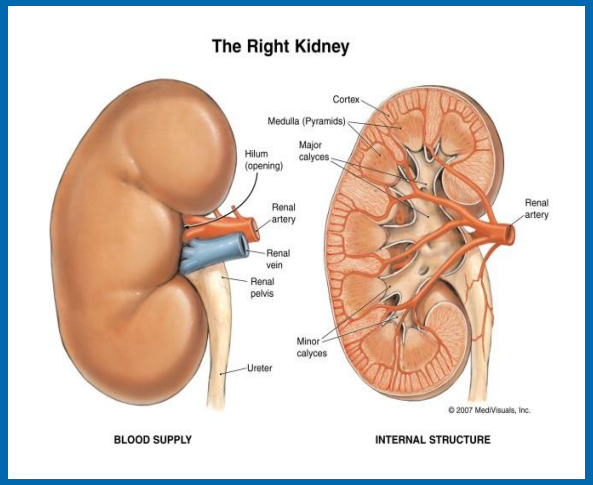
Experimental platforms to address mesangial repair: What has been accomplished

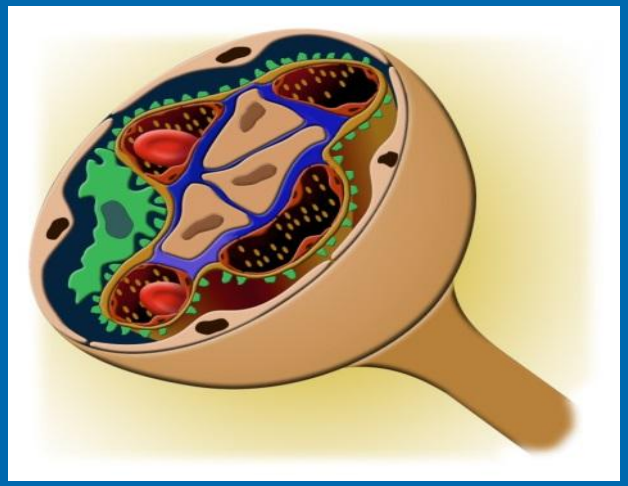
Jiamin Teng M.D., Ph.D.

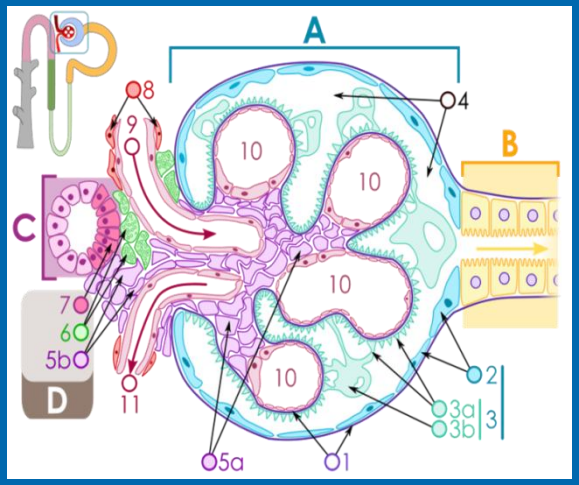
Department of Pathology and Translational Pathobiology
Louisiana State University Health Sciences Center
Shreveport, Louisiana, USA

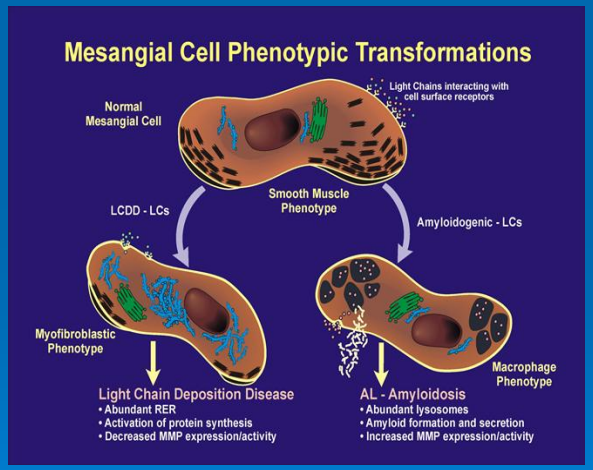
- Focused on mesangial repair and restoration of the mesangium after homeostasis has been altered, various experiment models including in vitro, ex vivo and in vivo have been used in our laboratory in the last 30 years.
- The most recent animal models have recreated the various mesangiopathies that are characteristic of glomerulonephritis.

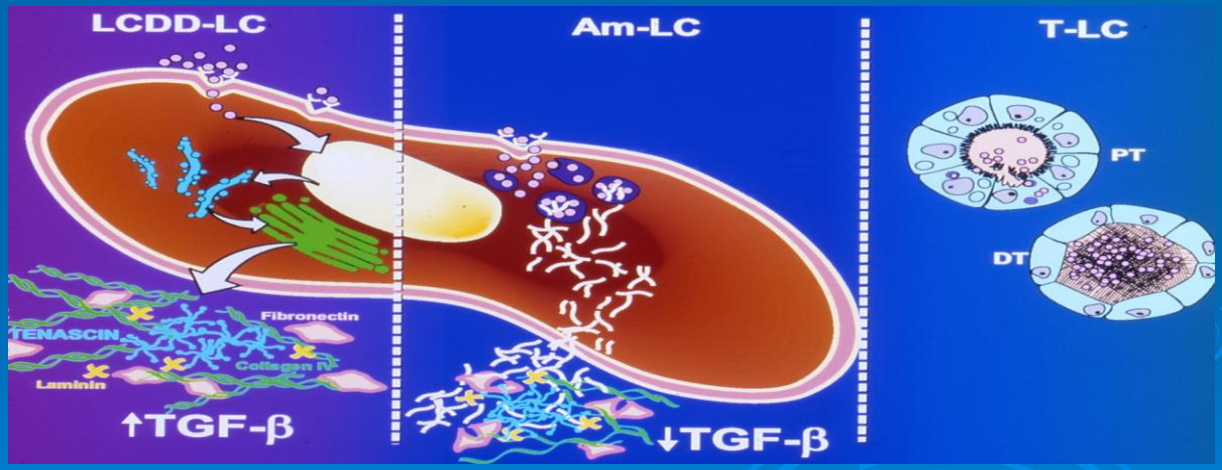
Nephron and Light chains





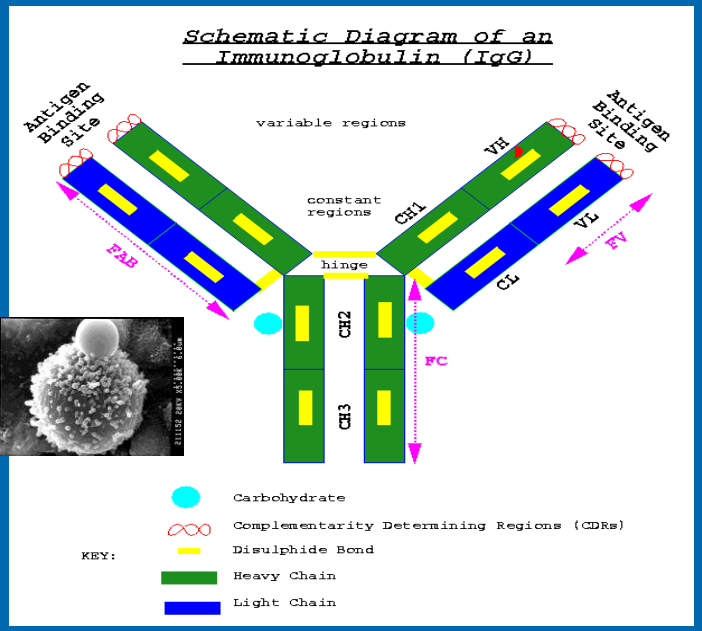


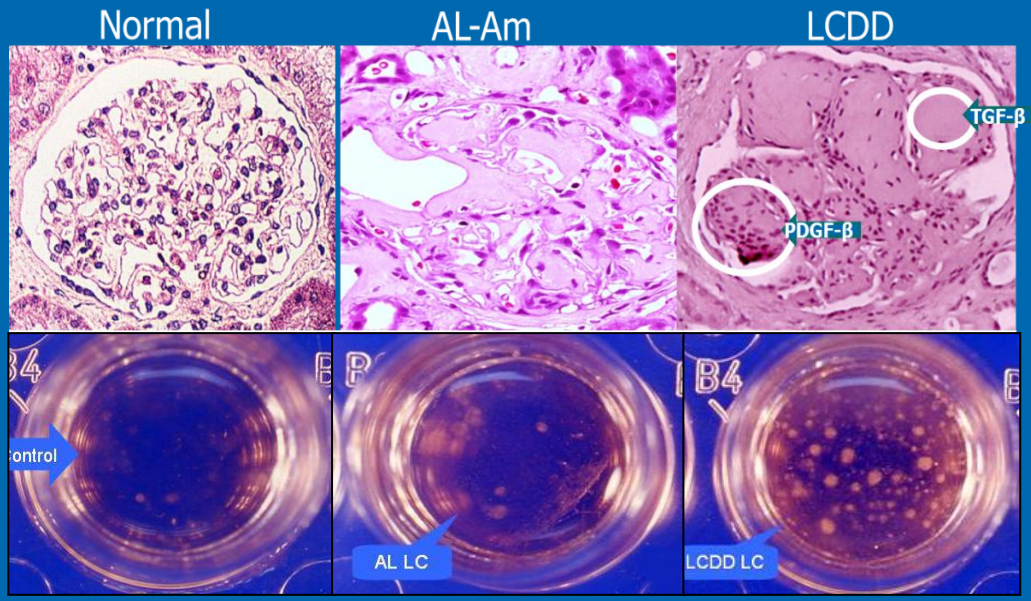


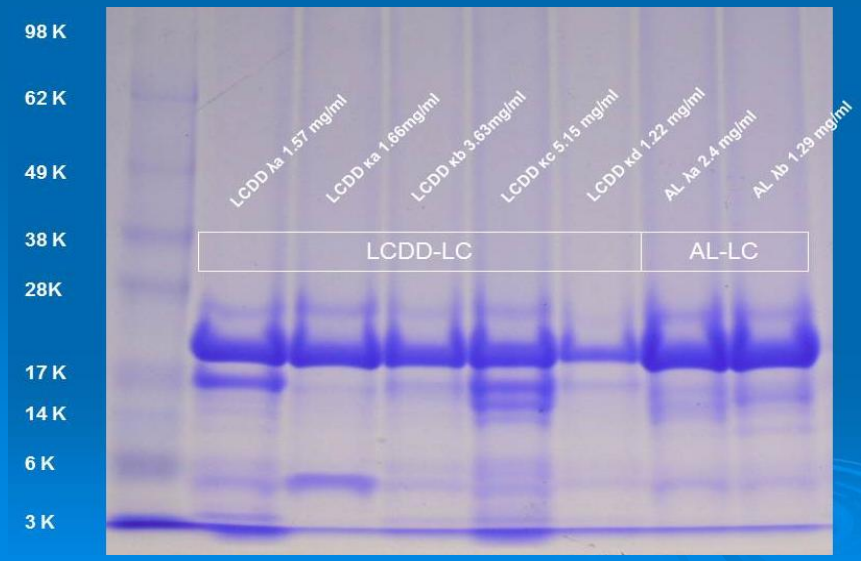


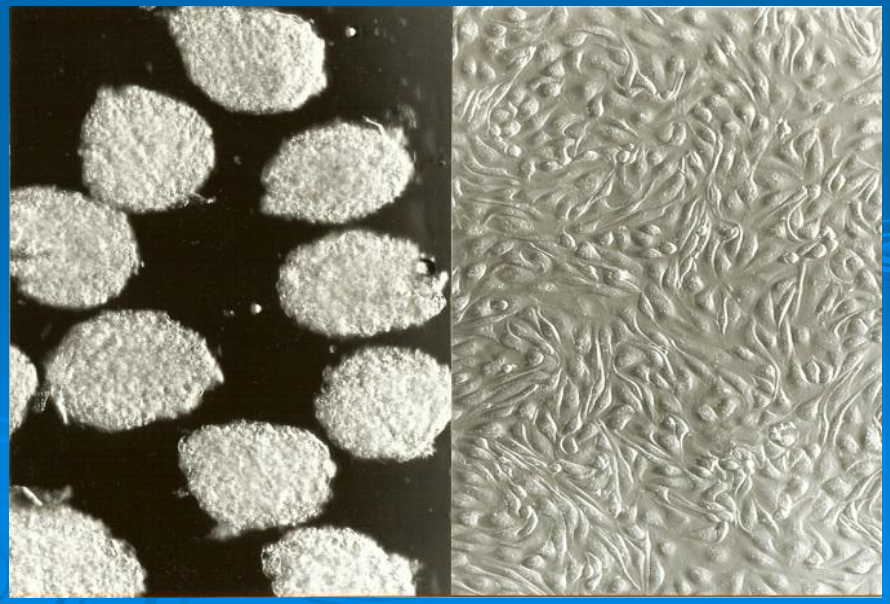
- Glomeruli composed of endothelial cells, visceral / parietal epithelial cells and mesangial cells,
- Normally, light chains pass through the capillary walls into urinary space and catabolized in proximal tubules.
- G-LCs interact with mesangial cells and alter mesangial homeostasis.

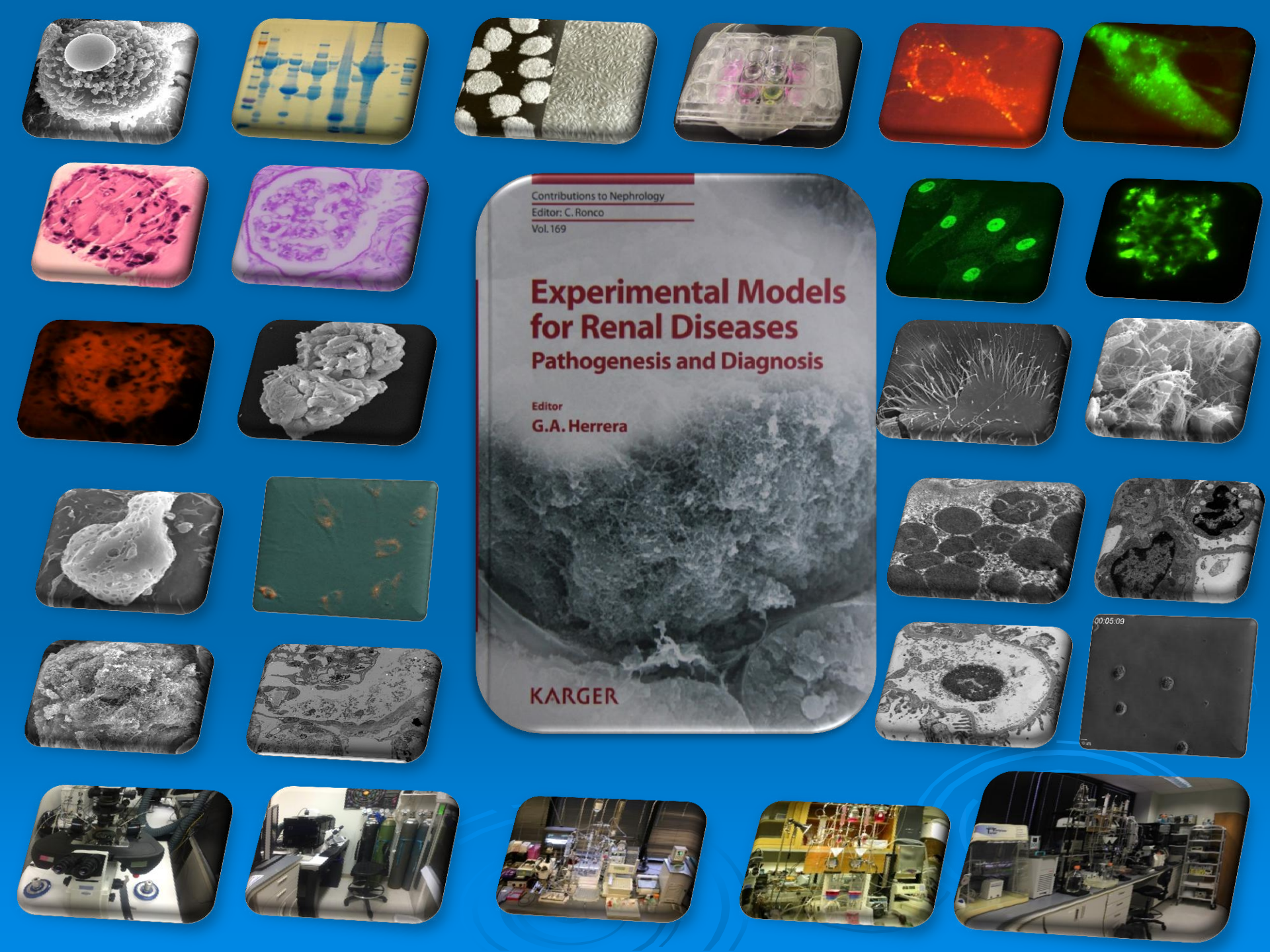
Mesangial cells and glomerulopathic light chains



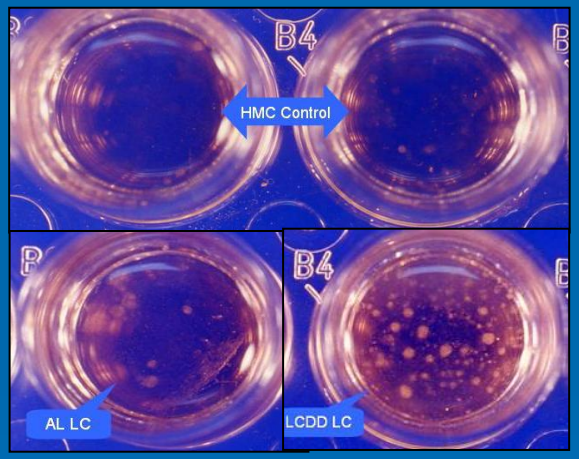


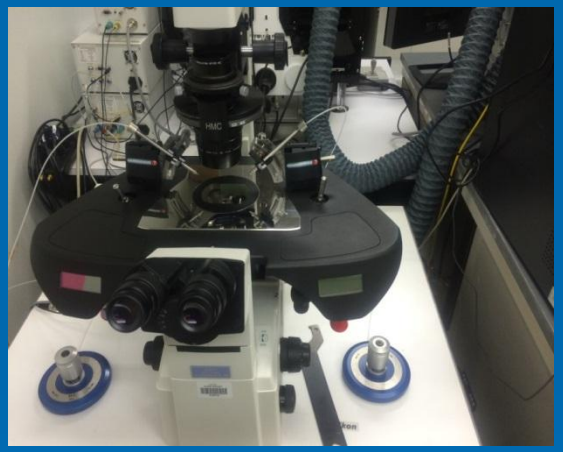


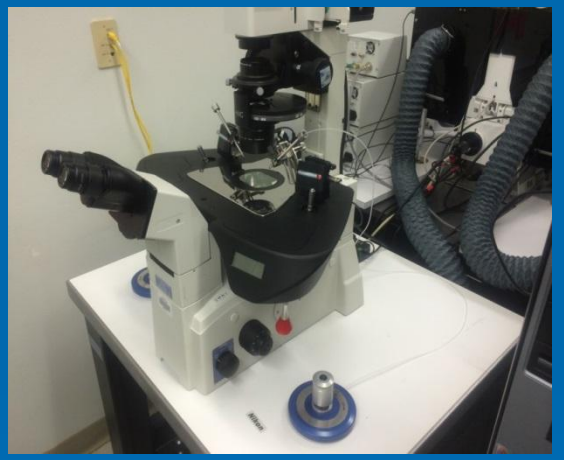



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Pathogenesis and Diagnosis
Editor
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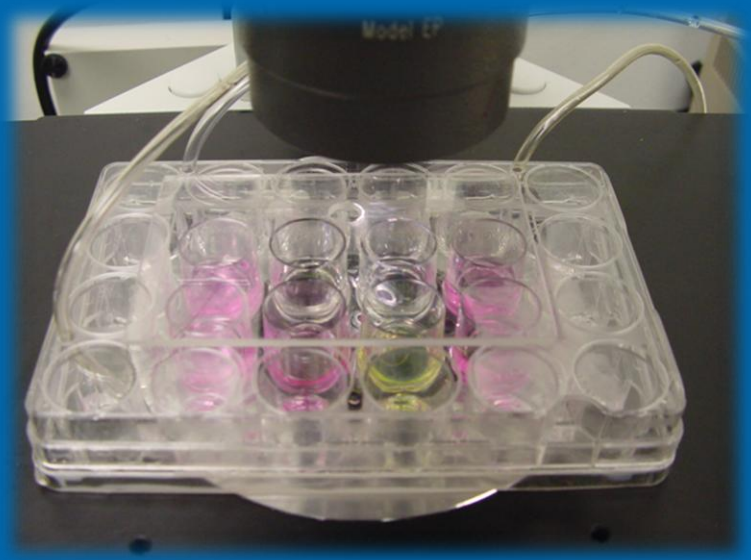
Live cell micromanipulator system





