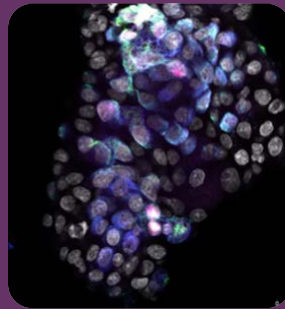
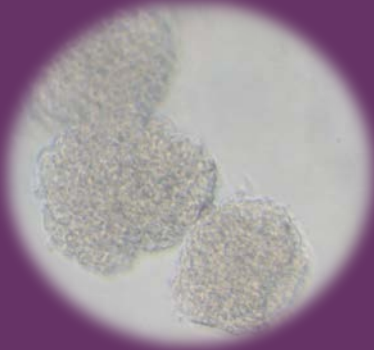
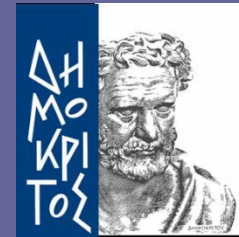




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“Demokritos”



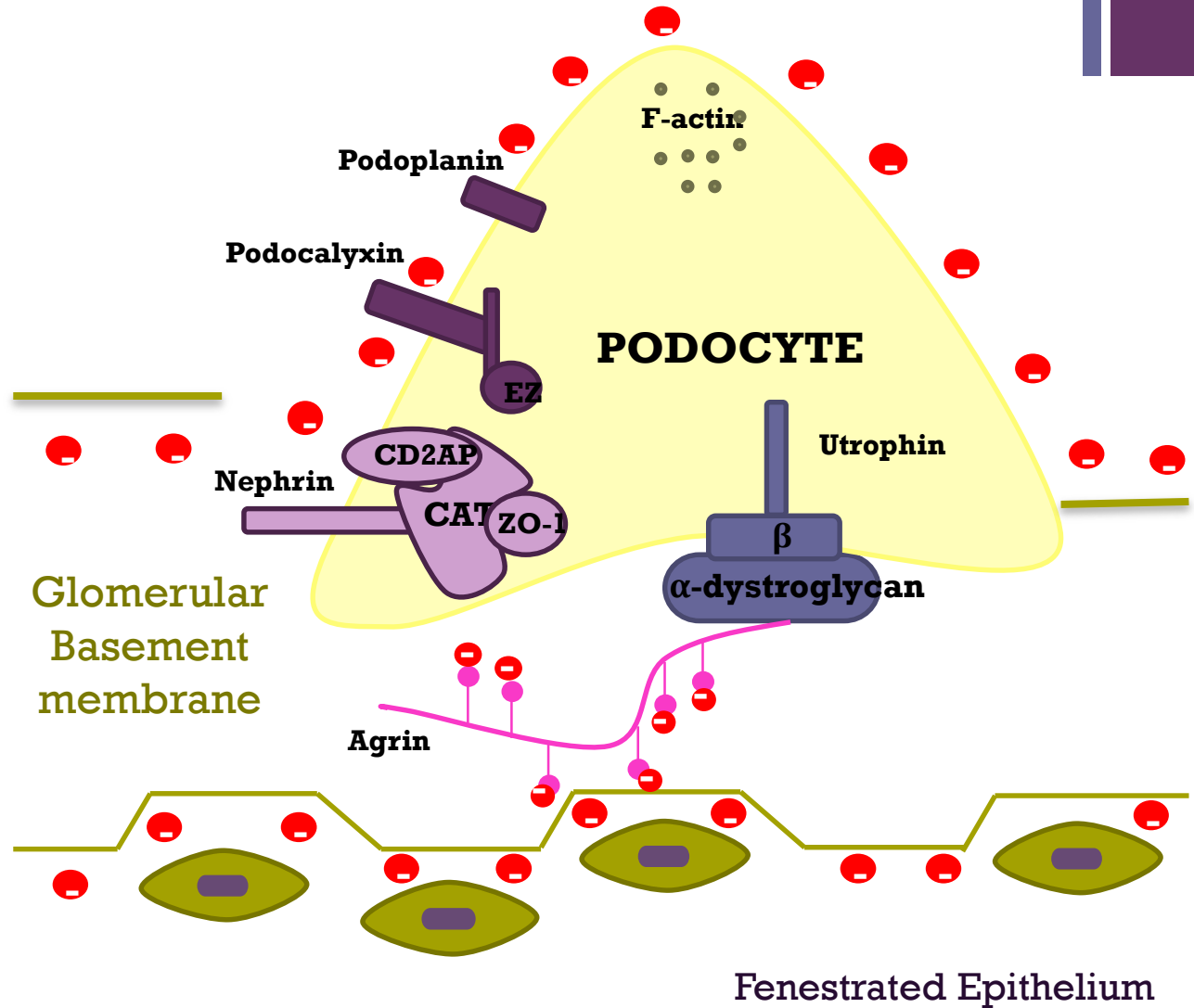
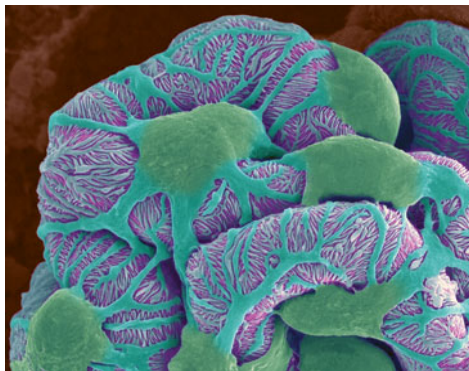
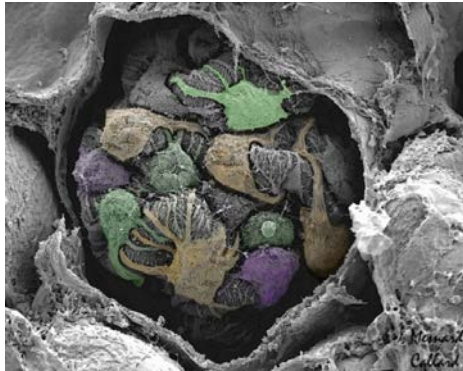
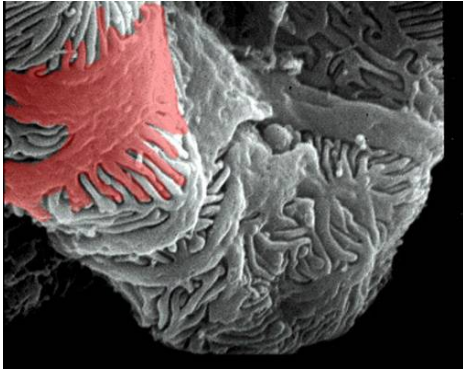
Laboratory of
Cell and
Matrix
Pathobiology

20o Hellenic
Nephrology
Conference
2018

Vitamin D₃ ameliorates podocyte injury through the nephrin signaling pathway in isolated rat glomeruli

