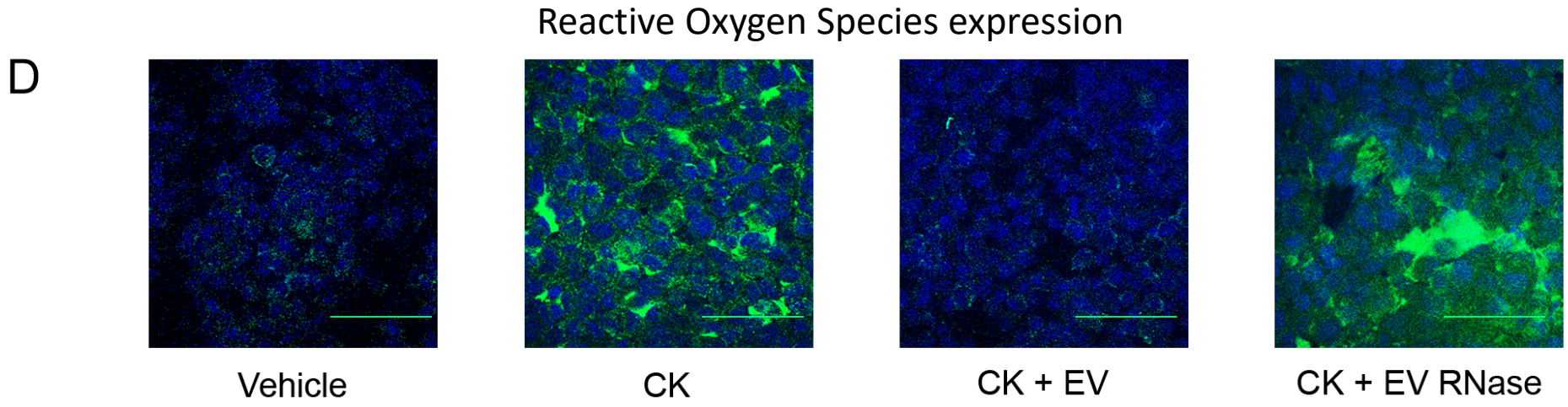



B

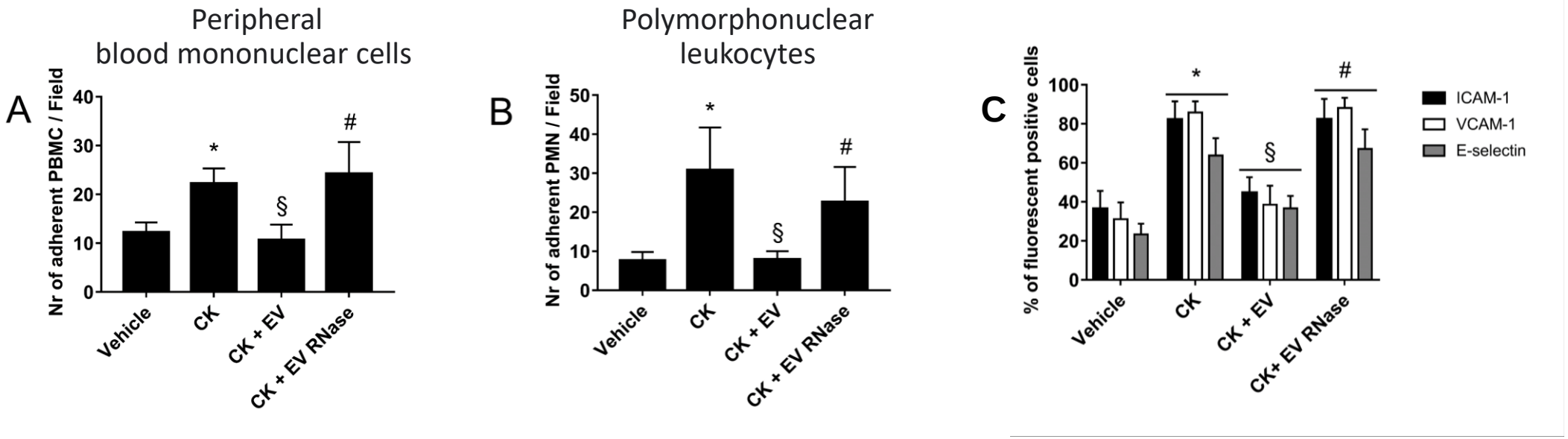
| List of miRNAs | Target genes |
|---|---|
| mir-137 | ITGAV, TGFB2, THBS1 |
| mir-142-3p | ITGAV |
| mir-142-5p | ITGAV, TGFB2 |
| mir-17-3p | EPHB4, NRP2, S1PR1, THBS1 |
| mir-17-5p | COL4A3, EPHB4, ITGAV, NRP2, S1PR1, TGFB2, THBS1 |
| mir-18a | HPSE, THBS1 |
| mir-19a | EPHB4, FGF2, S1PR1, THBS1 |
| mir-30a-3p | FGF2, THBS1 |
| mir-30e-3p | FGF2, THBS1 |
| mir-30a-5p | CXCL1, CXCL6, ITGAV, THBS1 |
| mir-30e-5p | CXCL6, ITGAV |
| mir-324-3p | HPSE |
| mir-425-5p | ITGAV, THBS1 |
| mir-484 | EPHB4, ITGAV, THBS1 |
| mir-485-3p | FGF1, THBS1 |
| mir-650 | THBS1 |