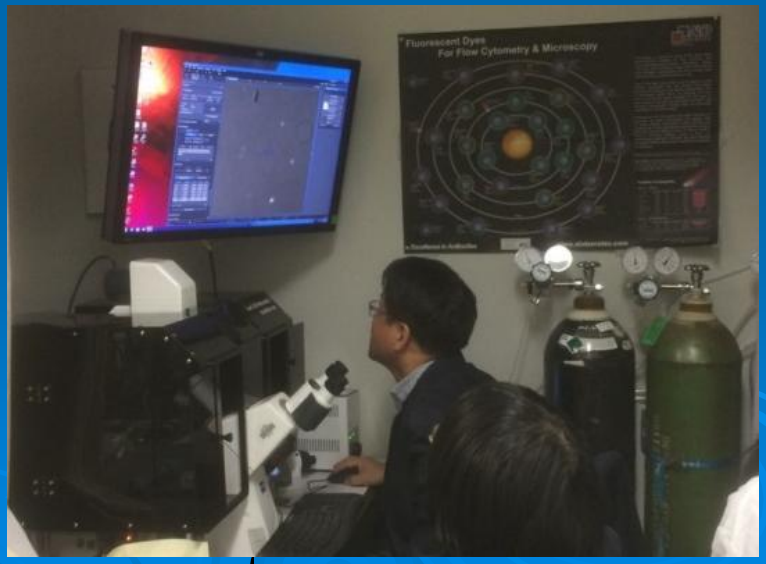


6-D Live cell imaging system



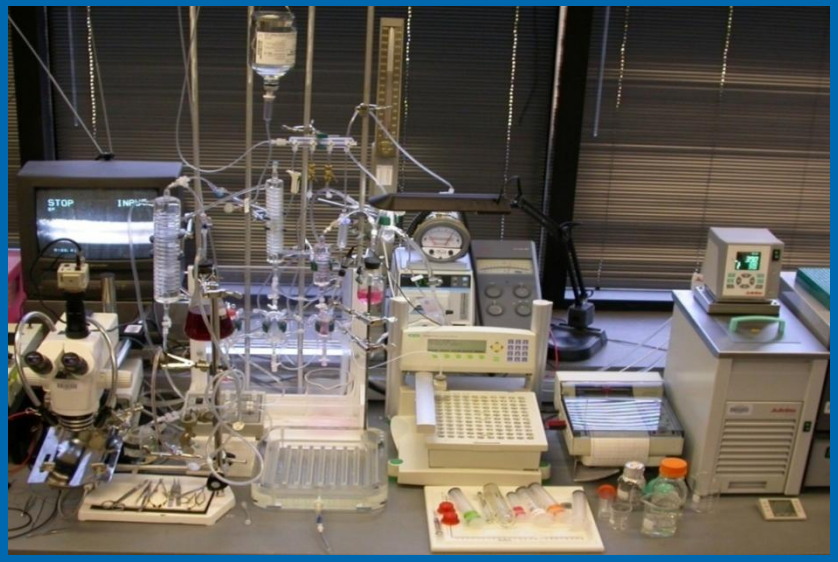


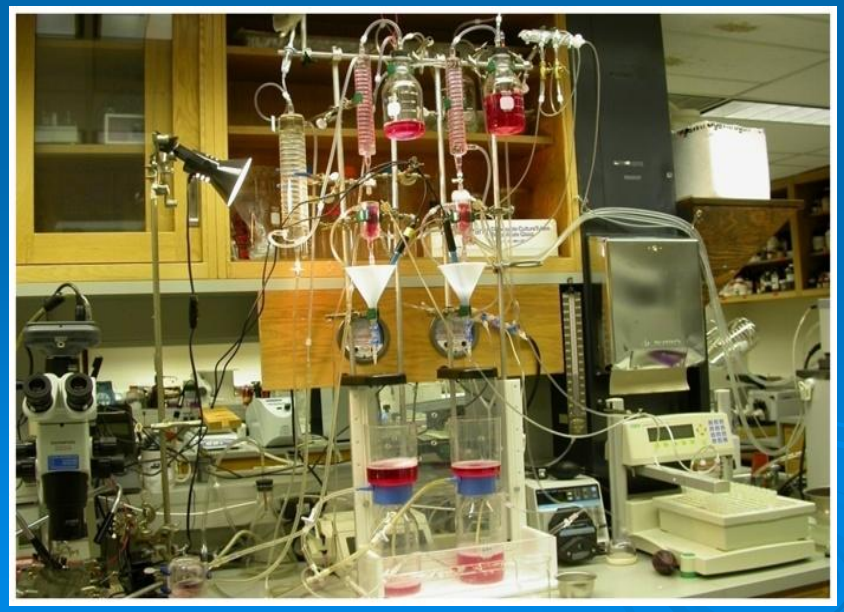


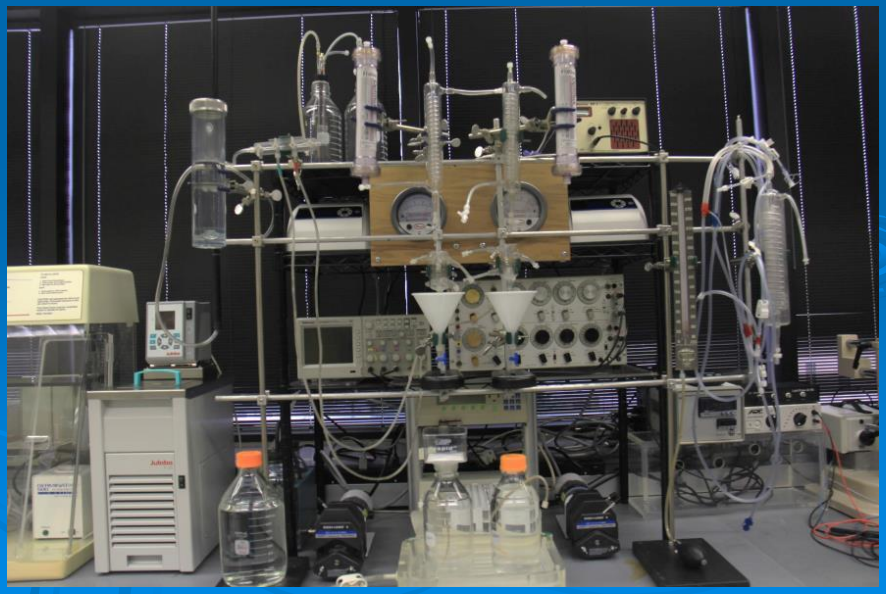


Ex-Vivo kidney perfusion system

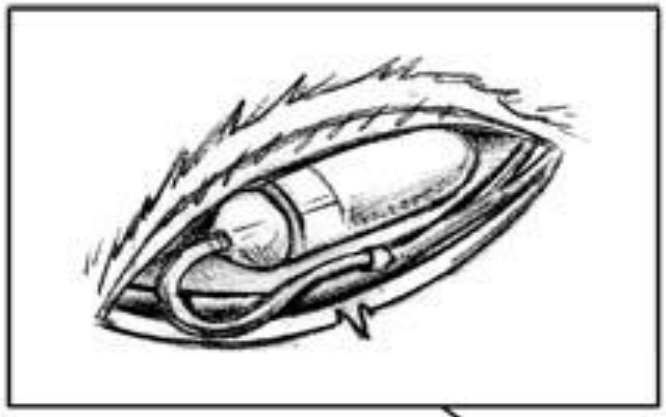




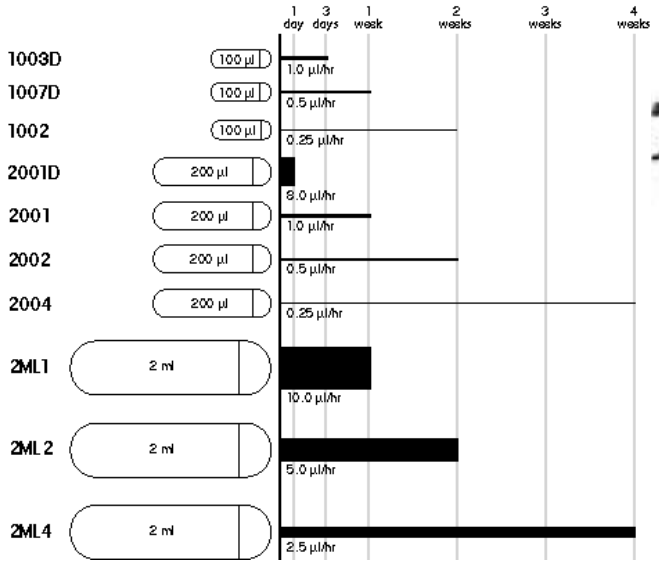




Osmotic pump administration model

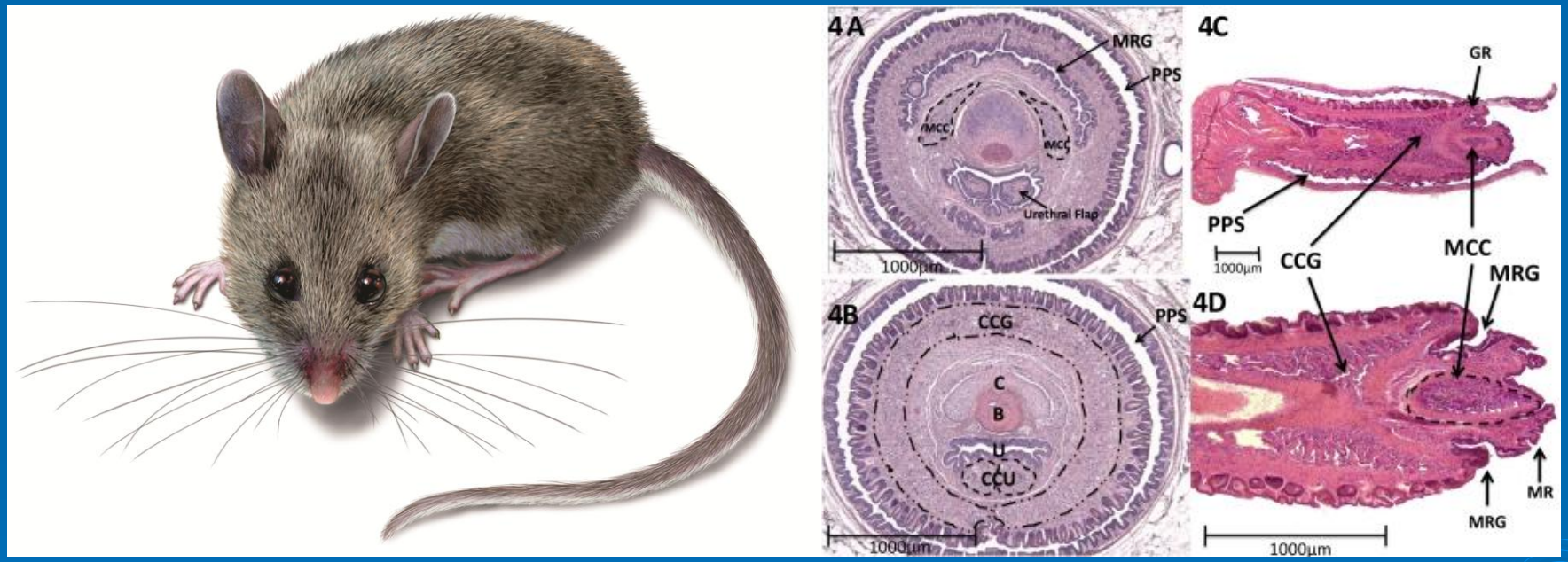




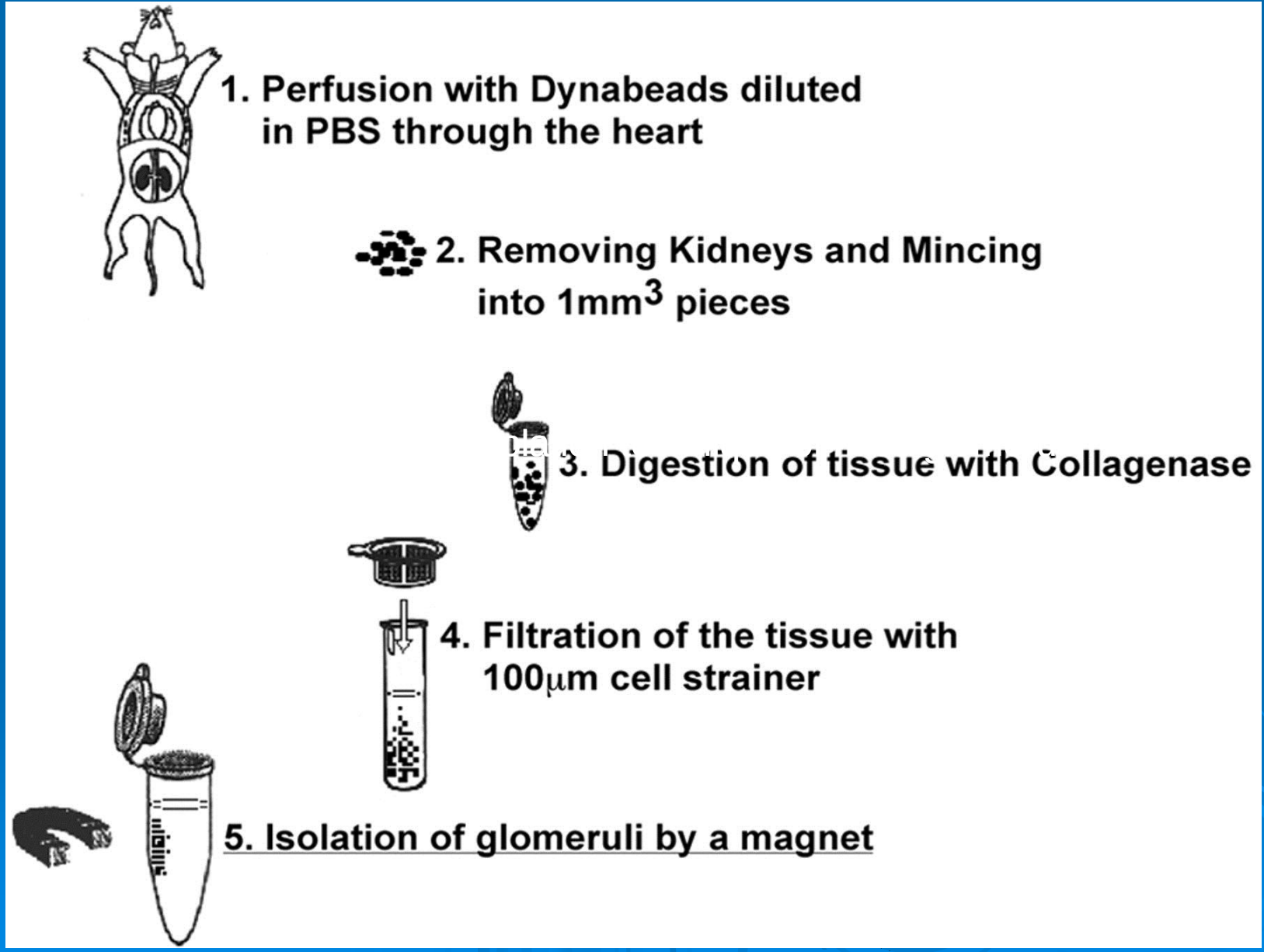




In vivo Mouse Penile injection model

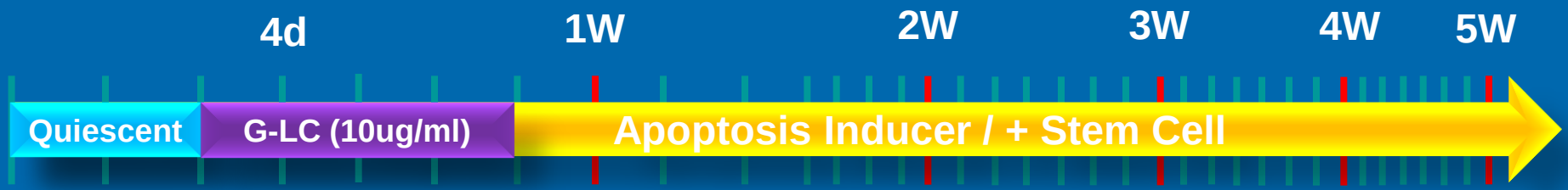


Isolation technique of mouse glomeruli

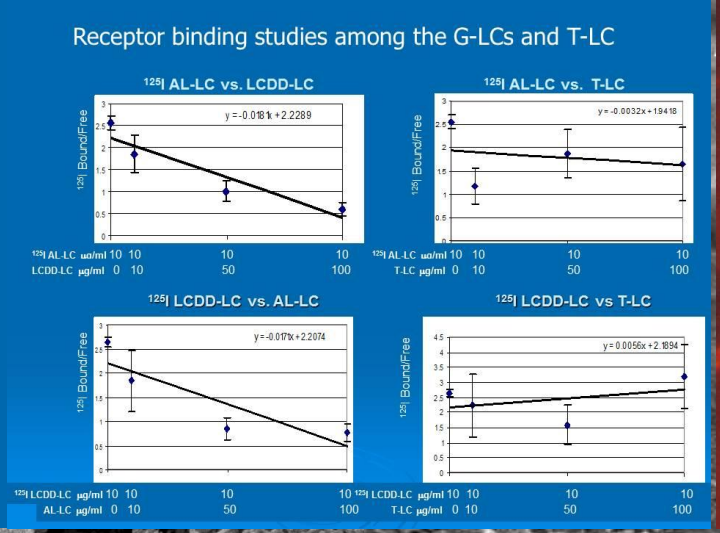


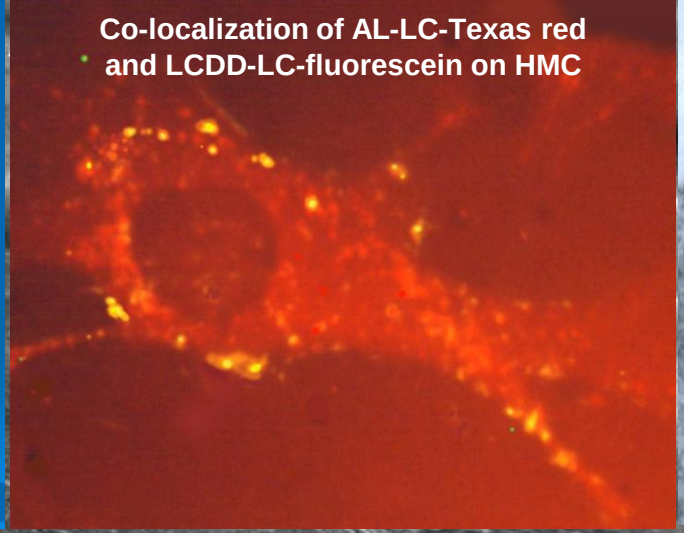
Takemoto M. et al: American Journal of Pathology. 161(3):799-805, 2002 Sep.