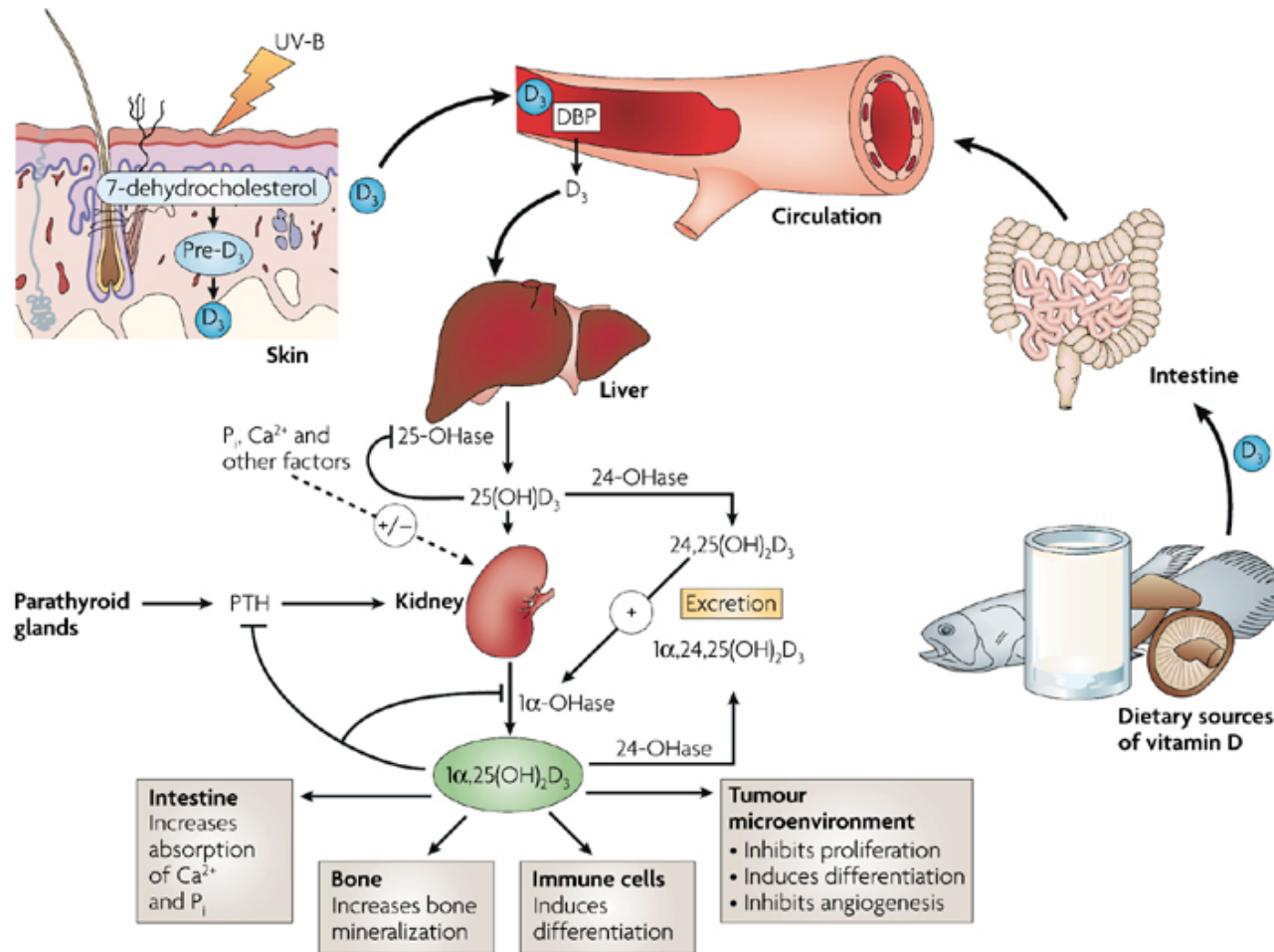
Trohatou O., Drossopoulou G., Tsilibary EC., Charonis A., and Iatrou C.