# EPC-derived EVs protect GECs in a inflammatory model with TNF-α and other cytokines (CK)

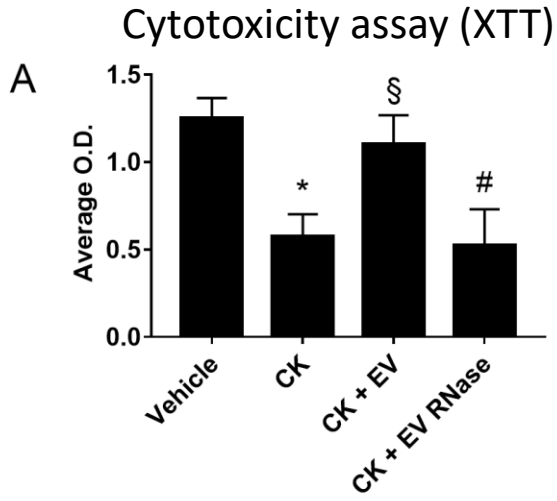


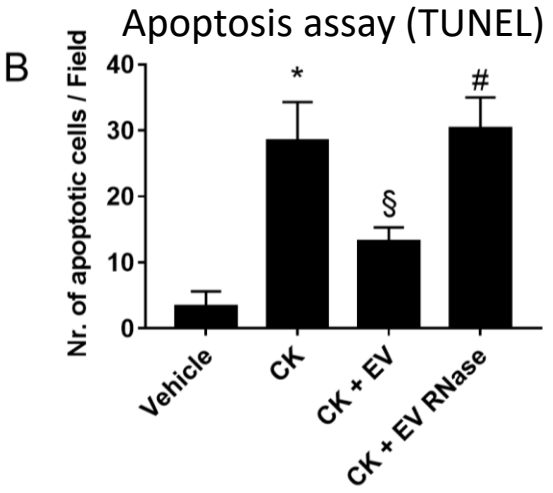



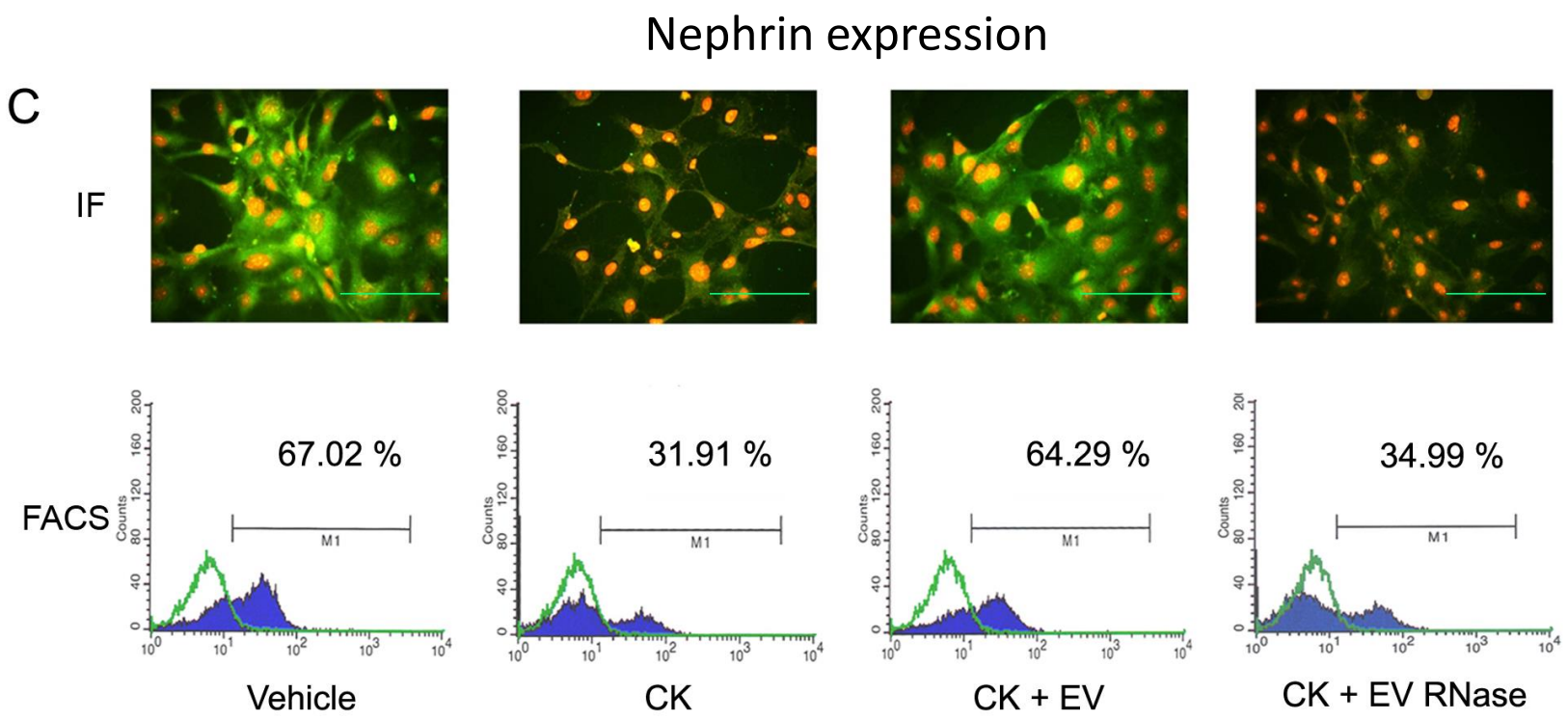
# EPC-derived EVs inhibit Leukocyte adhesion on GECs in a inflammatory model with TNF-α and other cytokines (CK)



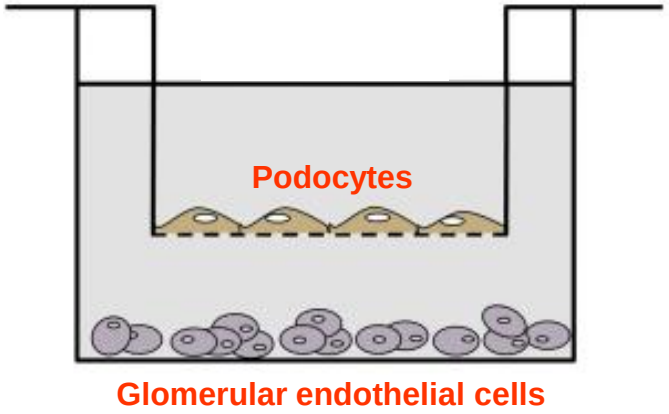
# EPC-derived EVs protect Podocytes in a inflammatory model with TNF-α and other cytokines (CK)