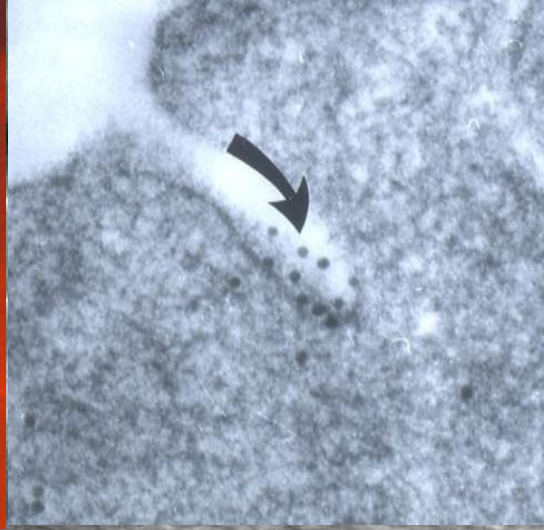
Experiment Design

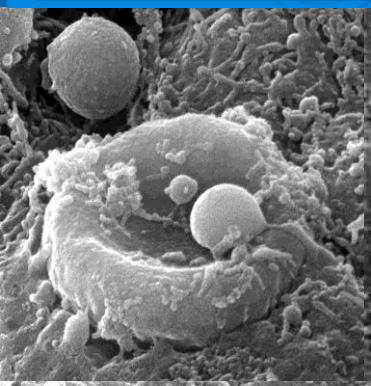


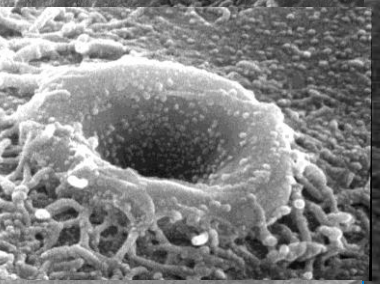
1. Cell level - 2D : MCs incubated as monolayer
2. Cell level - 3D: MCs growing on Matrigel
3. Cell level - 6D: Live cell imaging system
4. Glomeruli level - In vitro single glomerular system
5. Animal level- In-Vivo penile injection
6. Kidney level - Ex-vivo kidney regeneration system

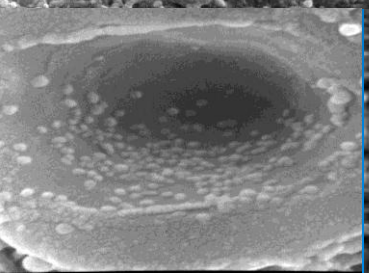


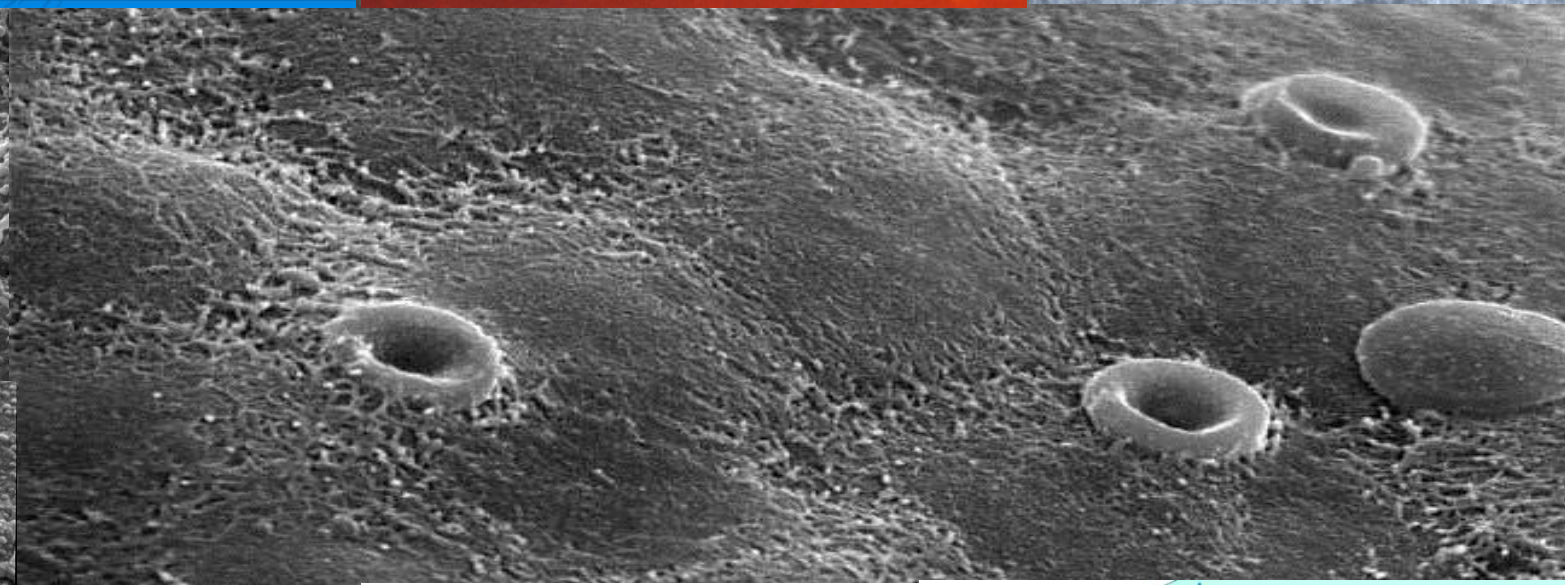


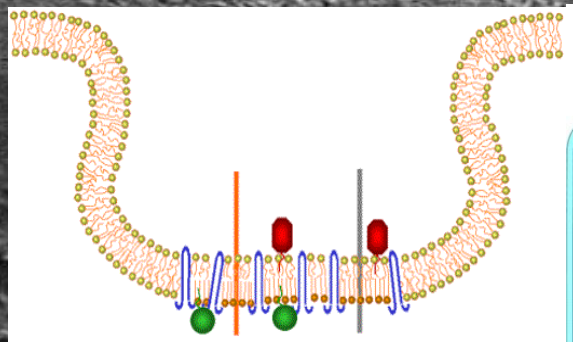


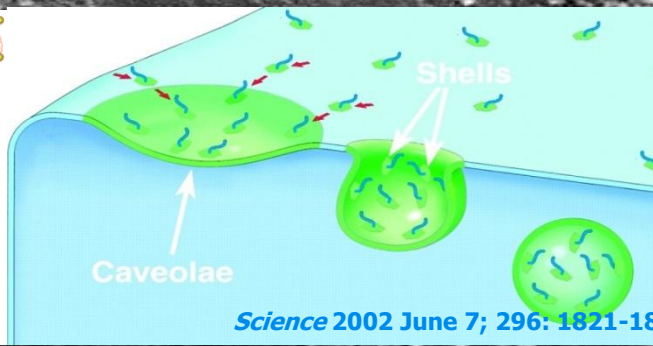






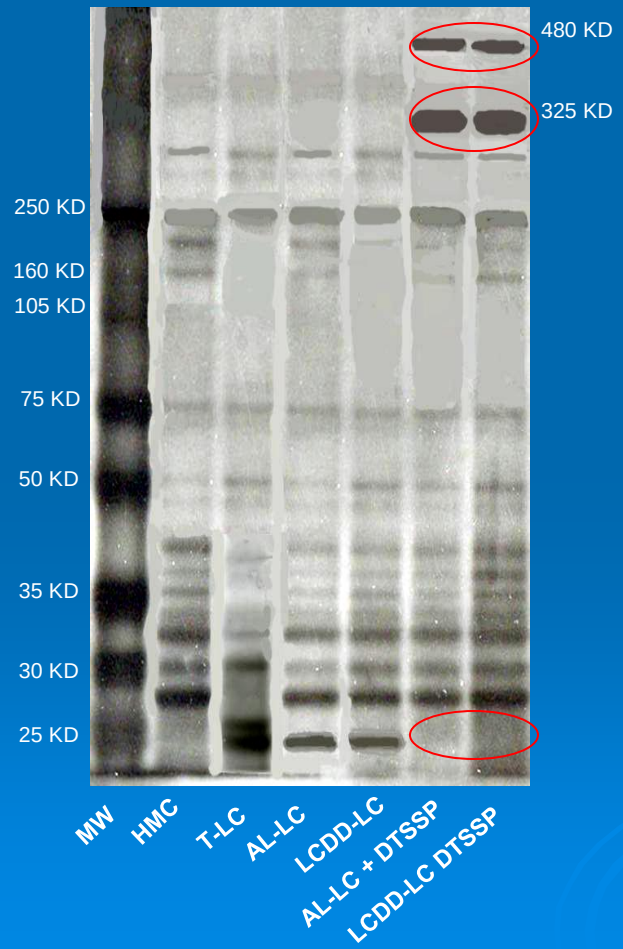


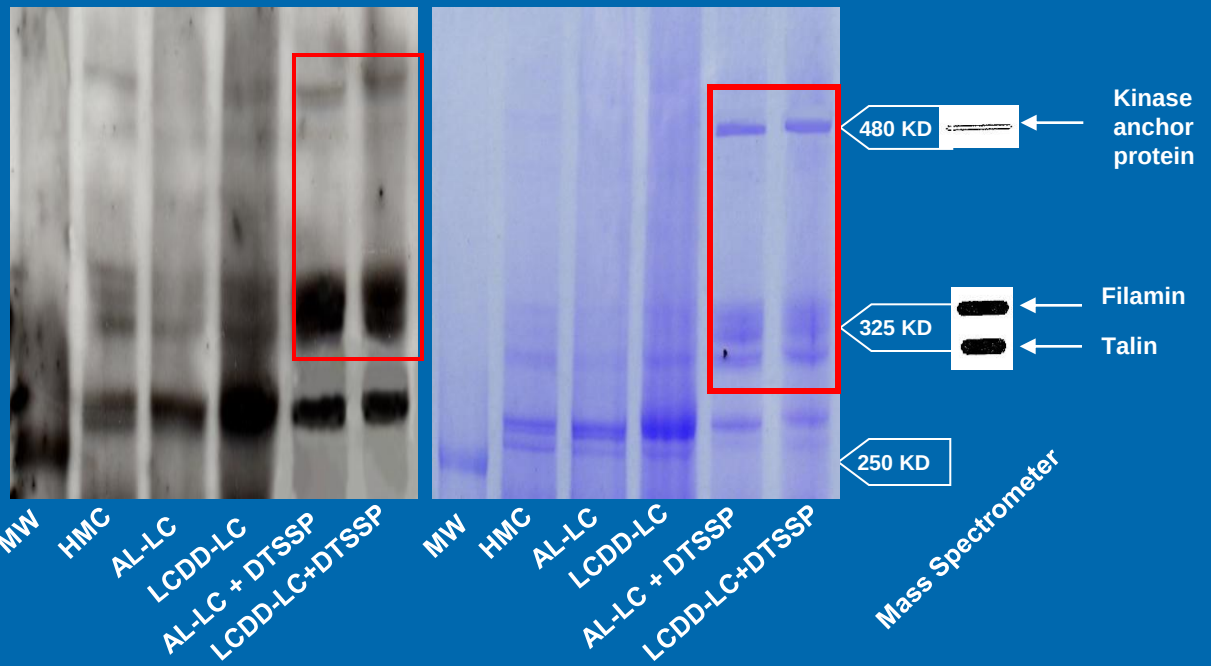


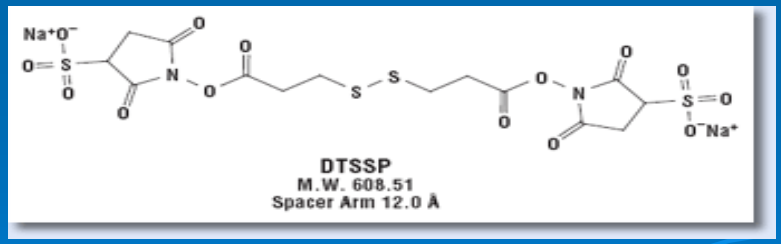


Science 2002 June 7; 296: 1821-18

Crosslinking of G-LCs to HMCs membrane

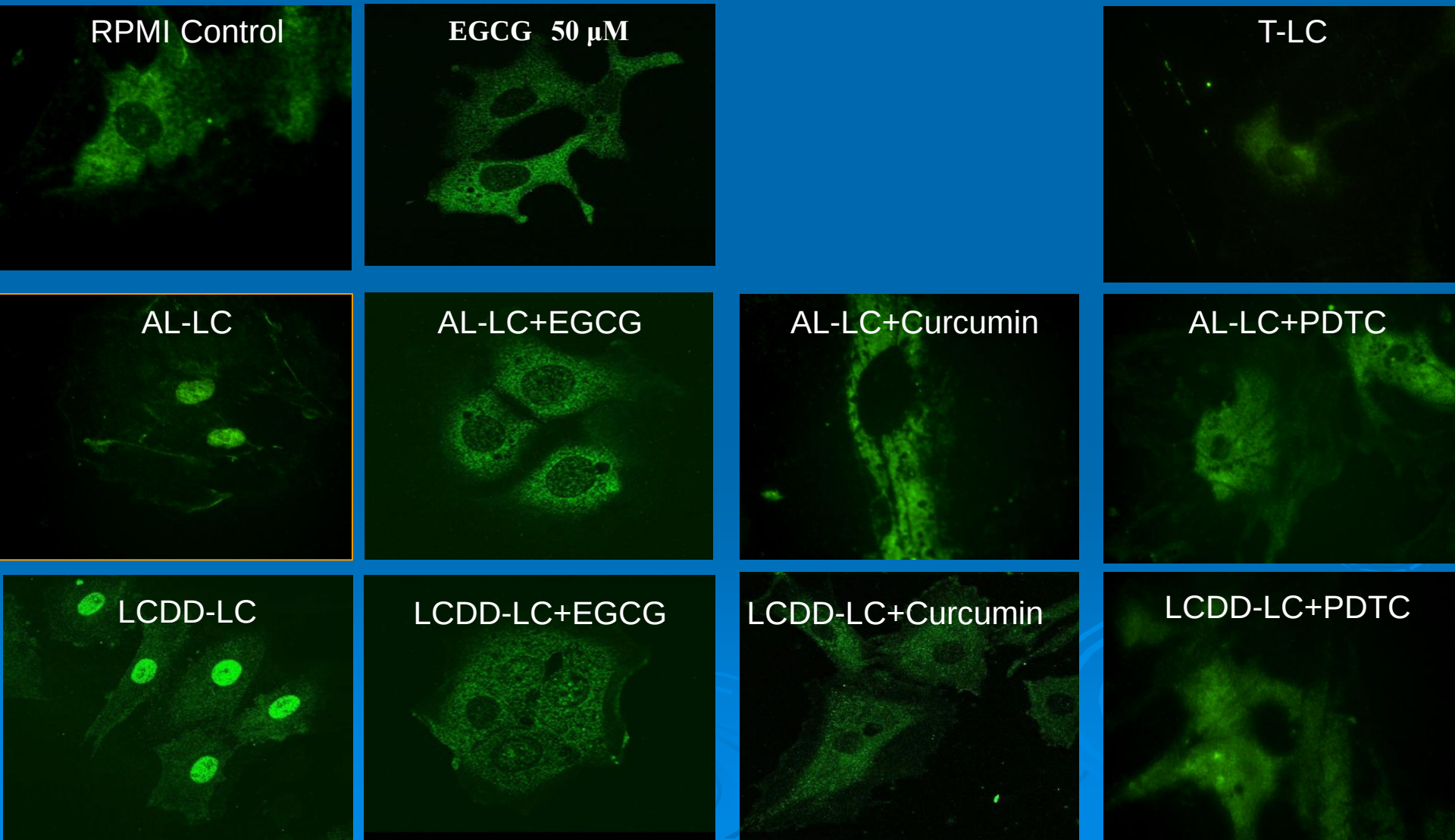




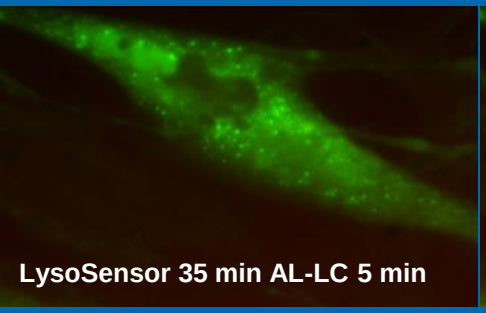


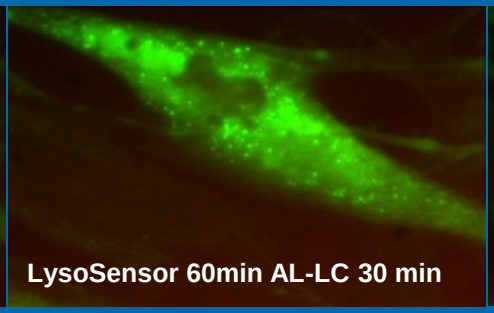
- DTSSP: 3,3´-Dithiobis (sulfosuccinimidylpropionate), is a water-soluble and membrane impermeable crosslinker.
- DTSSP is well used for cell-surface crosslinking.

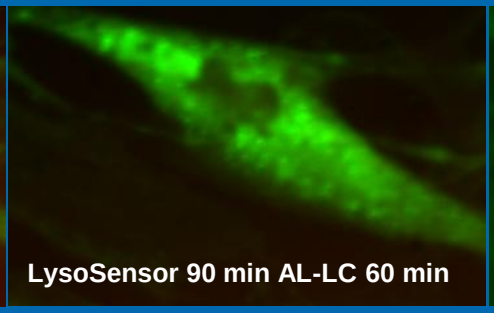
Curcumin, EGCG and PDTC prevented NFkB translocation in G-LCs treated HMC

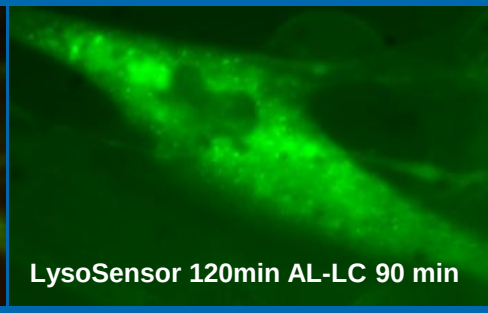


AL-LCs induced expansion of lysosomal compartment

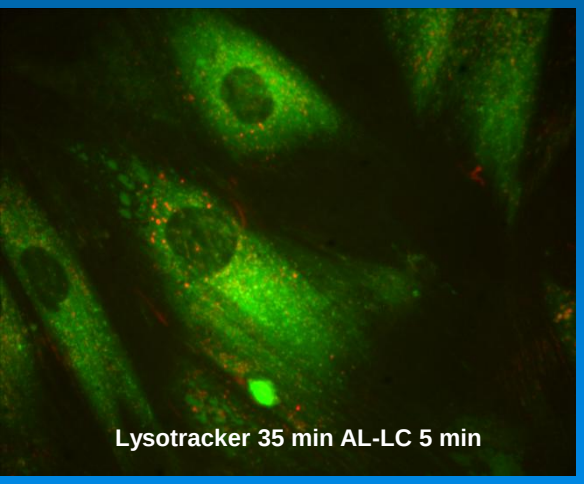


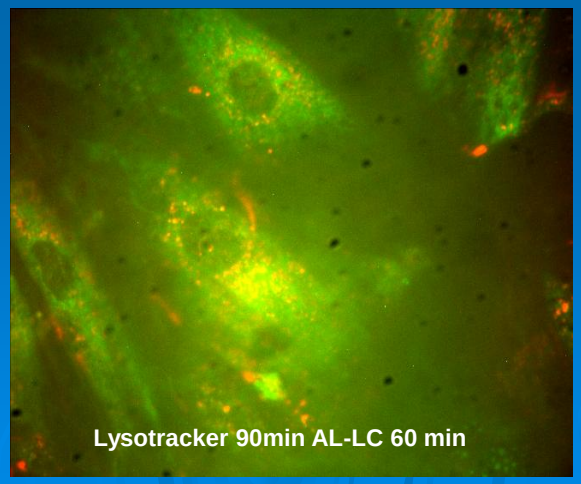


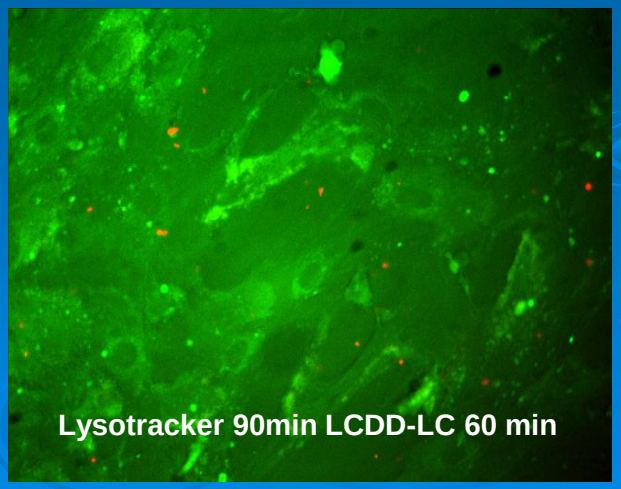




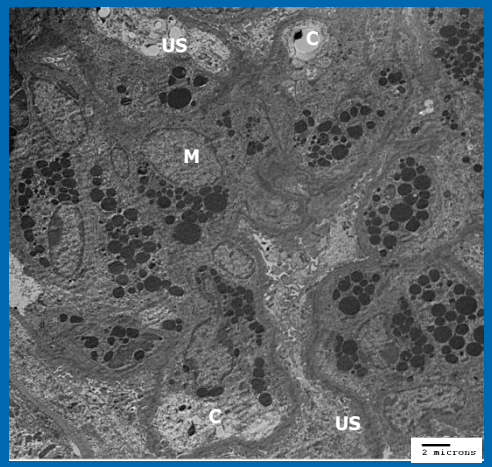
AL-LCs but not LCDD-LCs co-localized with mature lysosomes