+ Podocytes

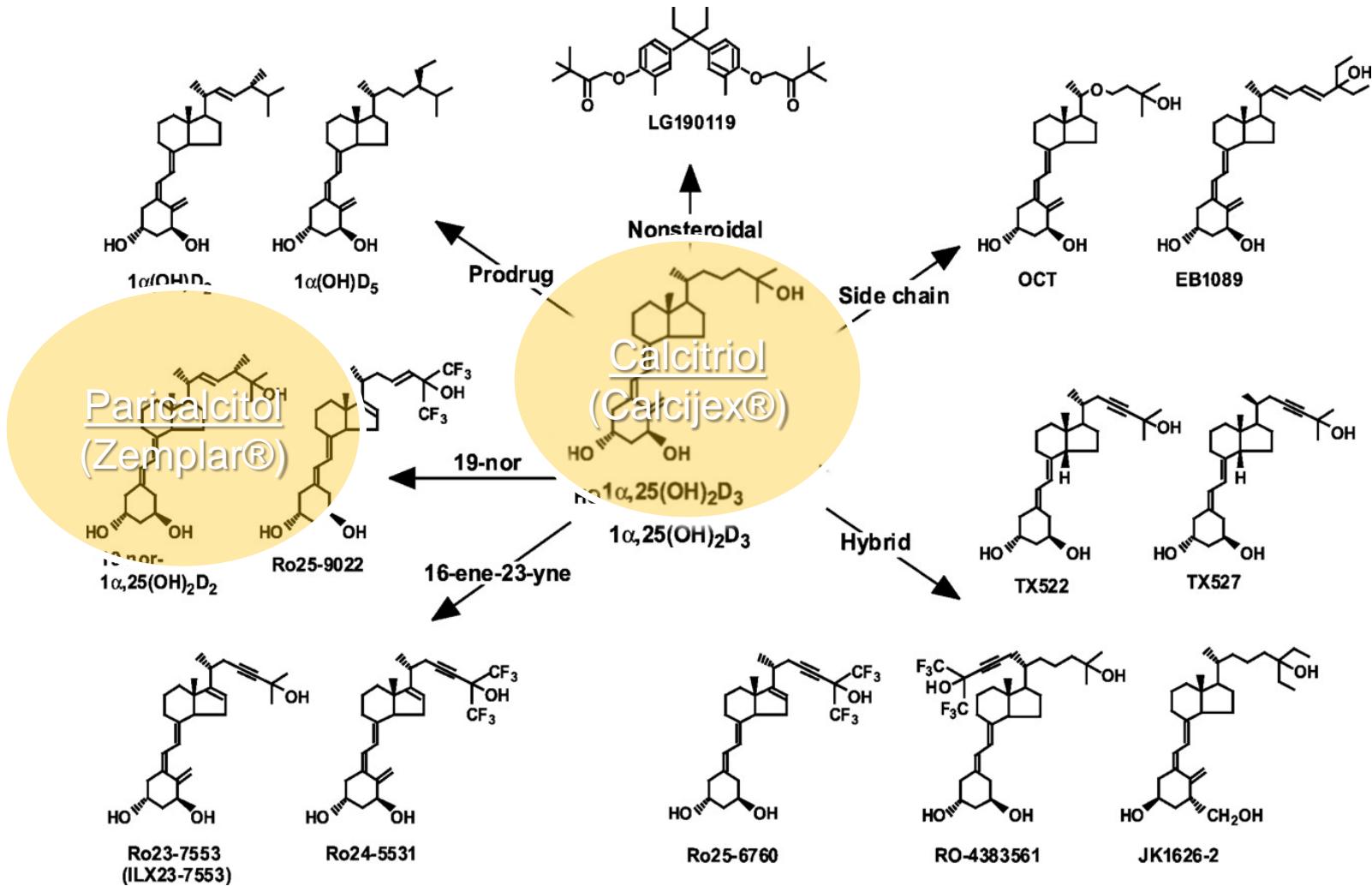




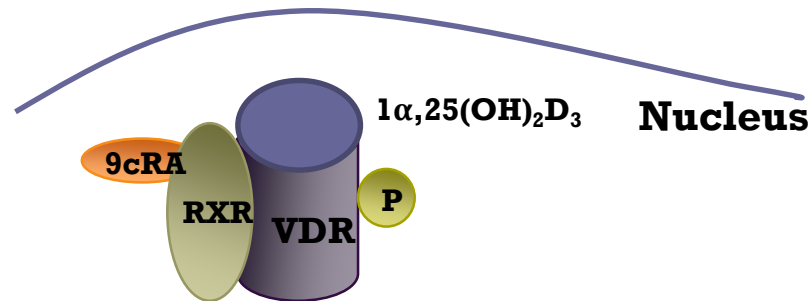
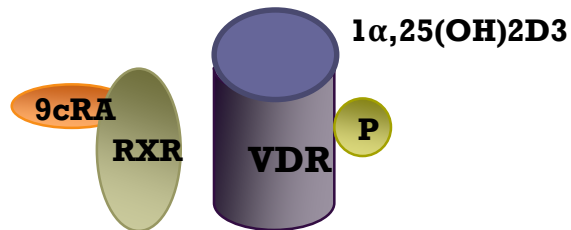
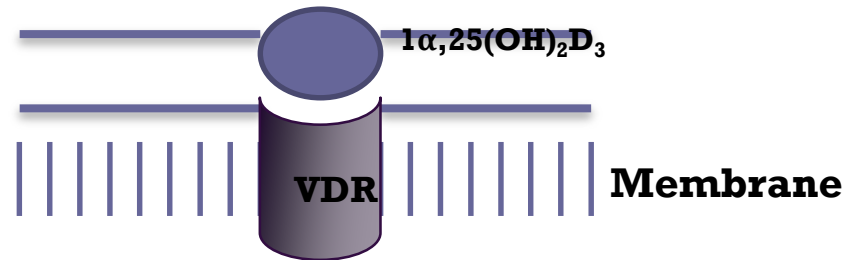
Synthesis and target organs for vitamin D



+ Vitamin D analogues



+ Active Vitamin D3 - VDR



- **Transcriptional activation**
- **Chromatin Remodelling**

+ Vitamin D3 effects in vitro

Vitamin D Receptor Activators Upregulate and Rescue Podocalyxin Expression in High Glucose-Treated Human Podocytes

S.N. Verouti^{a,b} E.C. Tsilibary^b E. Fragopoulou^c C. Iatrou^d C.A. Demopoulos^a
A.S. Charonis^e S.A. Charonis^f G.I. Drossopoulou^b

- HGEC-25mM exhibit sustained, reduced podocalyxin and nephrin expression compared to HGEC-5mM.
- Calcitriol and paricalcitol restore nephrin and podocalyxin expression in HGEC in a VDR dependent manner
- Calcitriol and paricalcitol induce nuclear translocation of VDR and co-localization with RXR, in HGEC

+ Aim

➤ Ex vivo

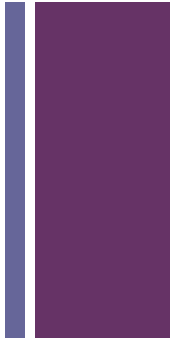
✓ **Treatment with paricalcitol (VDRA)**

- Isolation of rat glomeruli and culture in presence of normal or high glucose
- Expression levels of Nephtrin, PODXL and VDR
- Tunnel assay

➤ In vivo

✓ **Treatment with calcitriol and paricalcitol**

- STZ animal model
- Blood glucose levels, urine 24h and water uptake
- Expression levels of Nephtrin and VDR



+ Glomeruli isolation and culture



Wistar Rat



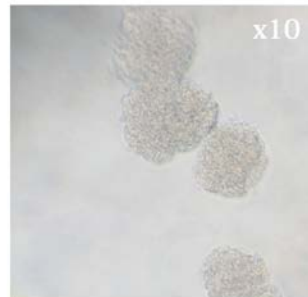
250µm

75µm

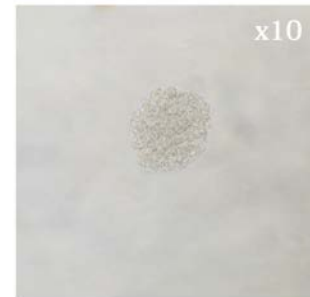


Glomeruli culture
~ 98% efficiency
In presence of
5mM or 25mM glucose

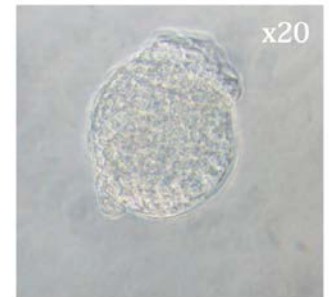
❖ **VDRA (VDR activator -
paricalcitol) treatment for 4
days**