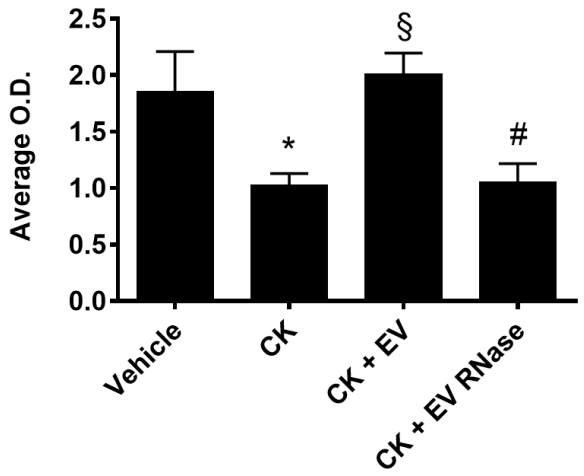



# Biological effect of EPC-derived EVs on a co-culture model of GECs and Podocytes



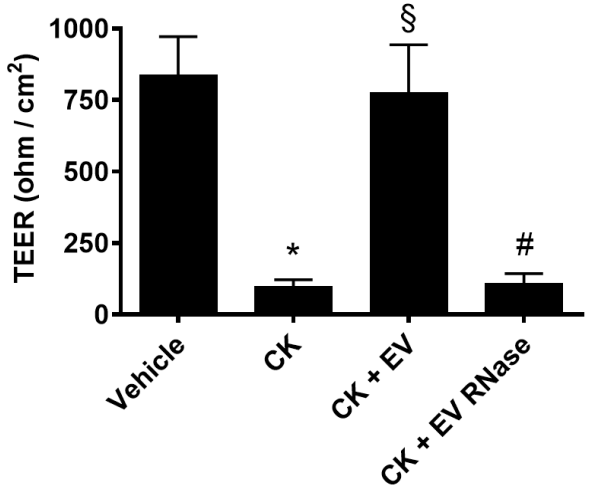

A

Cytotoxicity assay (XTT)



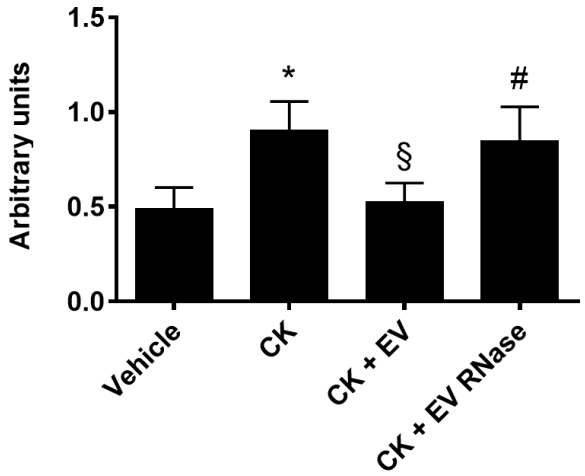

B

Trans-Epithelial Electrical Resistance




C

Permeability to Albumin



# Conclusions

1) EPC-derived EVs internalize in Glomerular Endothelial Cells (GECs) and Podocytes effectively through mechanisms mediated by various integrins (L-selectin)
2) EPC-derived EVs trigger an angiogenic program in GECs that regulates proliferation, migration, and remodeling of the Glomerular Basement Membrane
3) In an experimental model of inflammatory injury from TNF-$\alpha$ and other cytokines *in vitro,* EPC-derived EVs protect GECs and Podocytes
4) EPC-derived EVs stimulate GECs to release growth factors able to maintain the vitality and functionality of the Podocytes in inflammatory conditions
5) The role of mRNAs/microRNAs is confirmed by experiments using RNase-treated EVs.
6) EPC-derived EVs could be exploited as potential therapeutic approach in glomerular injury without the risks associated with whole stem cell transplantation (maldifferentiation and tumorigenesis).

# Special Thanks

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