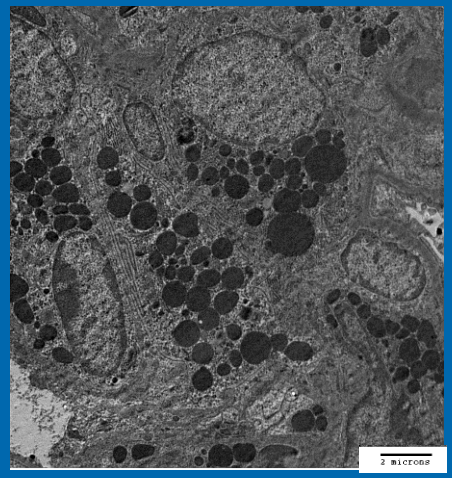


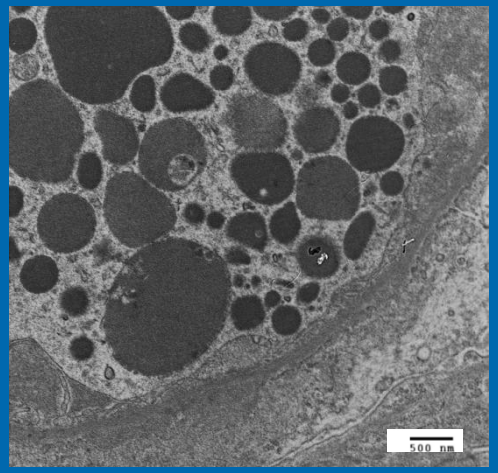


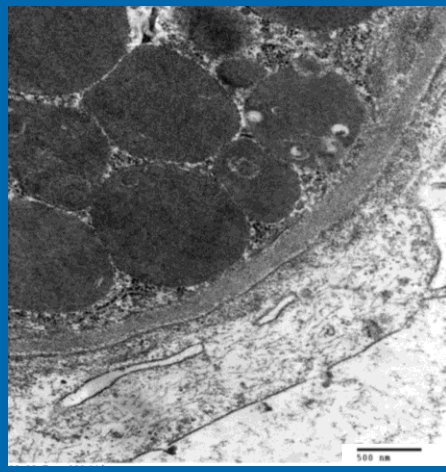


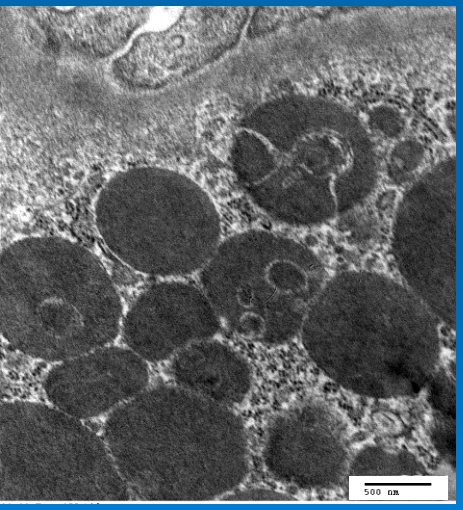
Numerous lysosome formed and amyloid fiber precursor processed in mouse glomerular lysosome after penile injection of ALLC for 2 weeks

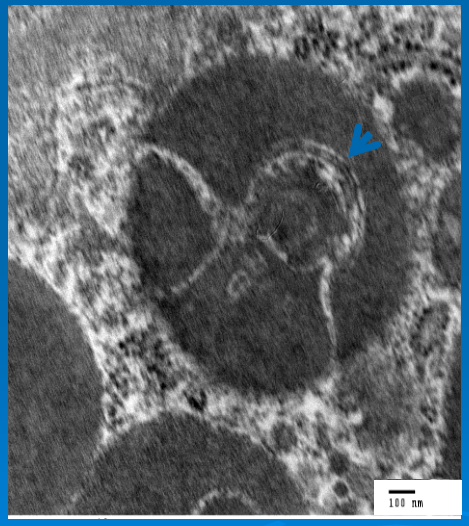


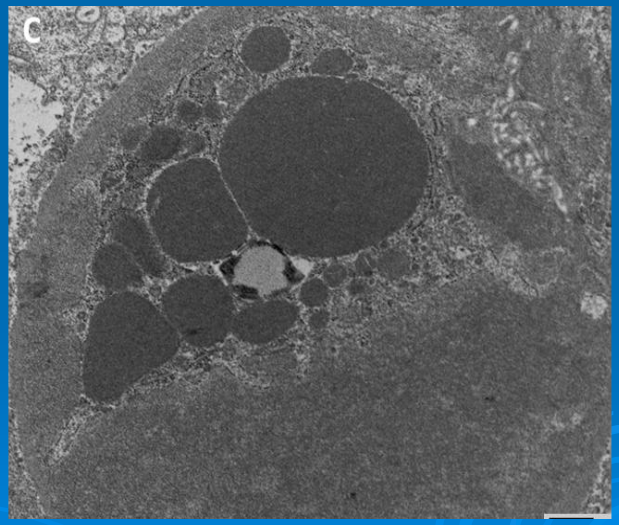


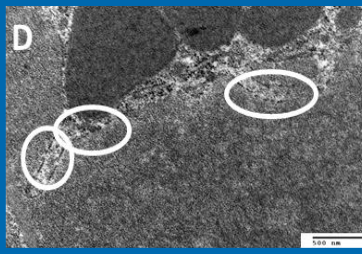


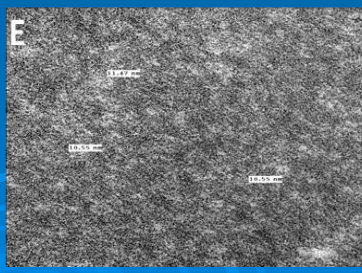




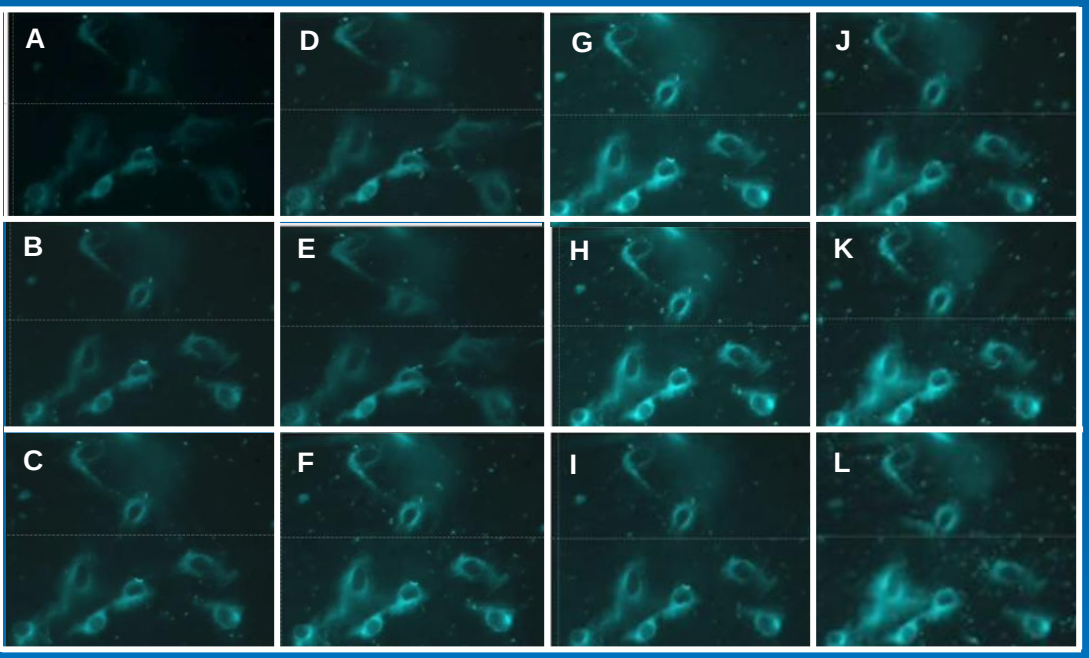


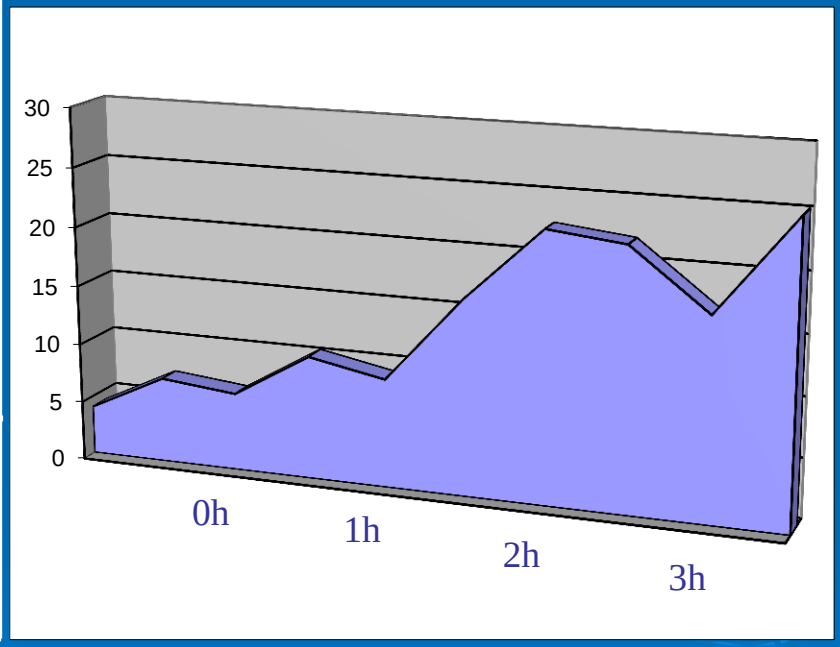






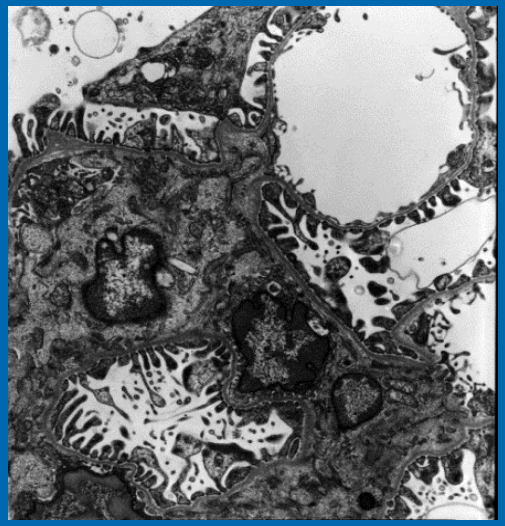
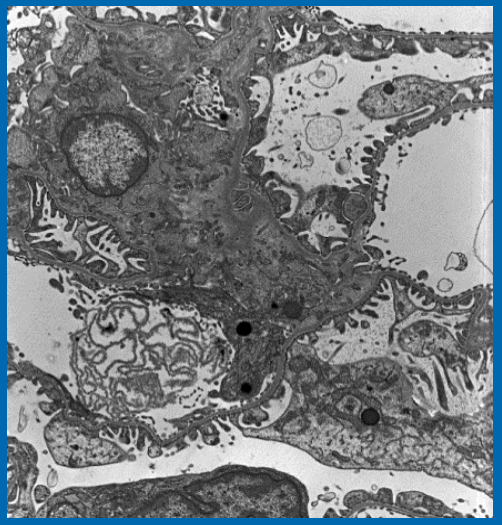
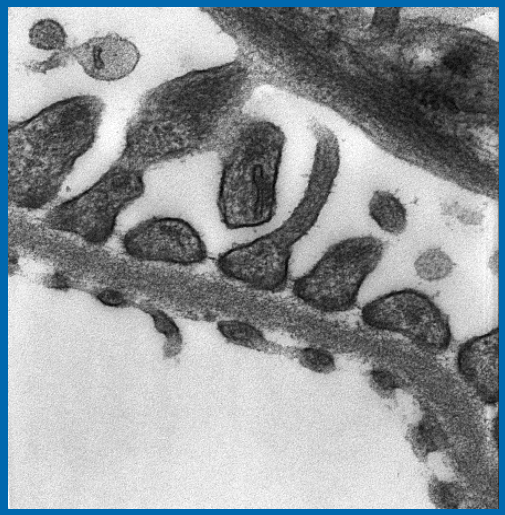
LCDD-LC induced ER expansion in HMCs



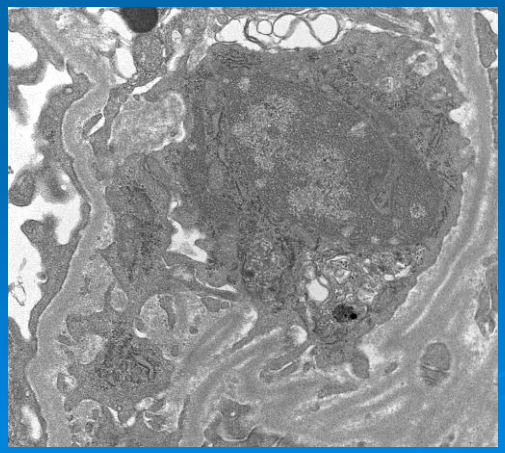
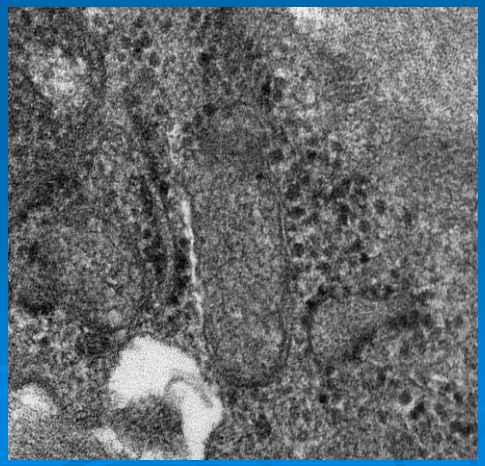
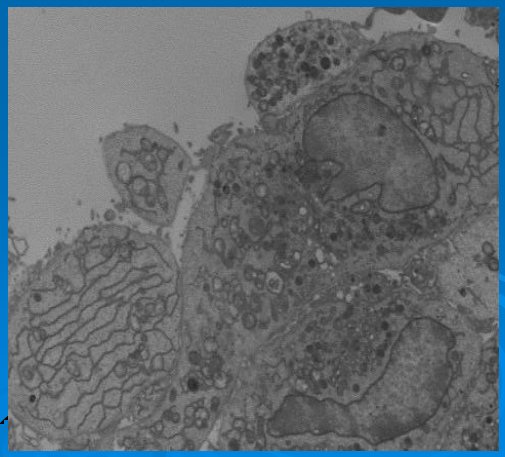


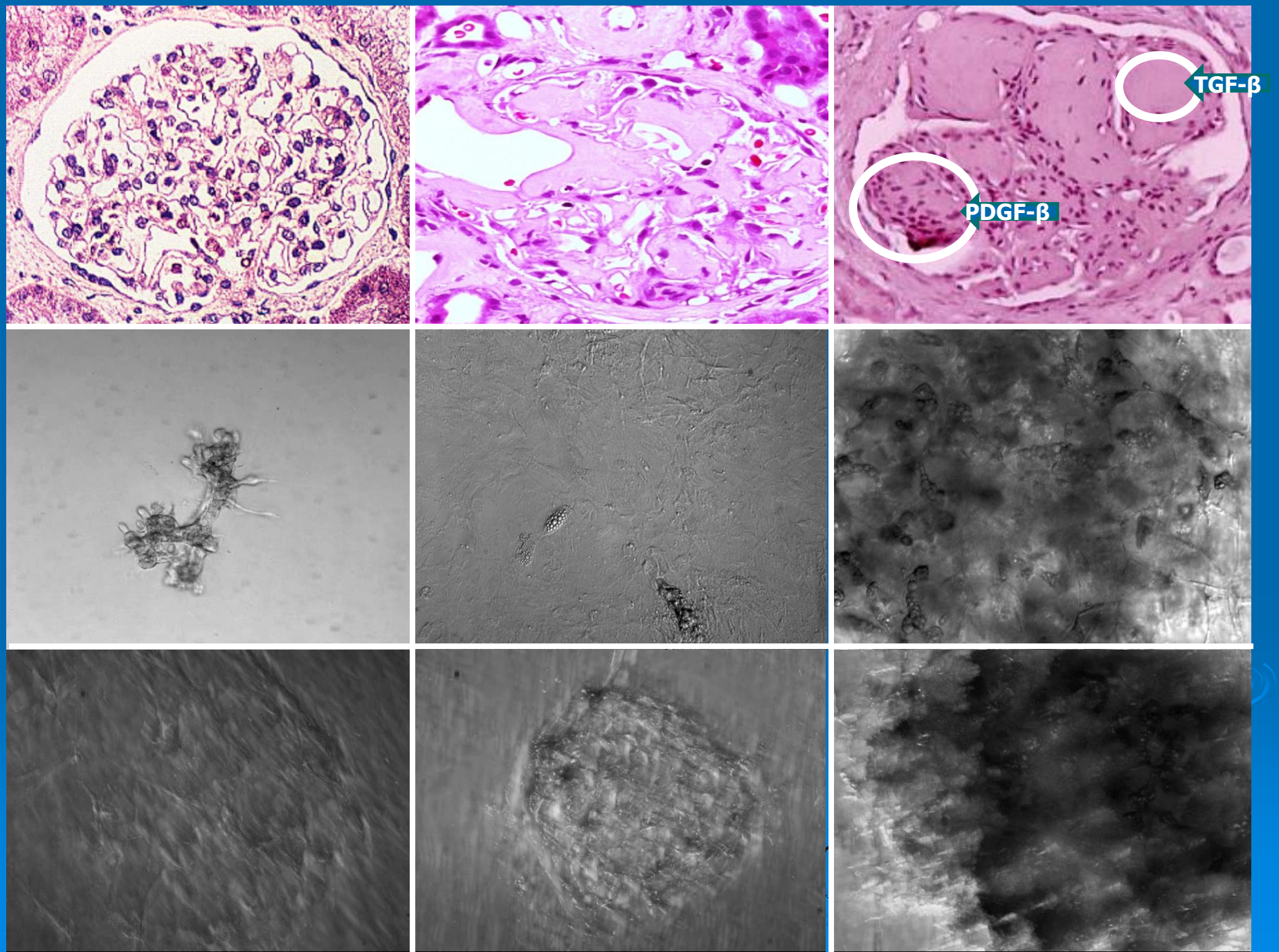
LCDD-LCs increased ribosomes and RER in HMCs