x10



x10

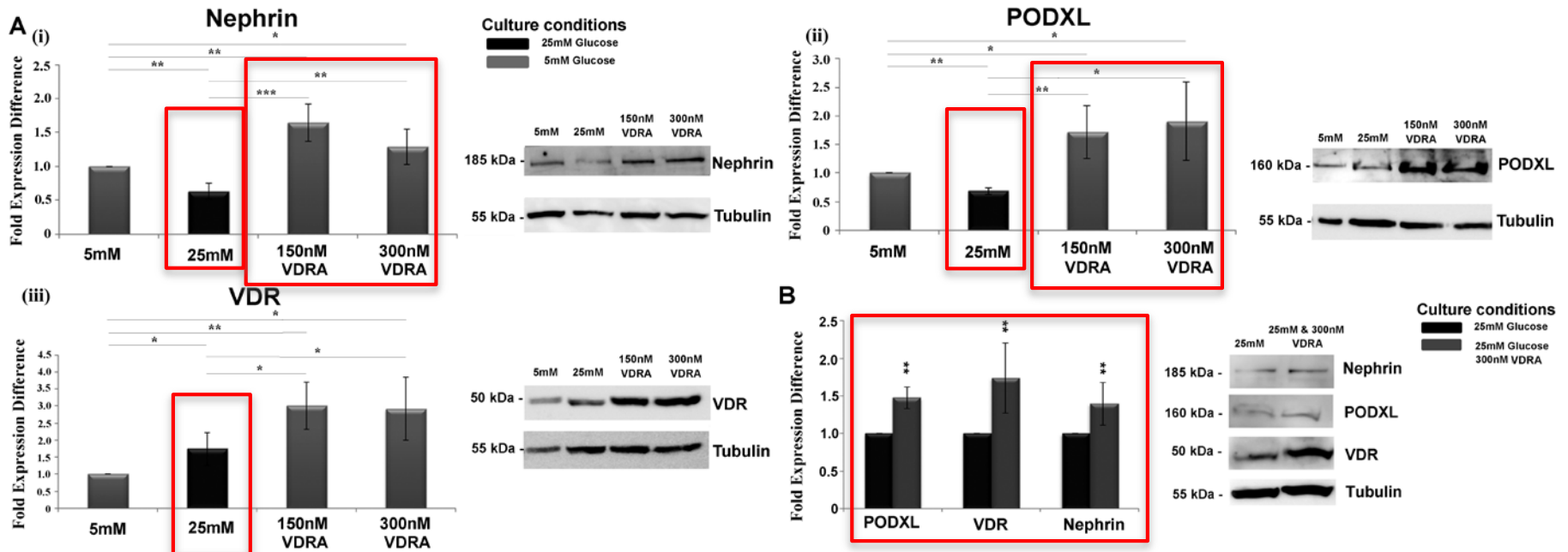


x20

Glomeruli

+ Results

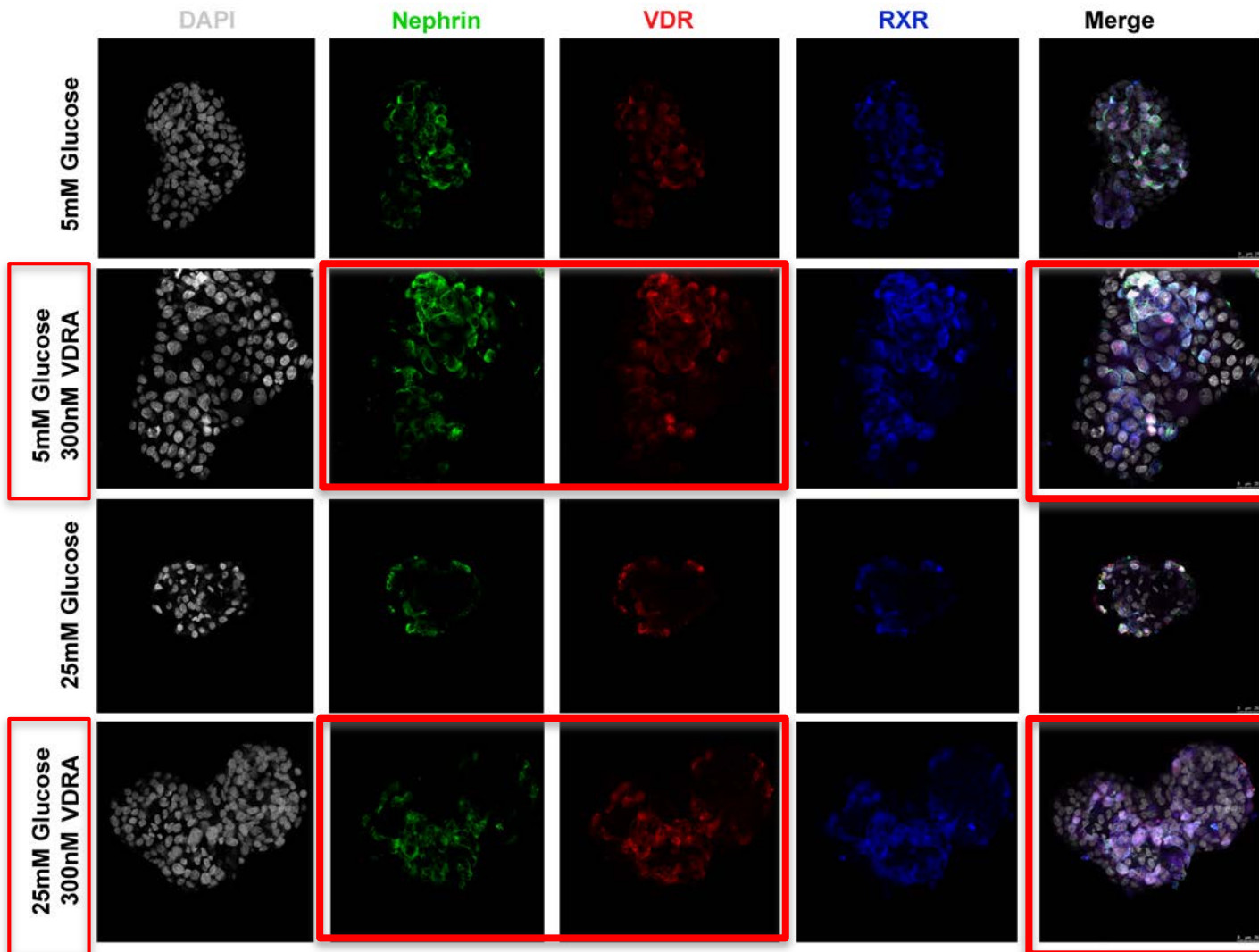
✓ Nephrin, VDR, PODXL expression levels



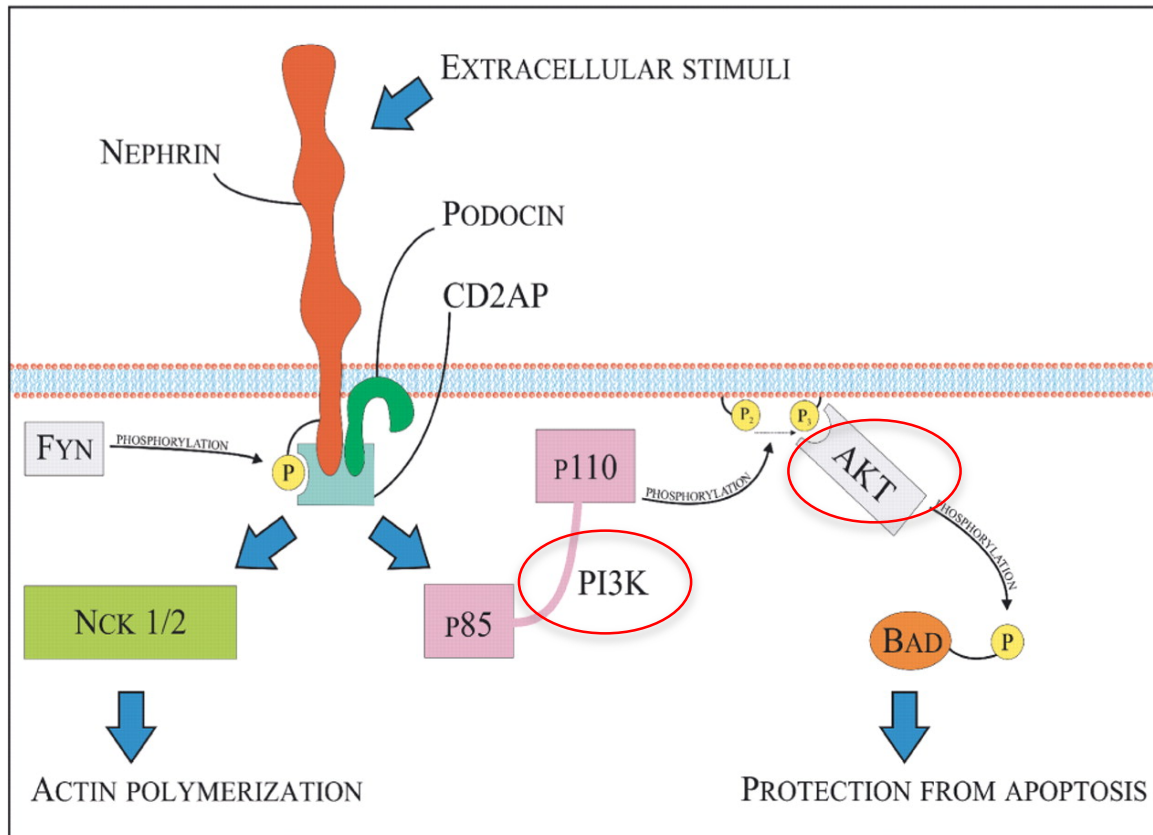
- ✓ Podocyte markers were **enhanced** in presence of **paricalcitol** in glomeruli cultured in **normal glucose**.
- ✓ Nephrin and PODXL were **reduced** in presence of **high glucose**.
- ✓ VDR expression was **enhanced** in presence of **high glucose**.
- ✓ VDRA **restored** the expression levels of Nephrin, VDR and PODXL after induce hyperglycemia