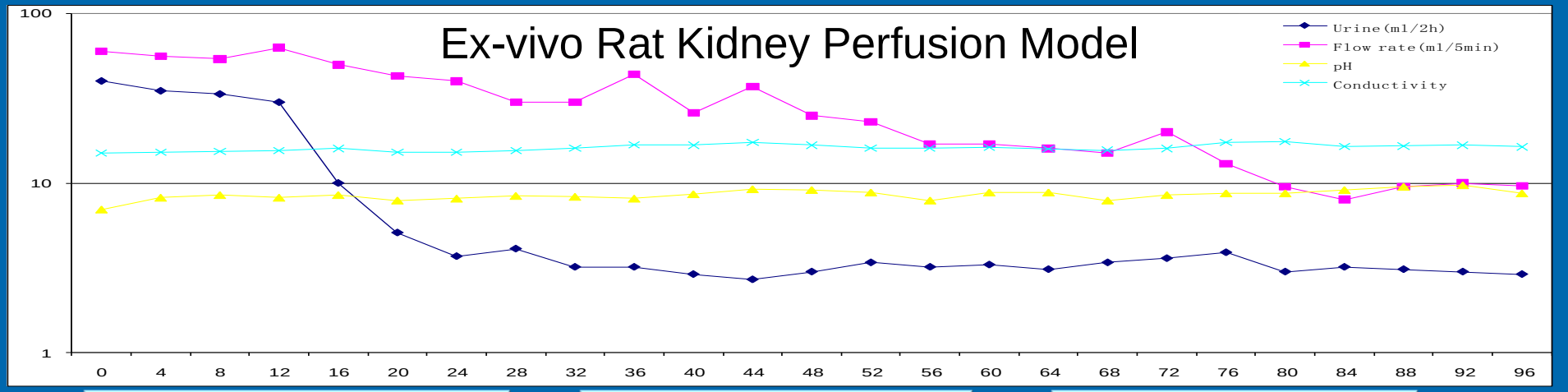
Rat kidney perfusion LCDD k 3h

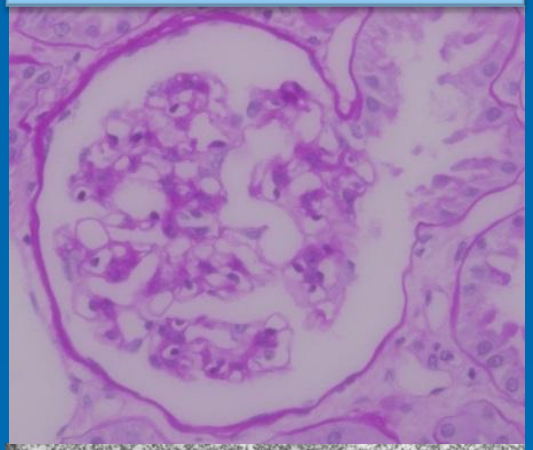
Rat kidney perfusion LCDD k 24h

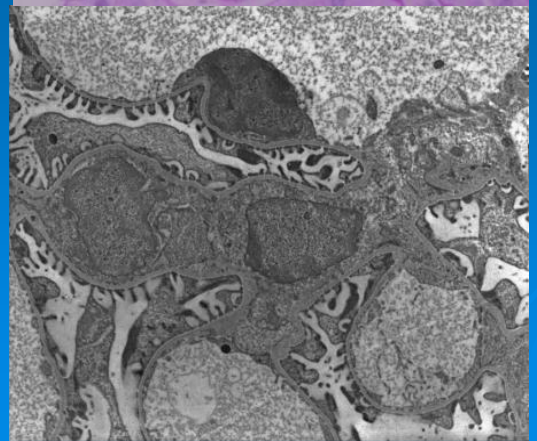





Normal
AL-Am
LCDD
TGF-β
PDGF-β

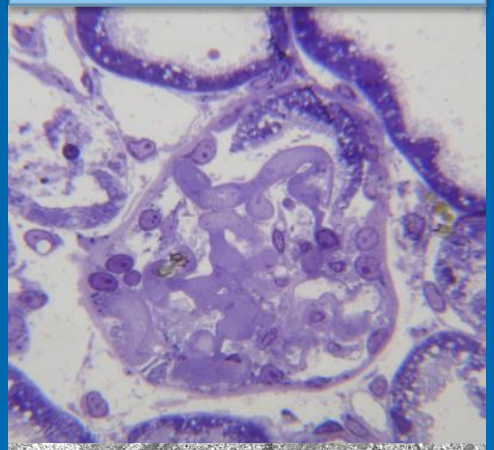


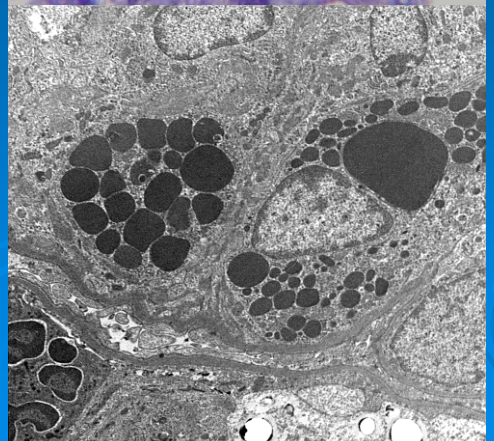
Control



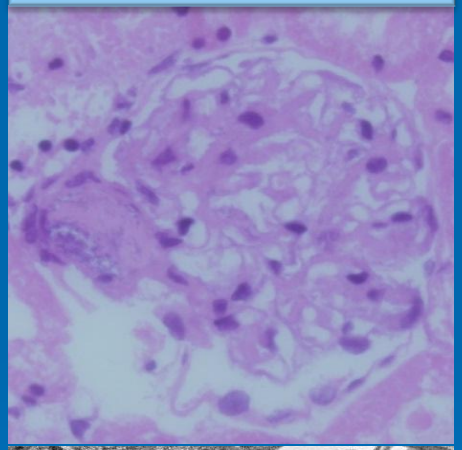


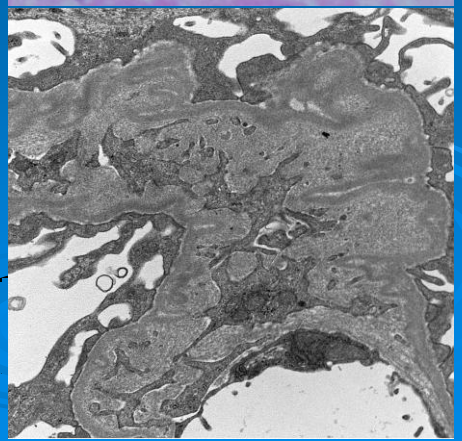
ALLC



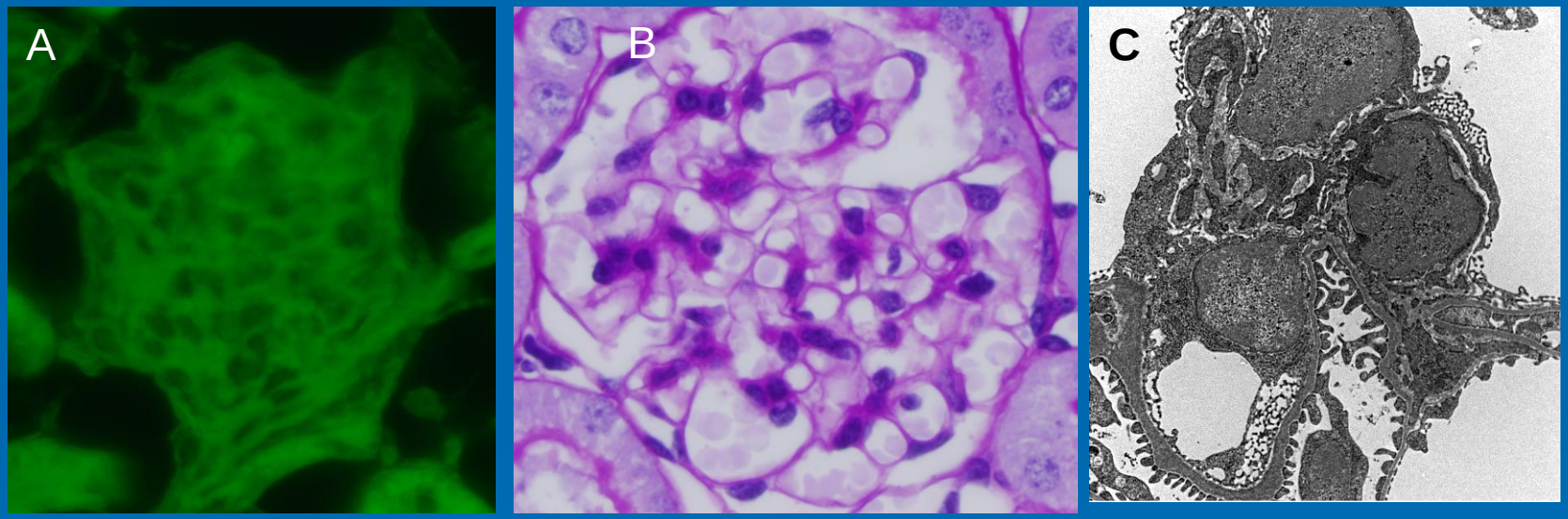


LCDDLC

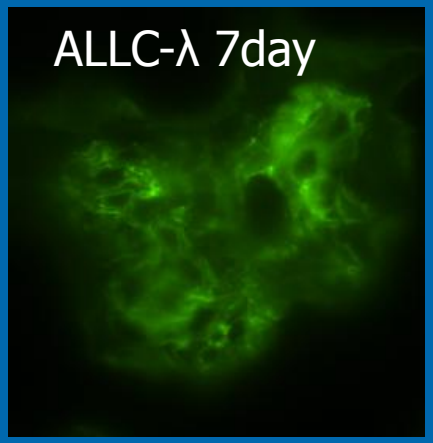


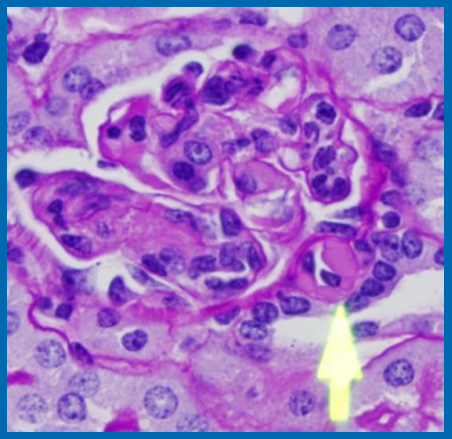


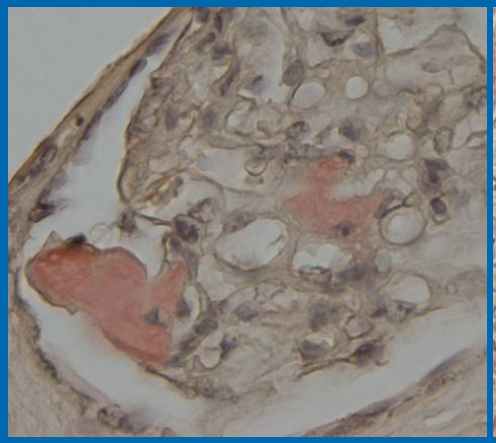
Penile injection control group

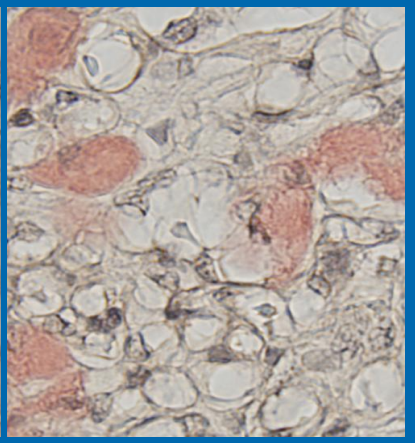


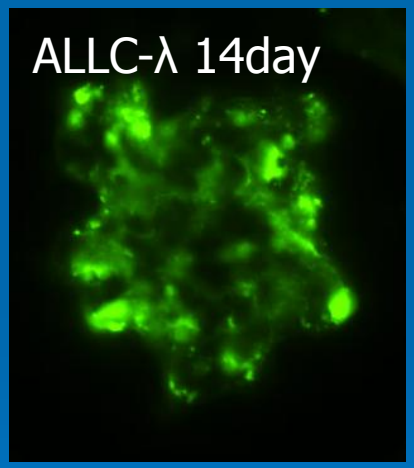
Penile injection ALLC group

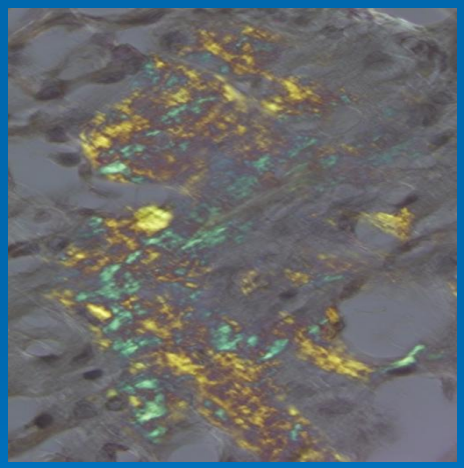


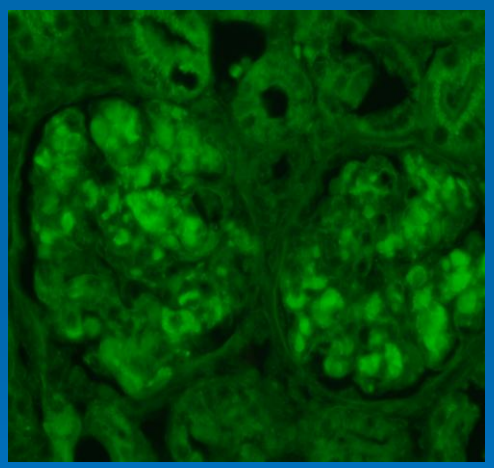


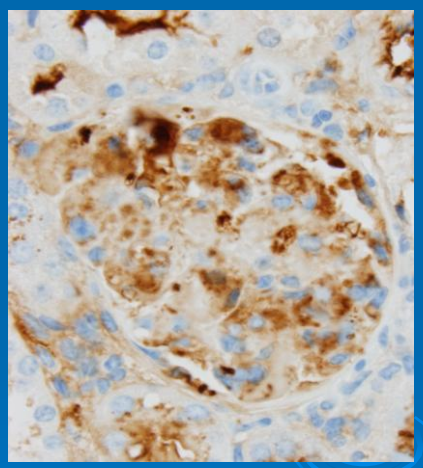




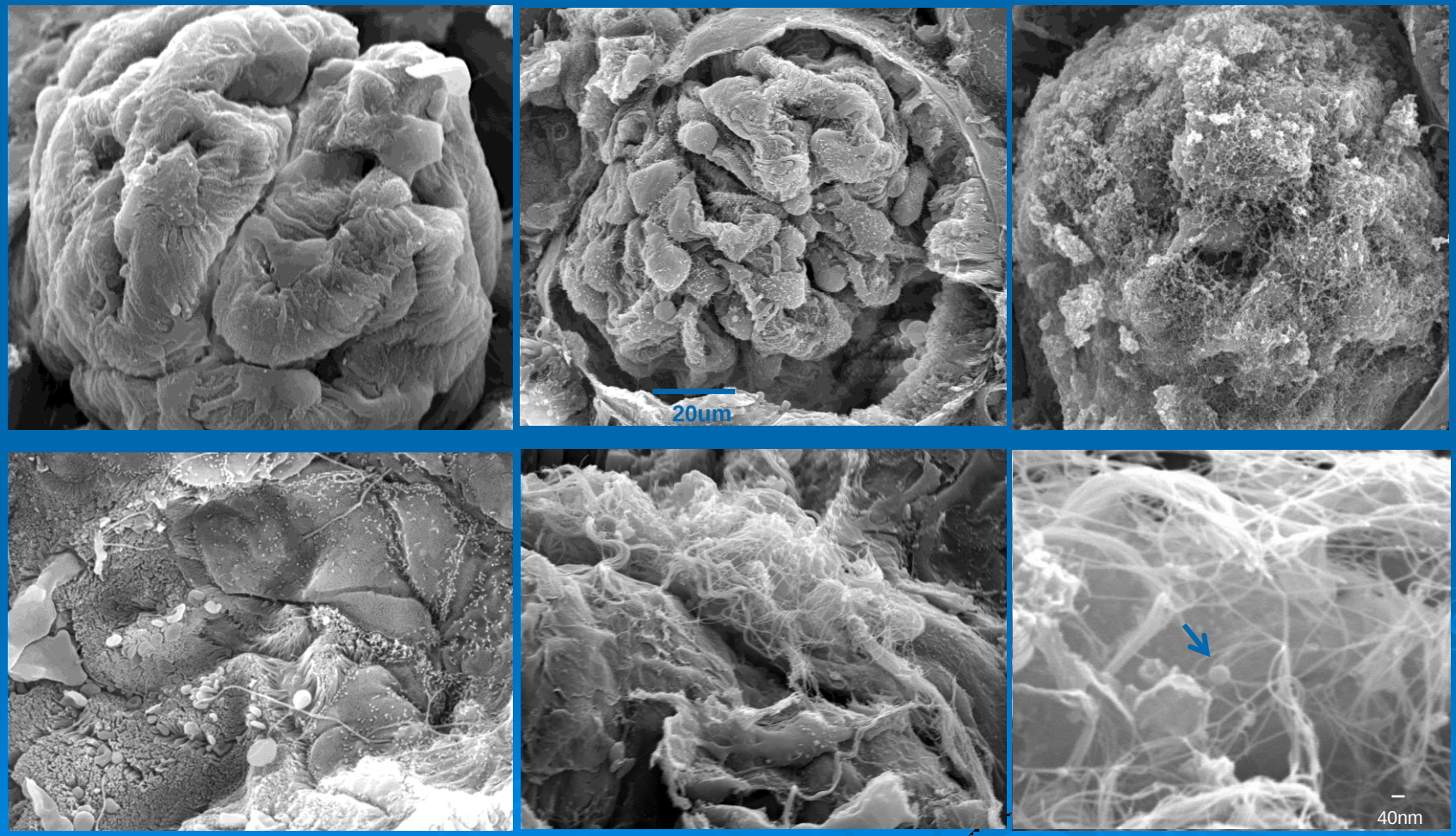






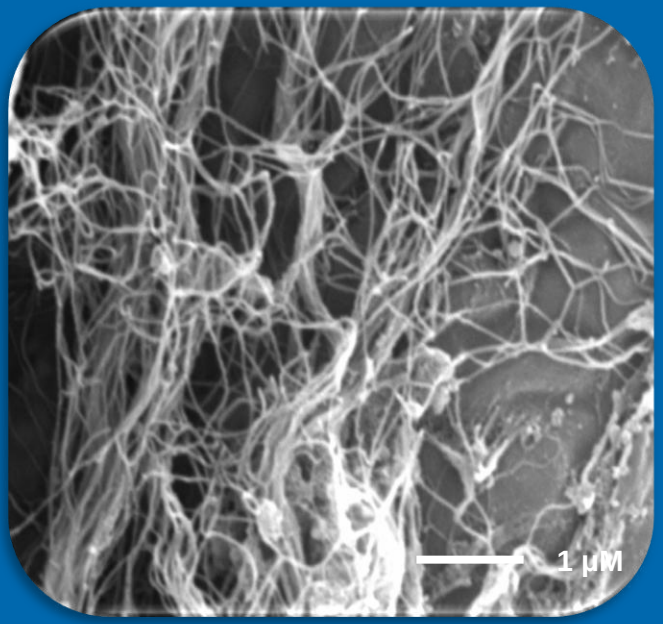


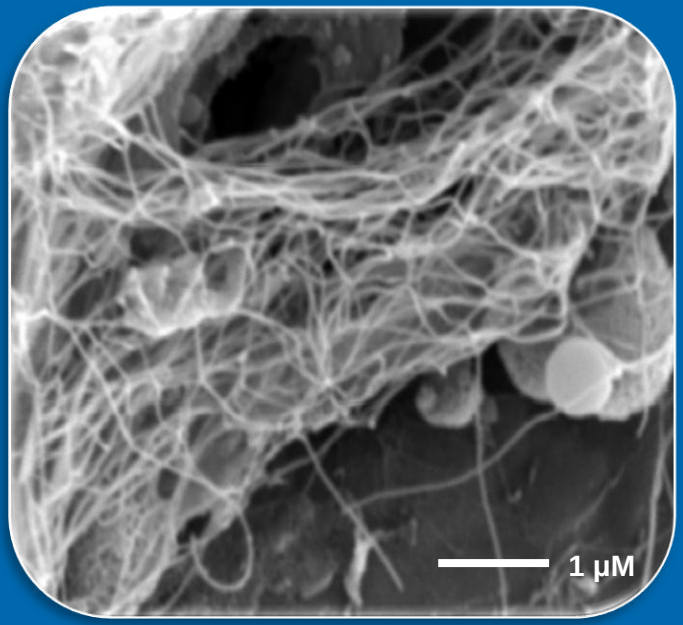
Mouse penile injection model SEM

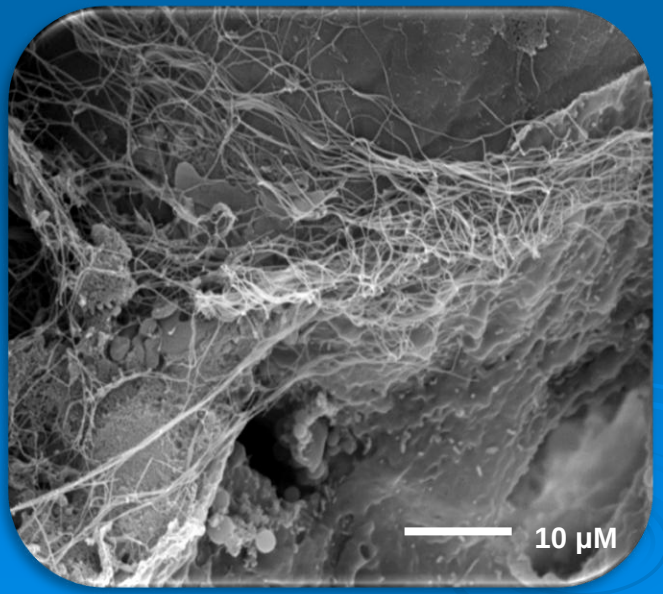


Control ALLC 1W ALLC 2W

Amyloid fiber formed in glomeruli of WT Mouse after penile injection of ALLC for 2 weeks





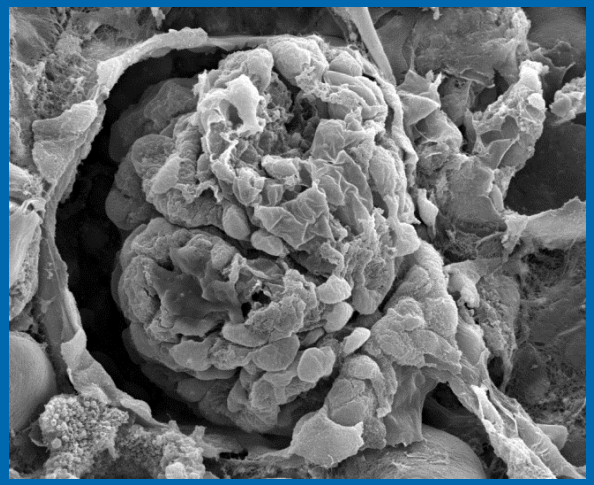


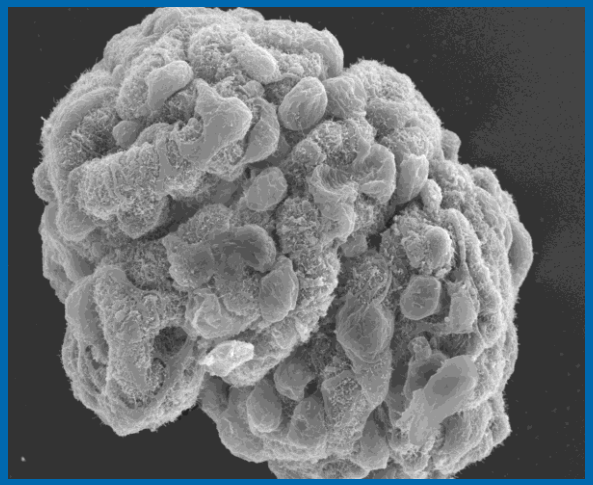


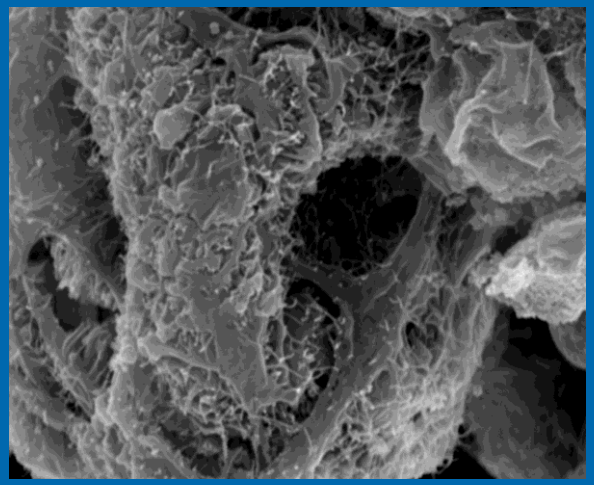
Ex vivo Kidney perfusion

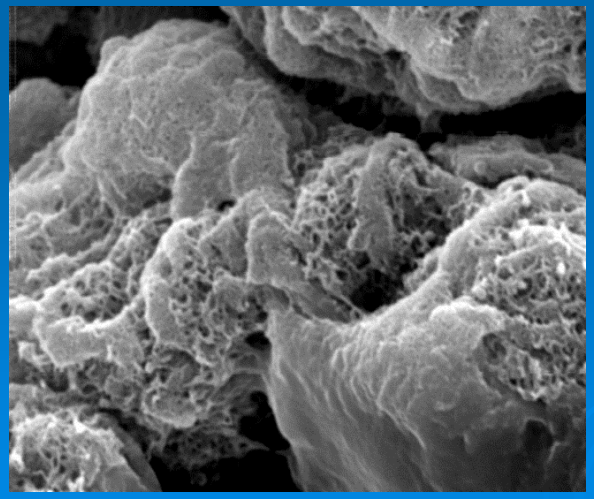
Control 24h

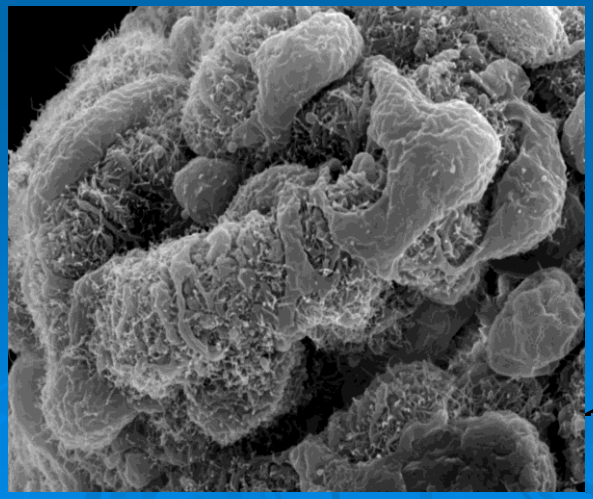
ALLC 24h

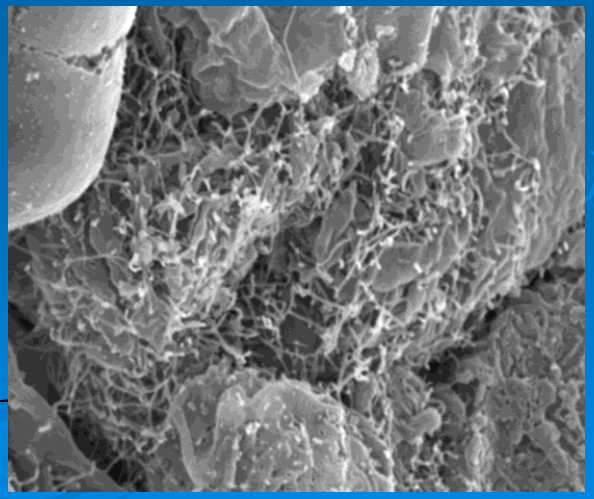




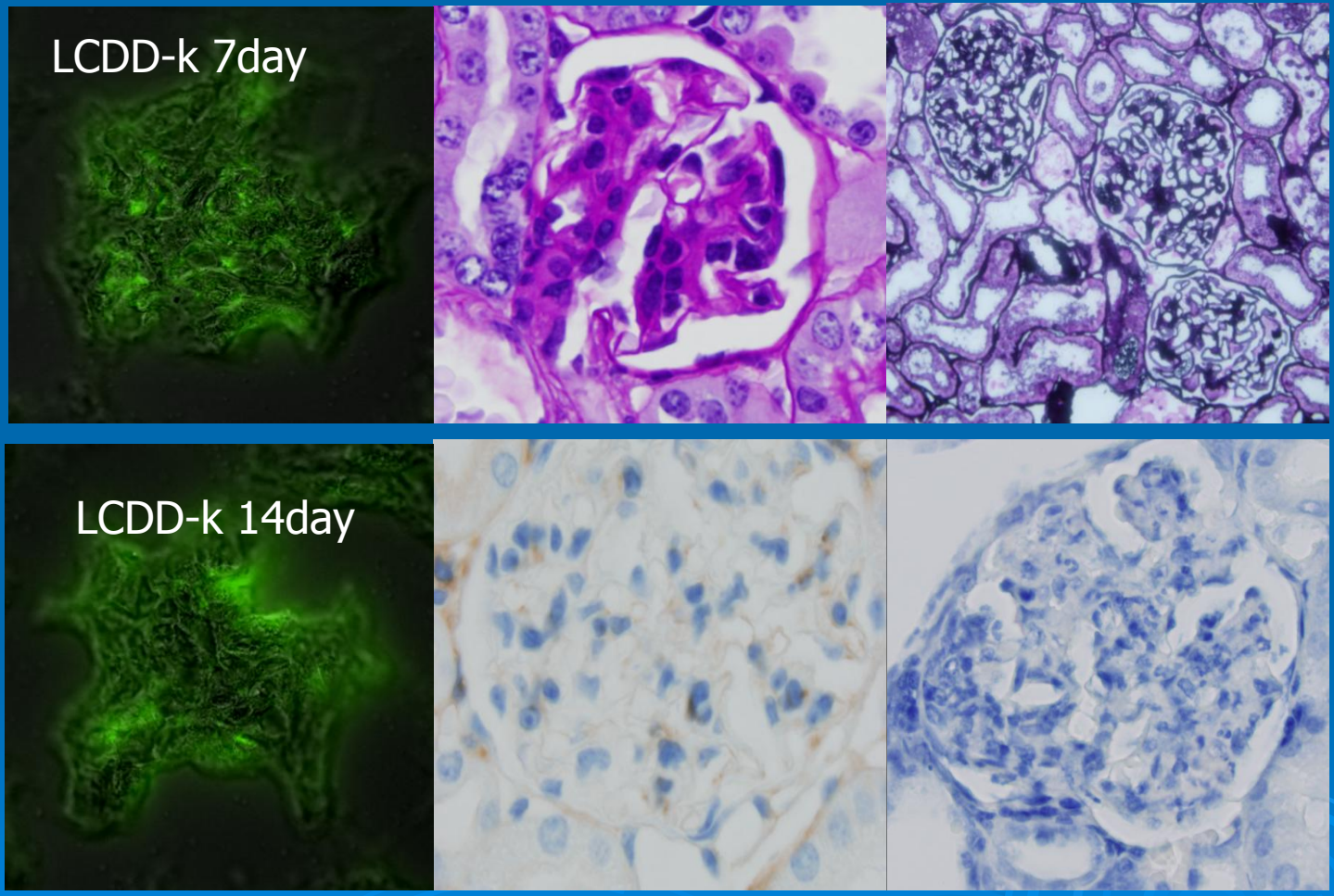






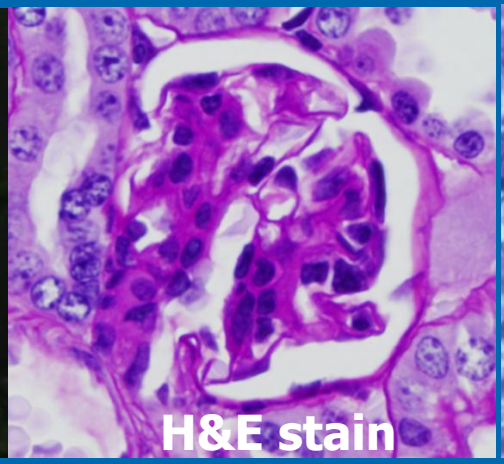


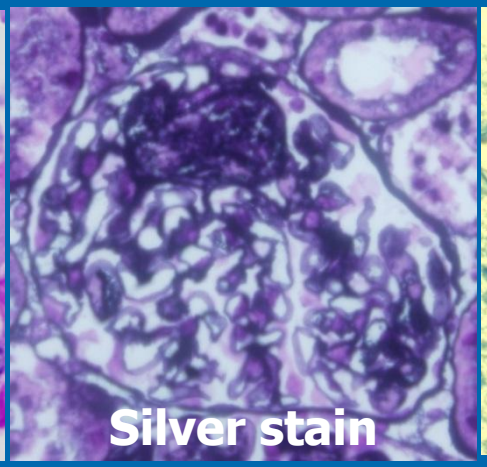
Penile injection LCDD-LC group



Penile injection LCDD-LC group

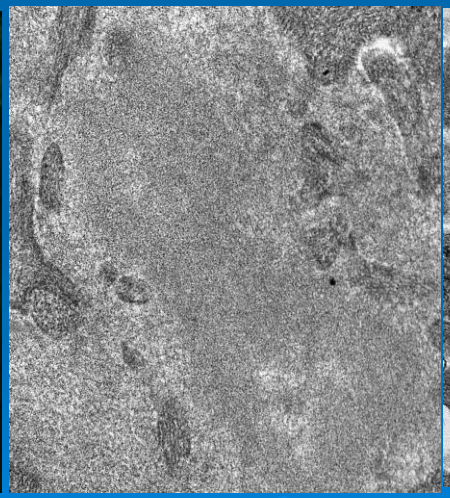


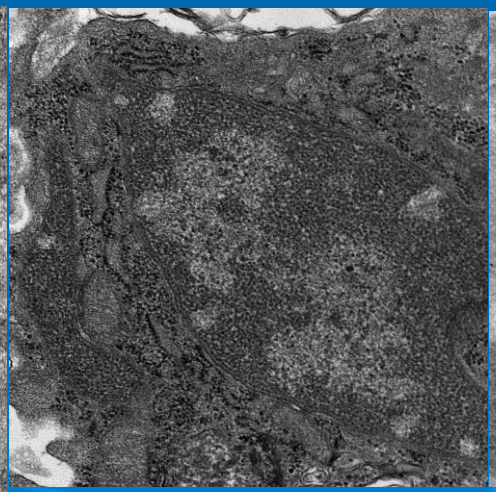


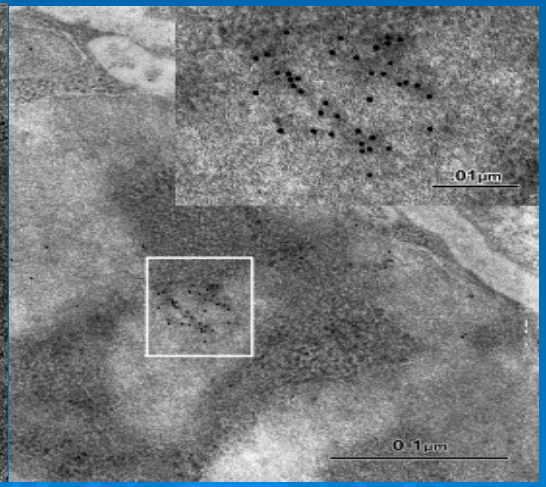




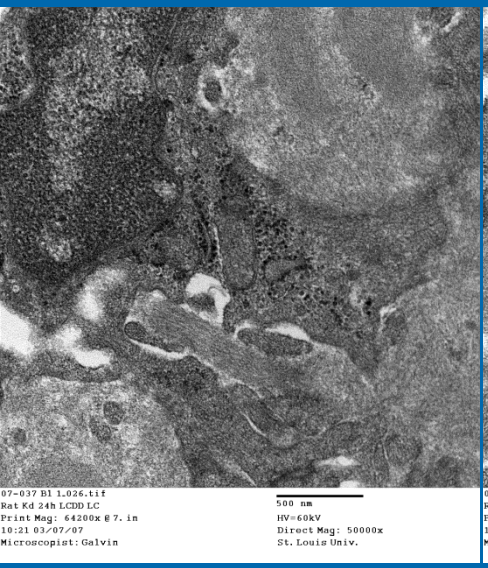


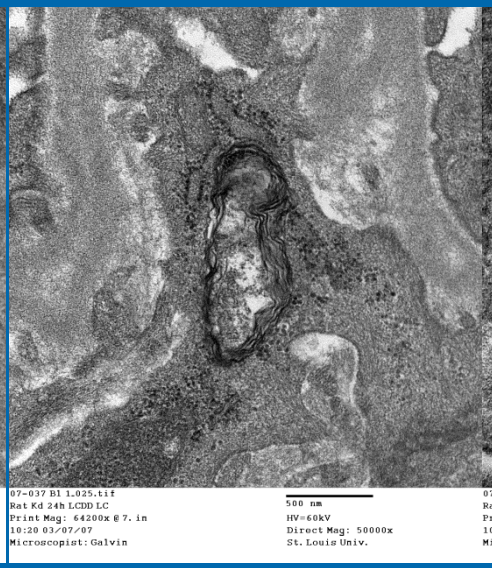


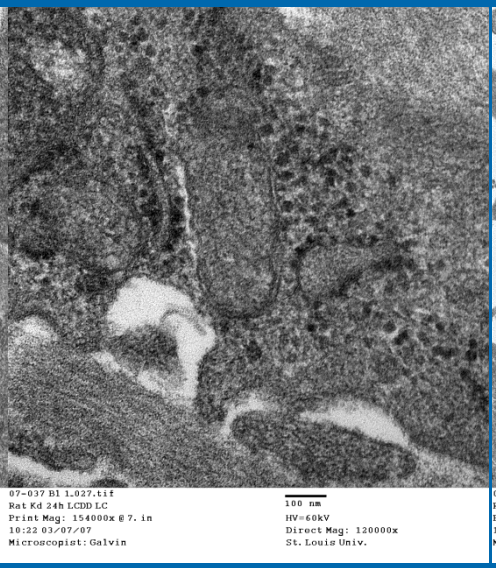


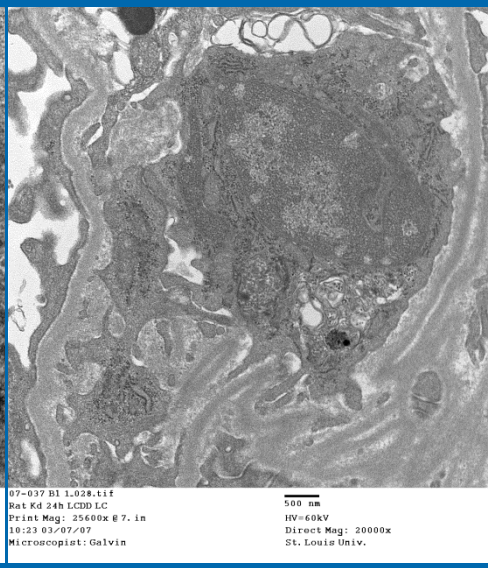


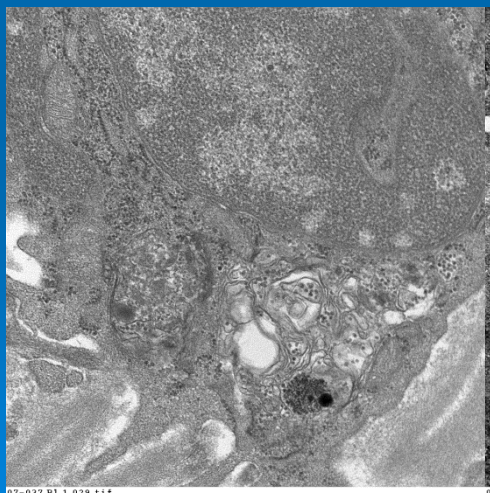
Kidney Perfusion Rat LCDD 24h

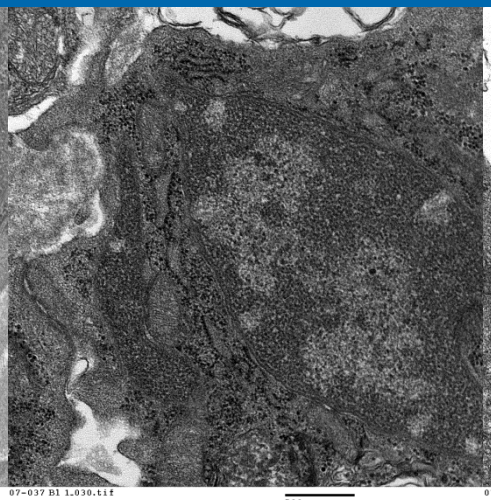


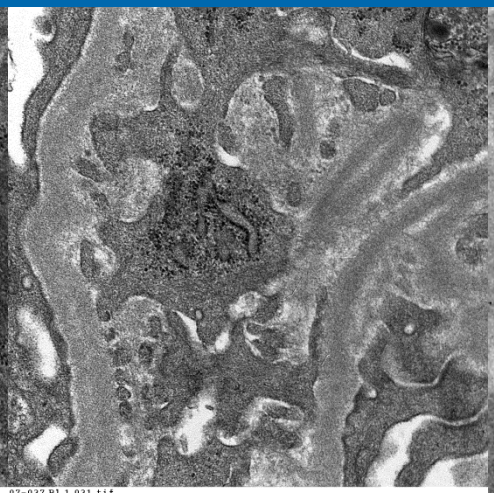


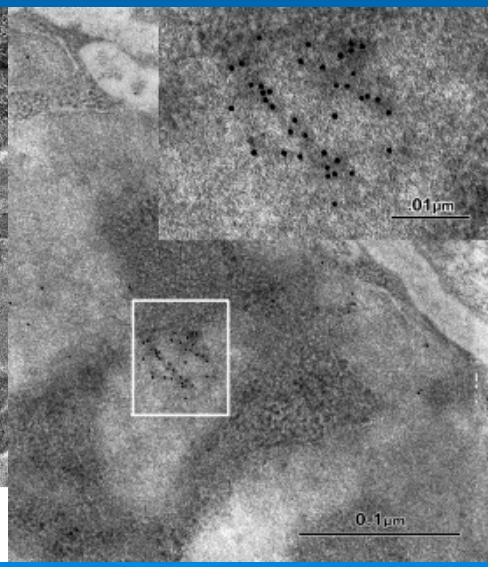




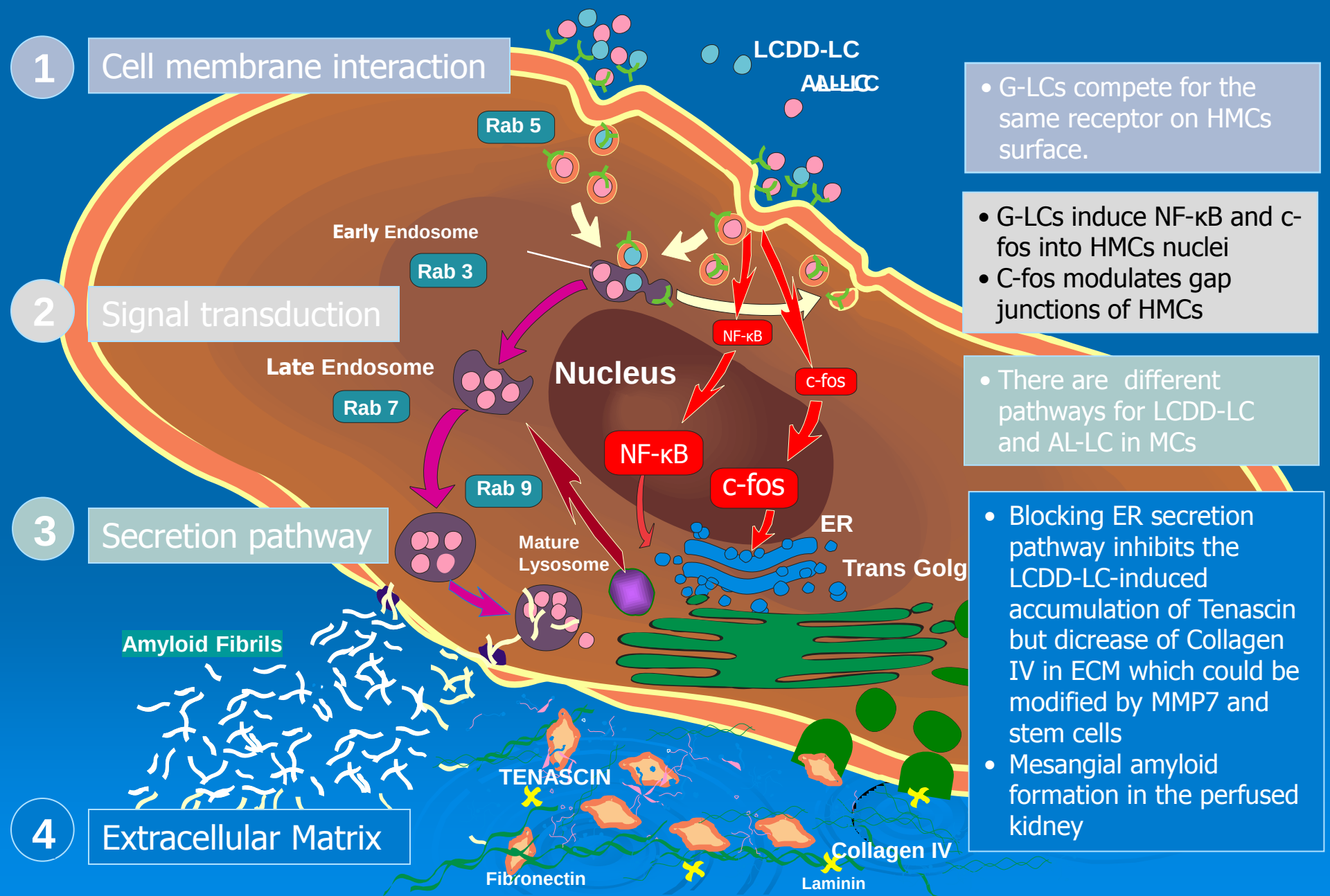








Mechanism of G-LC induced Glomerula Damage



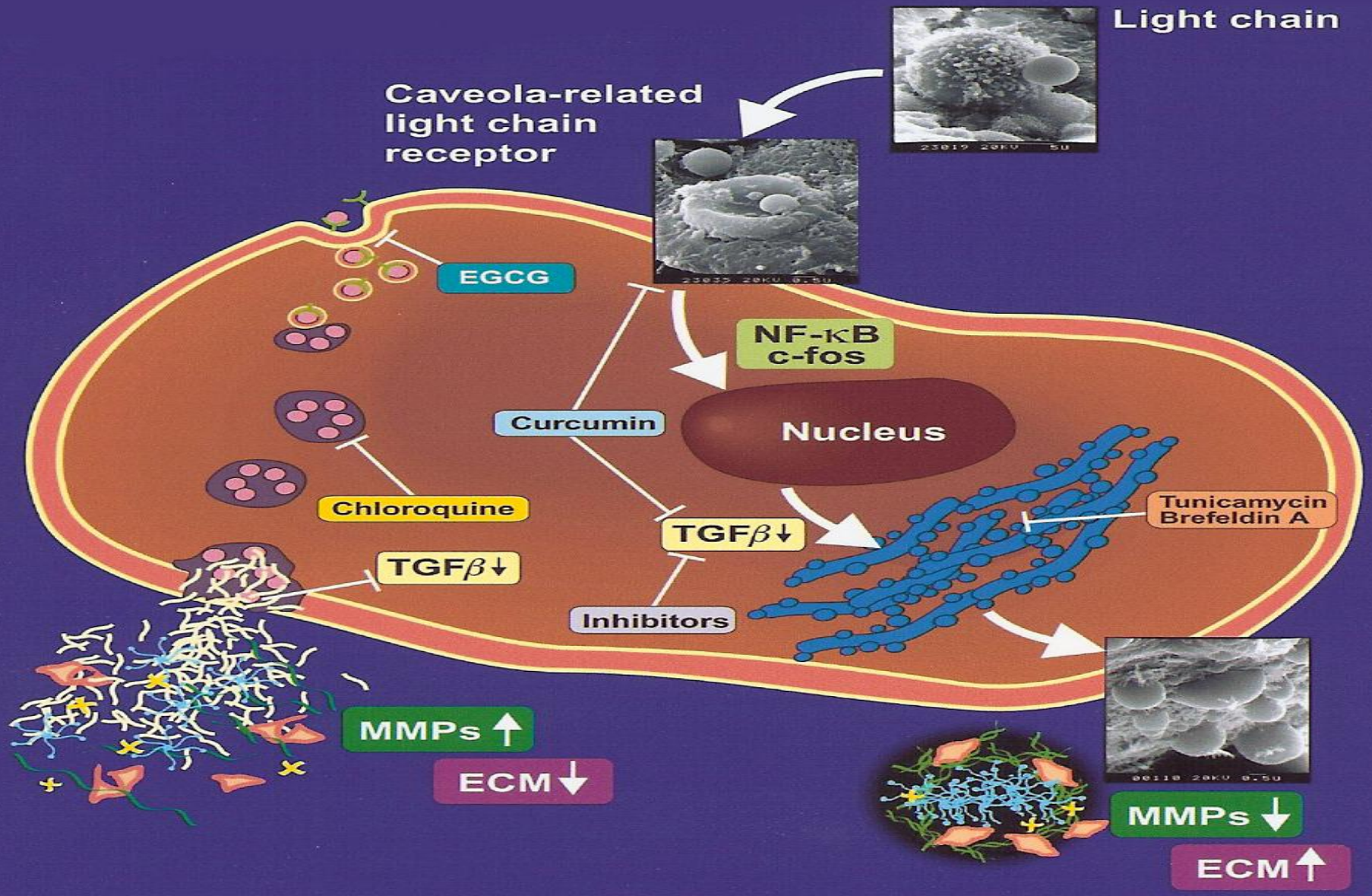
- G-LCs compete for the same receptor on HMCs surface.

- G-LCs induce NF-κB and c-fos into HMCs nuclei
- C-fos modulates gap junctions of HMCs