+ Results

✓ Nephrin, VDR and RXR



+ Nephrin survival pathway

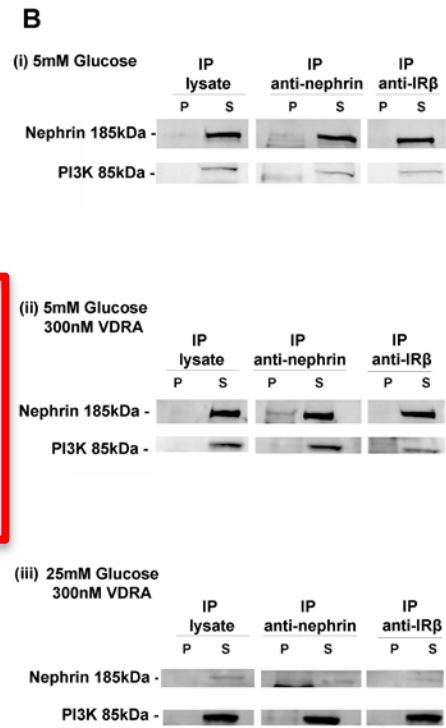
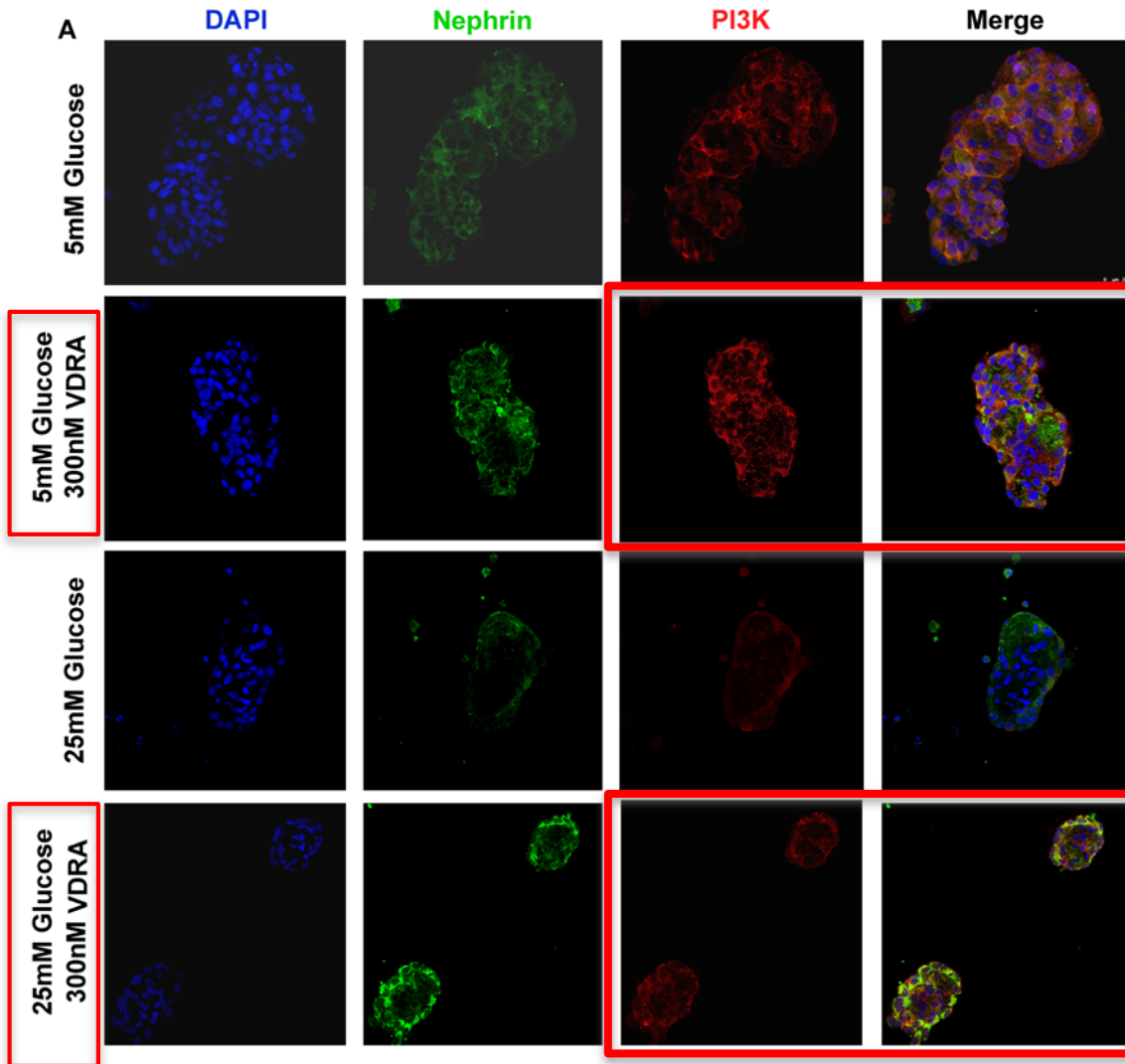


Nephrin and CD2AP interact with PI3K and stimulate PI3K-dependent AKT signaling.