- There are different pathways for LCDD-LC and AL-LC in MCs

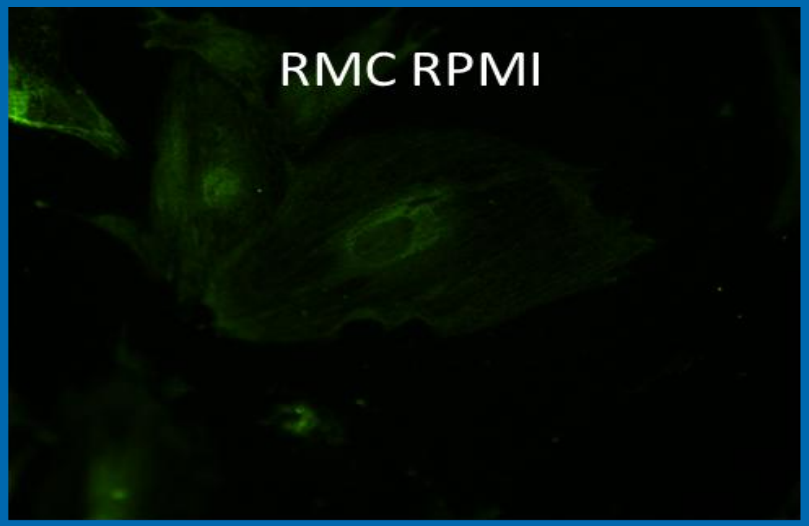
- Blocking ER secretion pathway inhibits the LCDD-LC-induced accumulation of Tenascin but dicrease of Collagen IV in ECM which could be modified by MMP7 and stem cells
- Mesangial amyloid formation in the perfused kidney

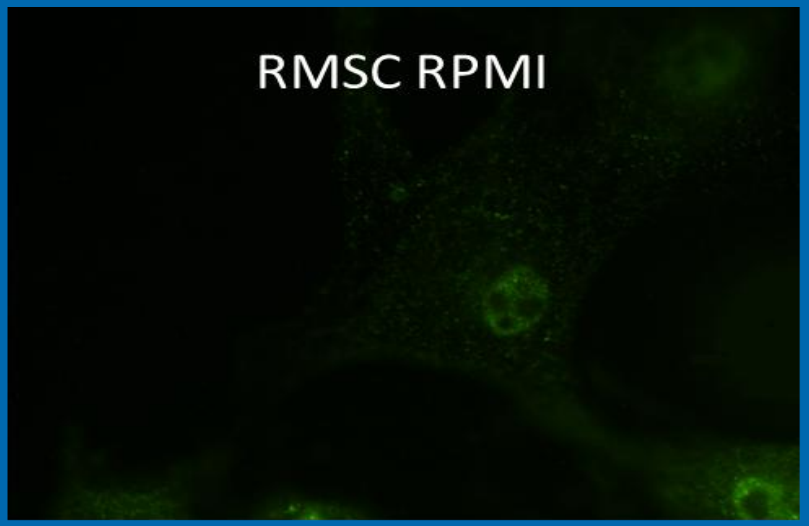
G-LCs and glomerulopathies

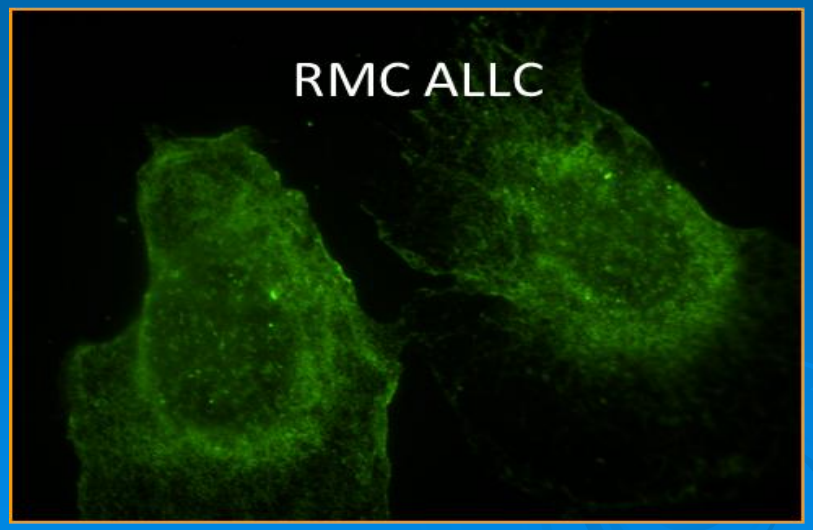


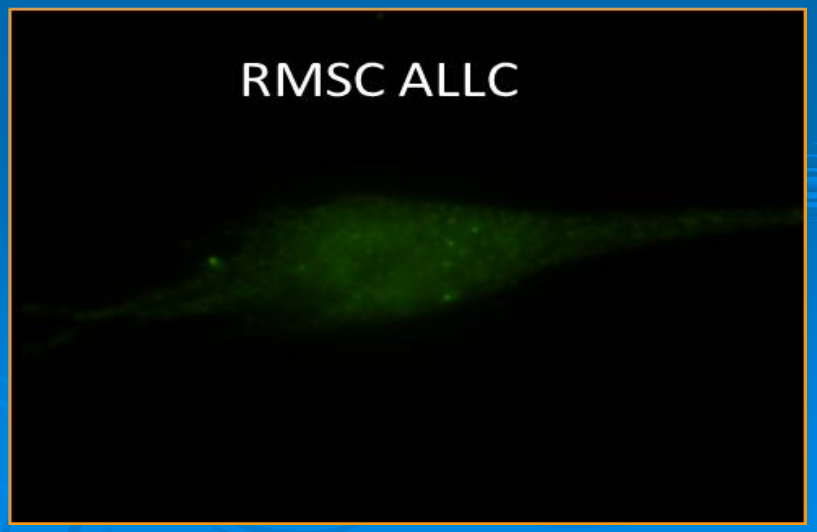
- Stem cell differentiation, restoration to functionality and cellular regeneration

CD 68 expression enhanced in HMCs and RMCs but not in RMSCs after ALLC treatment for 96h

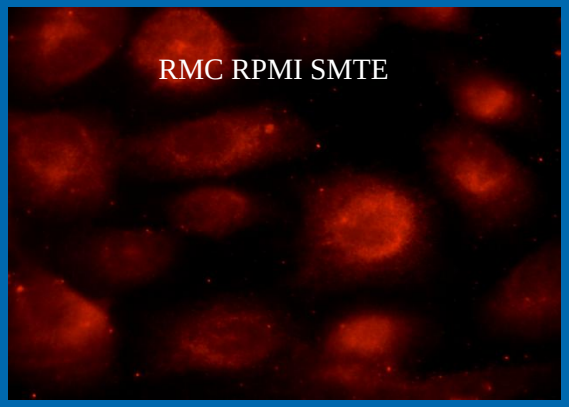




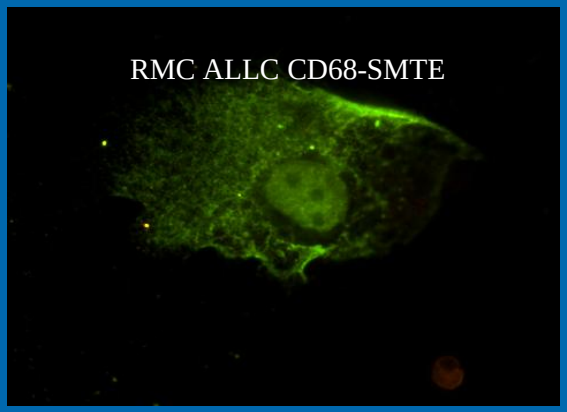




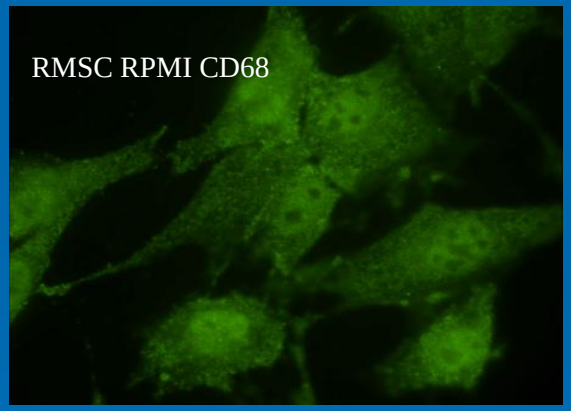
ALLC treated RMCs change from cytoplasmic smoothelin^{+} to CD68^{+} and reversed staining for RMSCs

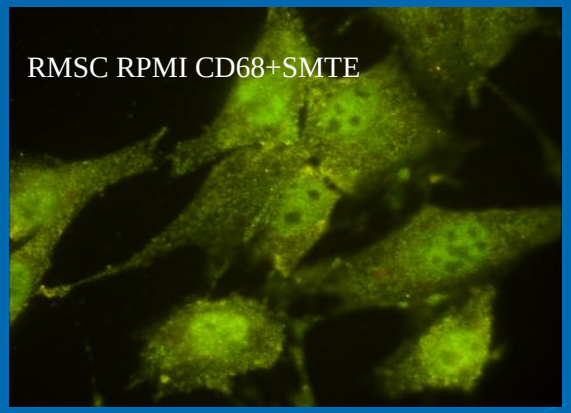


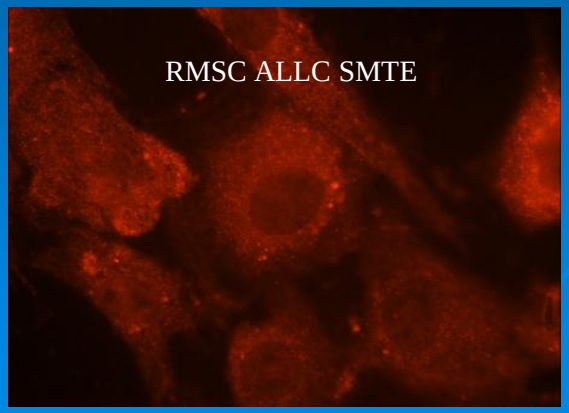


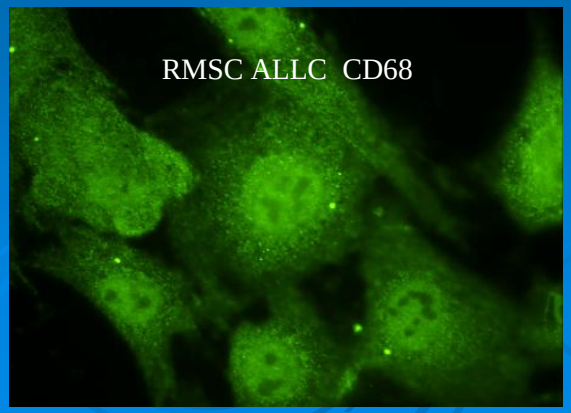


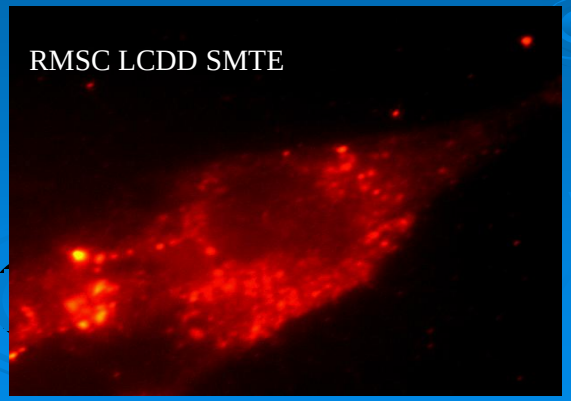




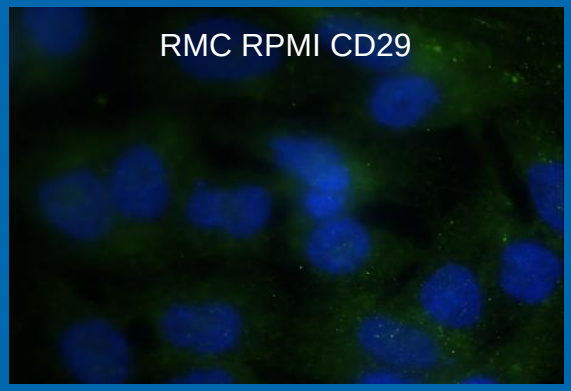


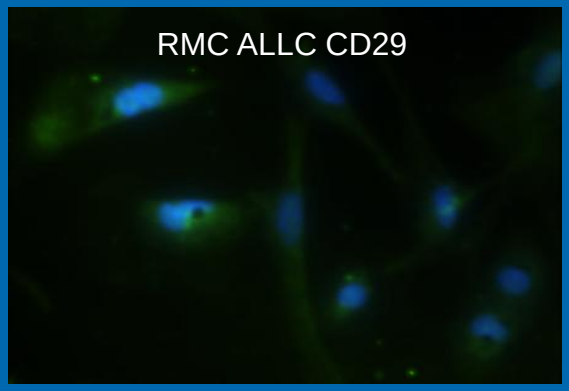


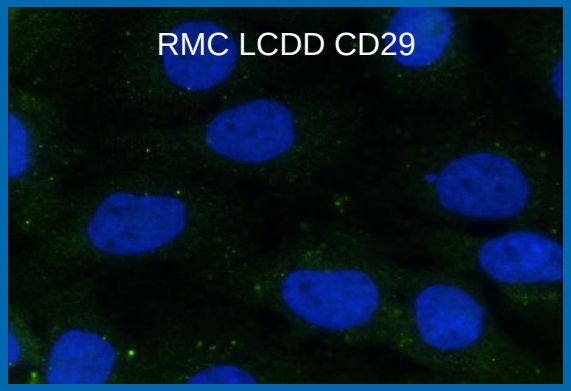


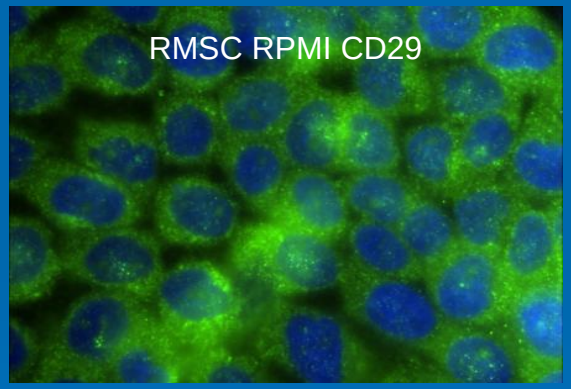


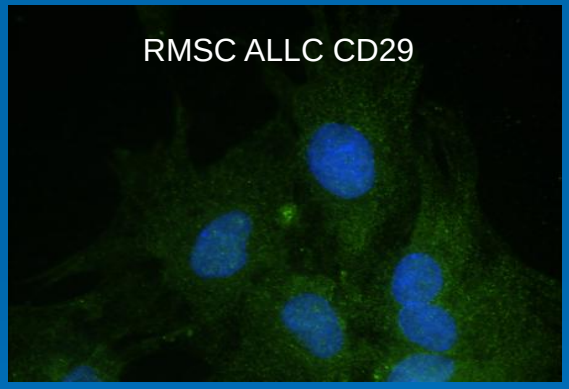
CD 29 not expressed in RMCs and faded away in RMSCs treated with G-LCs