Results

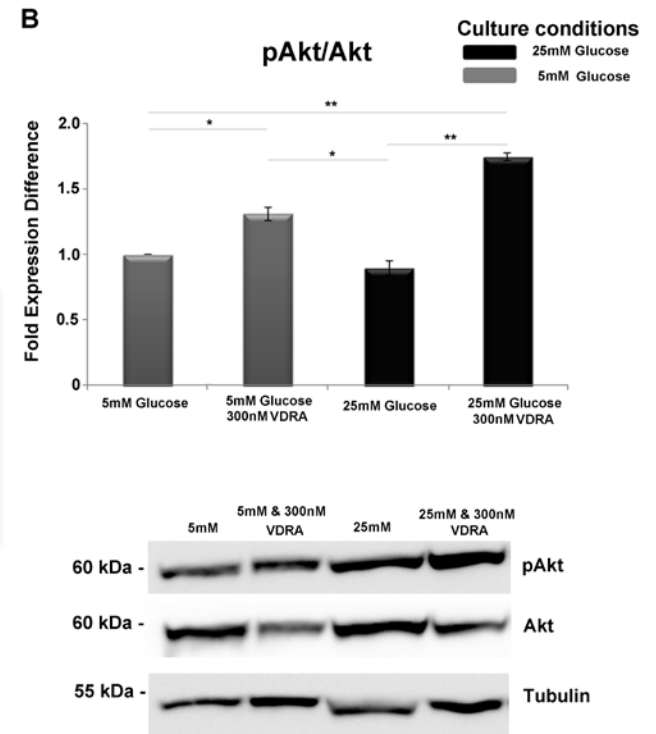
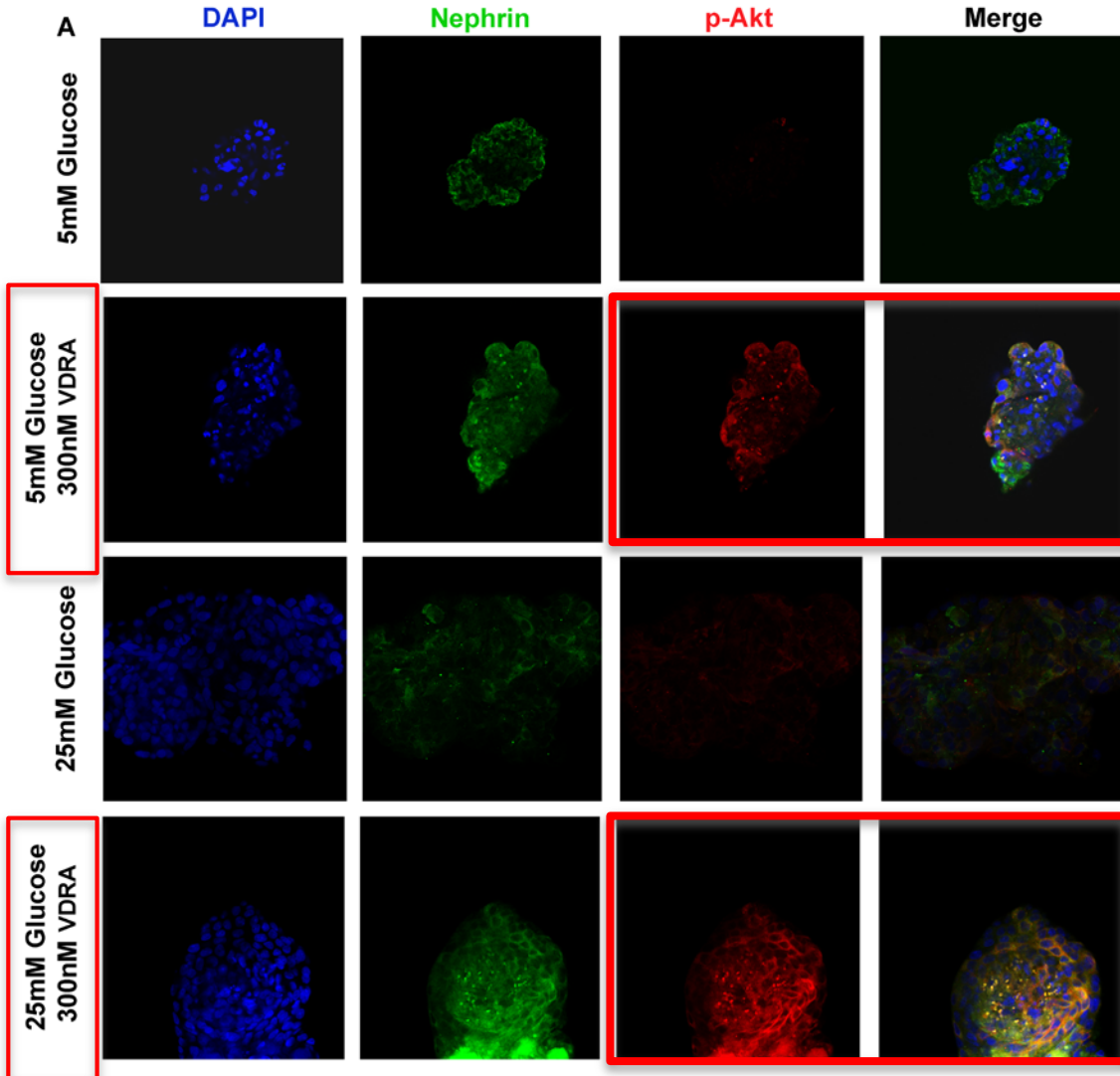
✓ Nephrin and PI3K





Results

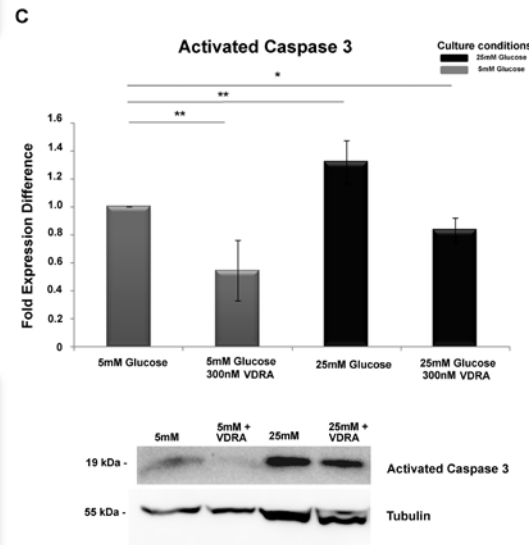
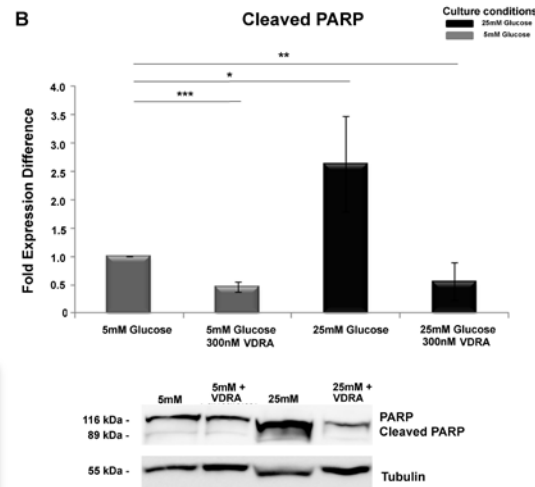
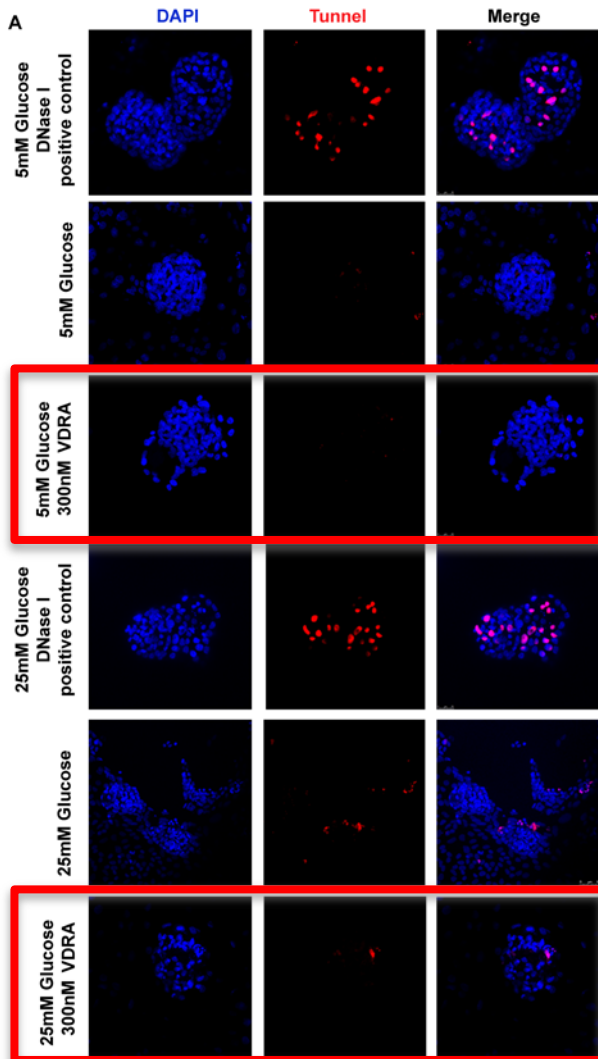
✓ Nephrin and p-Akt





Results

✓ Tunnel assay - Activated Caspase 3 & Cleaved PARP



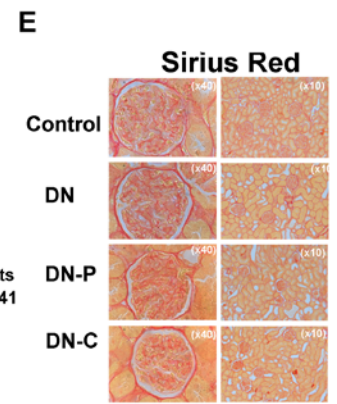
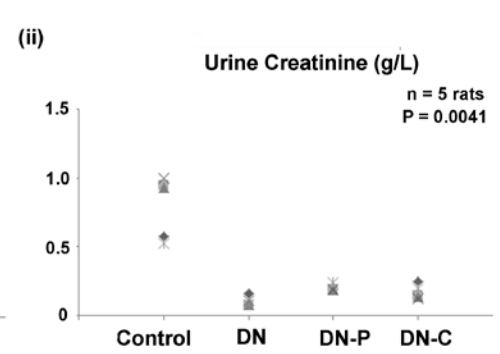
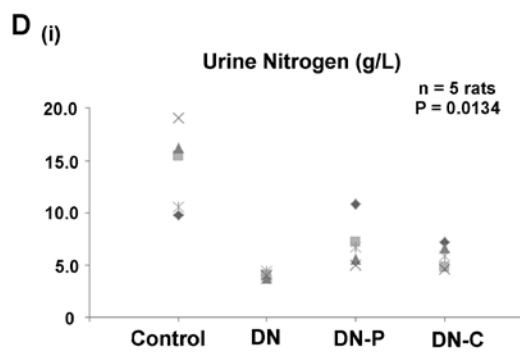
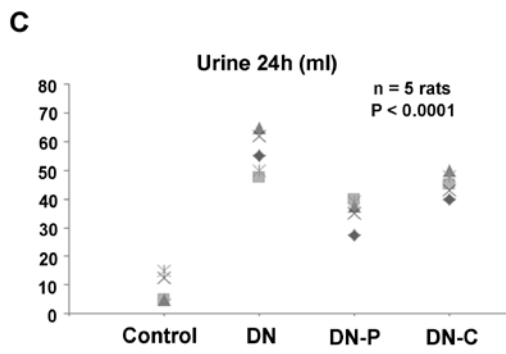
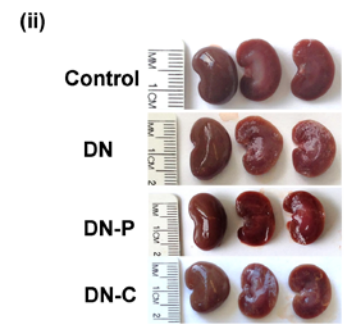
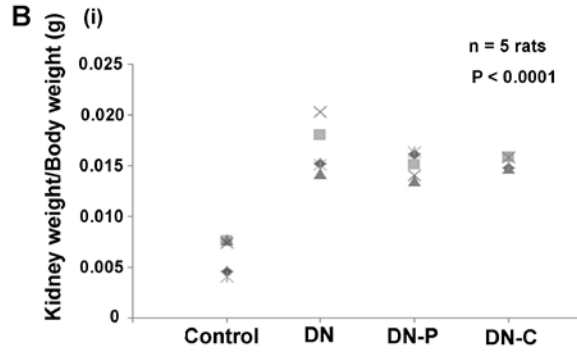
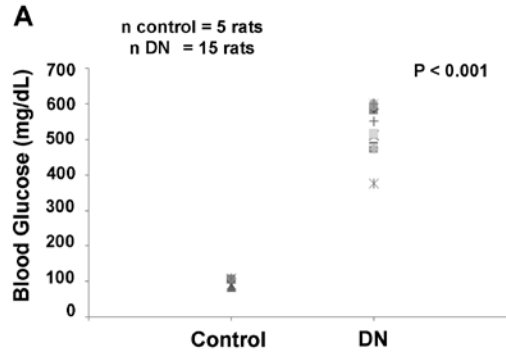
✓ **Paricalcitol** in presence of **normal or high glucose**, reduced the expression levels of activated caspase 3 and cleaved PARP.

+ **STZ animal model**

- ✓ **200g Wistar Rats**
- ✓ **65mg STZ/kg IP injection**
- ❖ **Diabetic Rats:** >370mg/dL glucose levels
- ❖ **After 3days:** 400ng/Kg/day Paricalcitol or
100ng/Kg/day Calcitriol
- ❖ **5 weeks**
- ❖ **After 10 days:** Insulin (10IU)

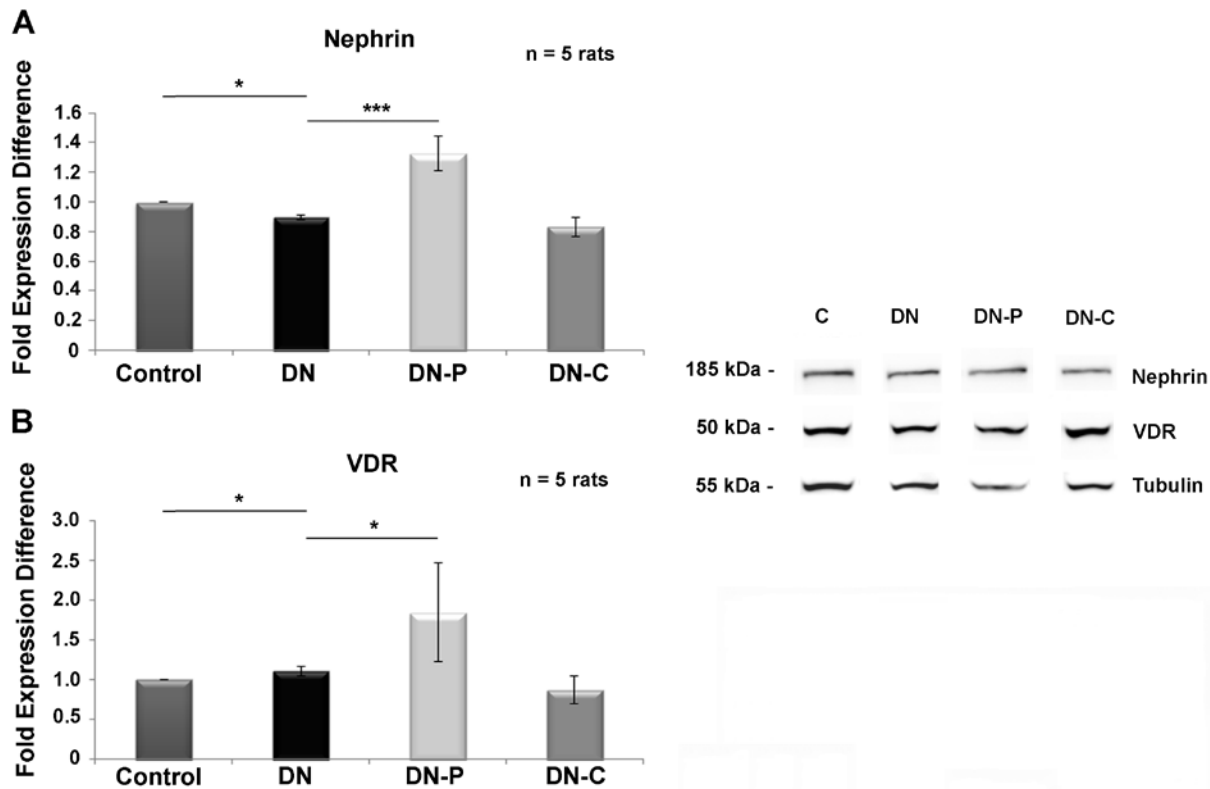


+ Results



+ Results

✓ Nephrin and VDR expression levels in isolated glomeruli after 5 weeks



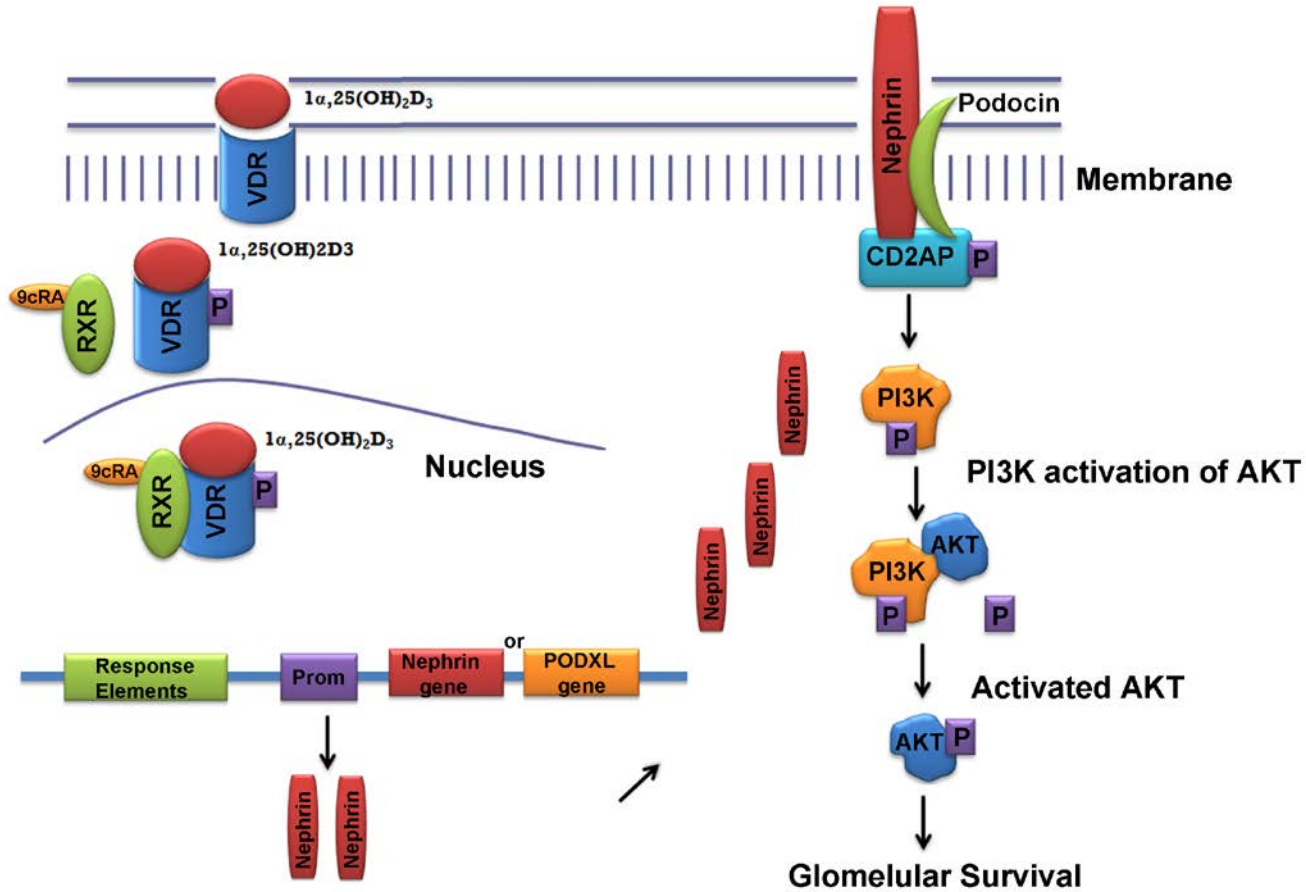
✓ **Nephrin and VDR were enhanced** in DN-animal model after 5 weeks treatment.

+ Summary

- ✓ Podocyte markers are **downregulated** in presence of **high glucose**.
- ✓ **Paricalcitol** restores Nephrin and PODXL expression levels.
- ✓ VDR expression is **enhanced** in presence of **high glucose** and is **activated** in presence of **paricalcitol**.
- ✓ **Upregulation of PI3K and pAKt** in presence of paricalcitol.
- ✓ Paricalcitol treatment ameliorates high glucose induced apoptosis.



+ Summary



❖ Vitamin D3 and its analogue, paricalcitol may have beneficiary effects in diabetic nephropathy.

+ Acknowledgement

Nephrology Center "G.Papadakis",
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STAVROS NIARCHOS
FOUNDATION