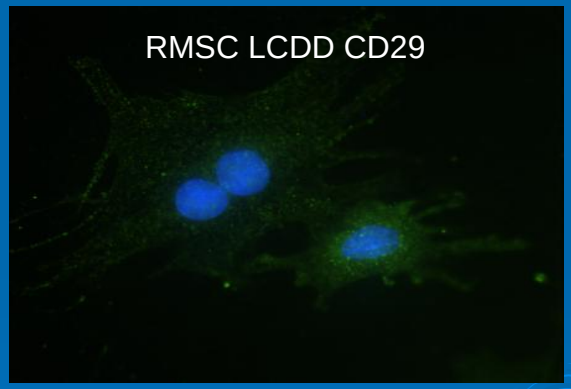


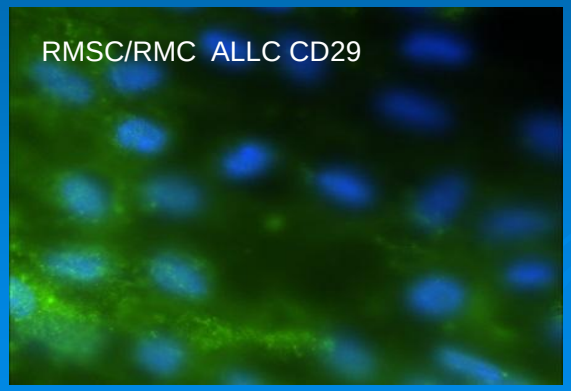


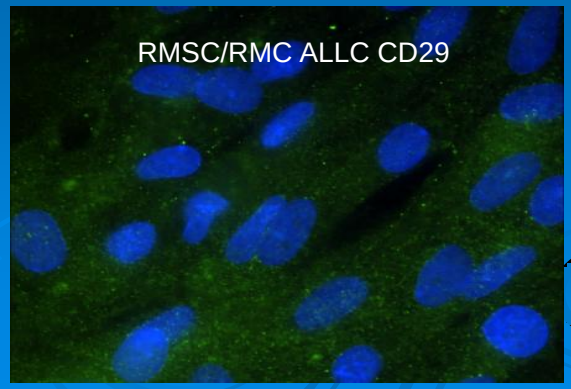


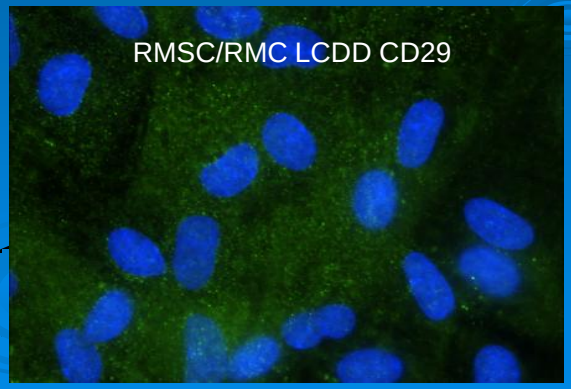




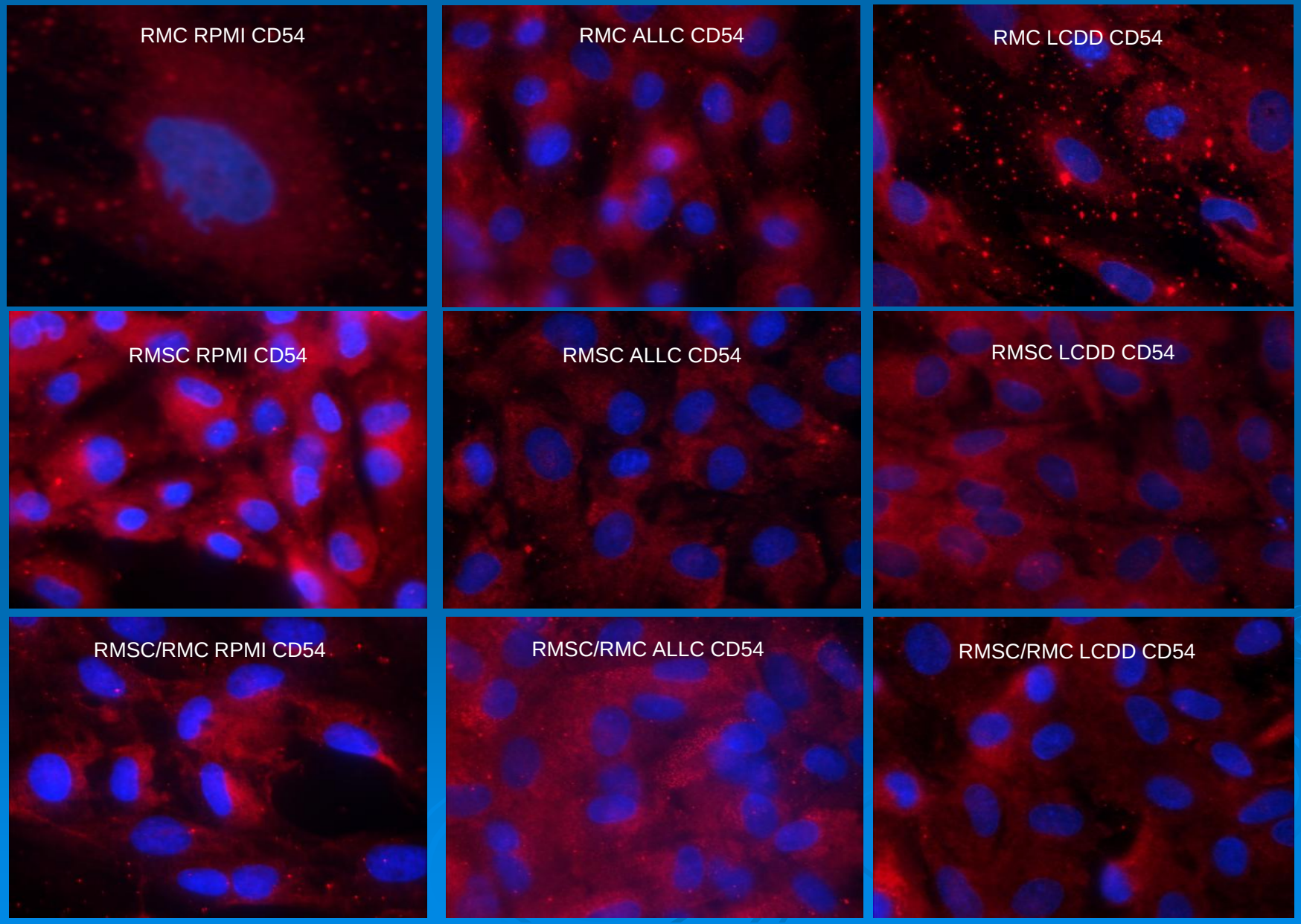








CD 54 expressed in RMSCs but not in RMCs, and decreased in the G-LC treated RMSCs



- Excellular matrix homeostasis restoration

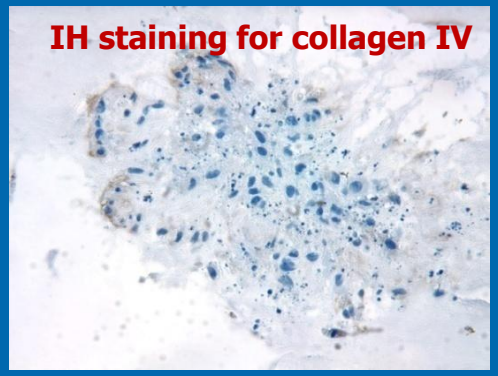
LCDD-LCs induce mesangial ECM over-production and nodule formation

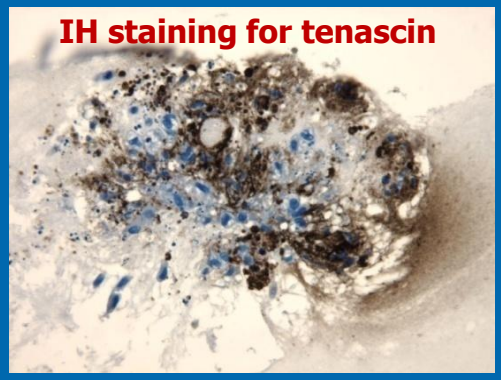
IN-VITRO:
Hman, rat, mice glomerular cells and/or MSCs remodeling or reconstruction in Matrigel model

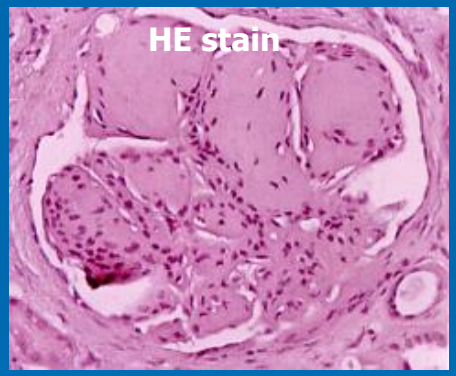
EX-VIVO:
Rat/mice perfusion model

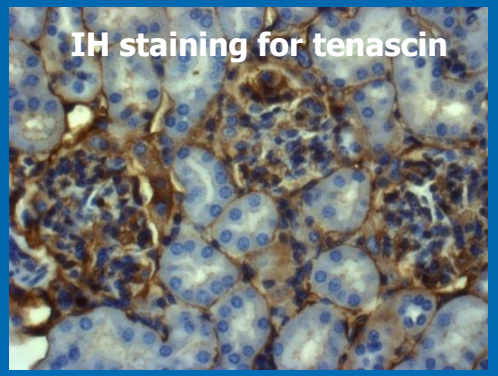
IN-VIVO:
Mice penile injection model

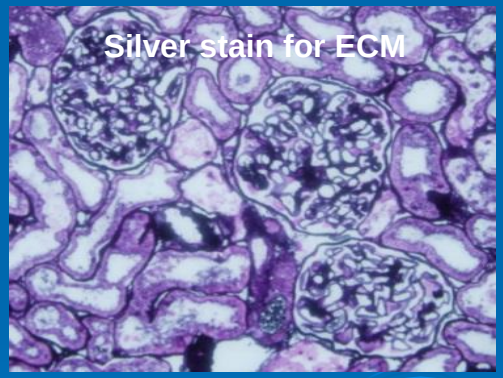


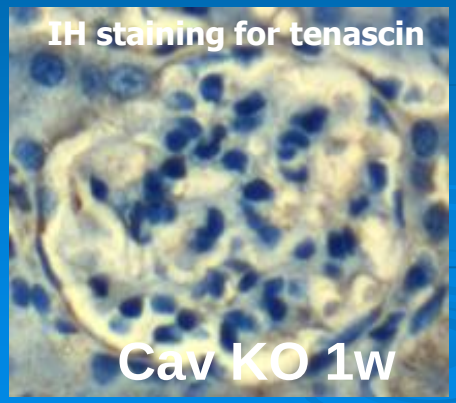


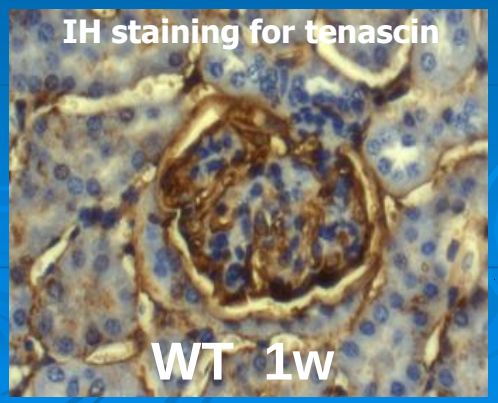


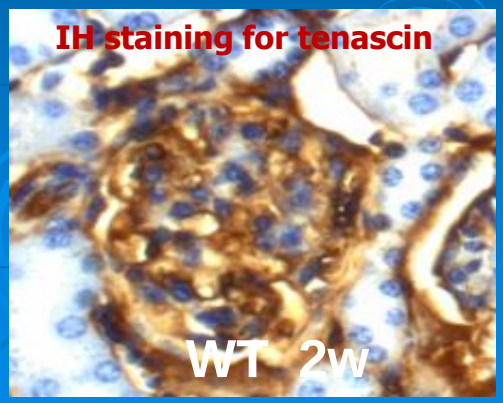


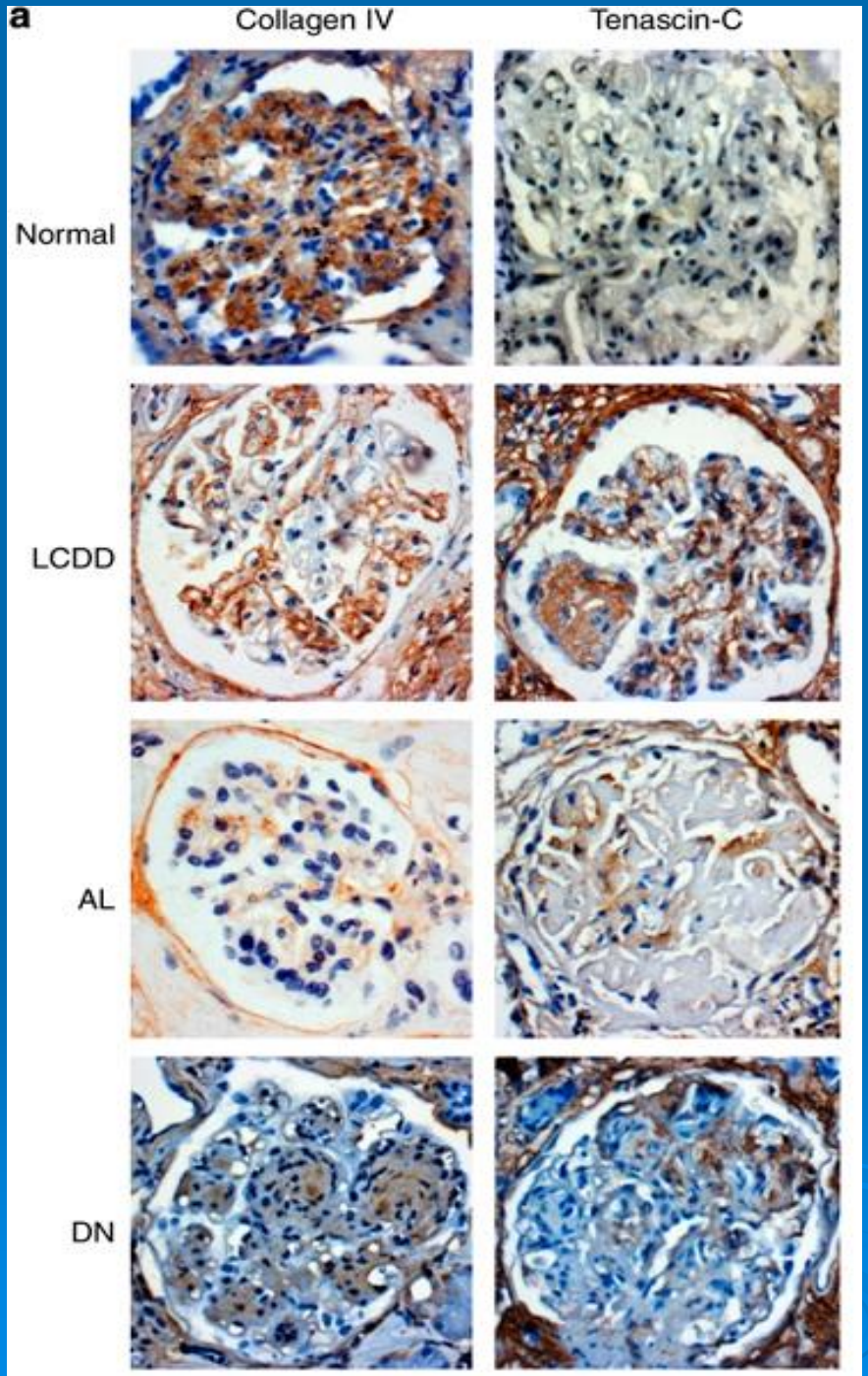


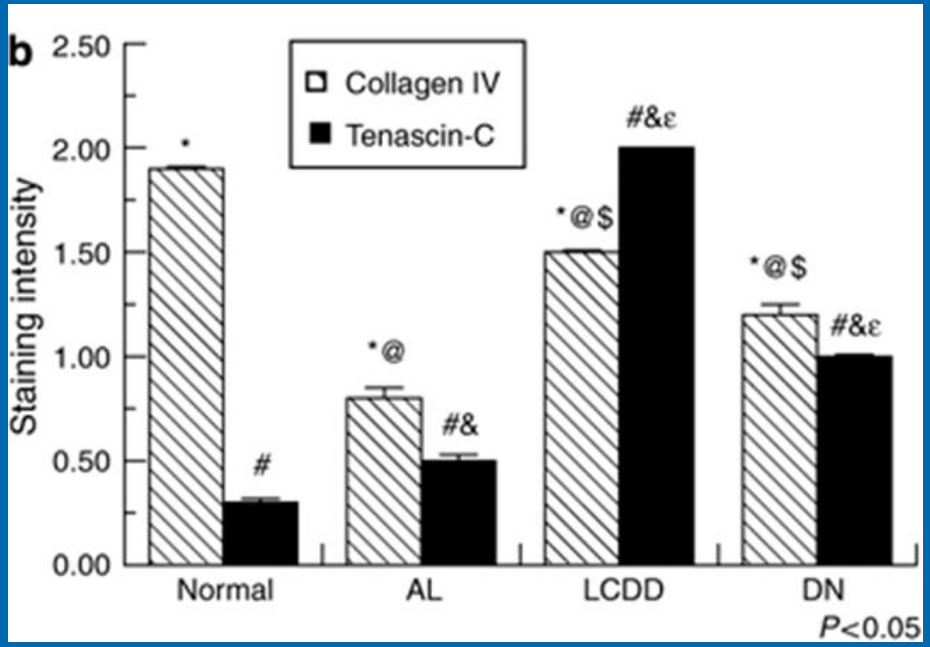








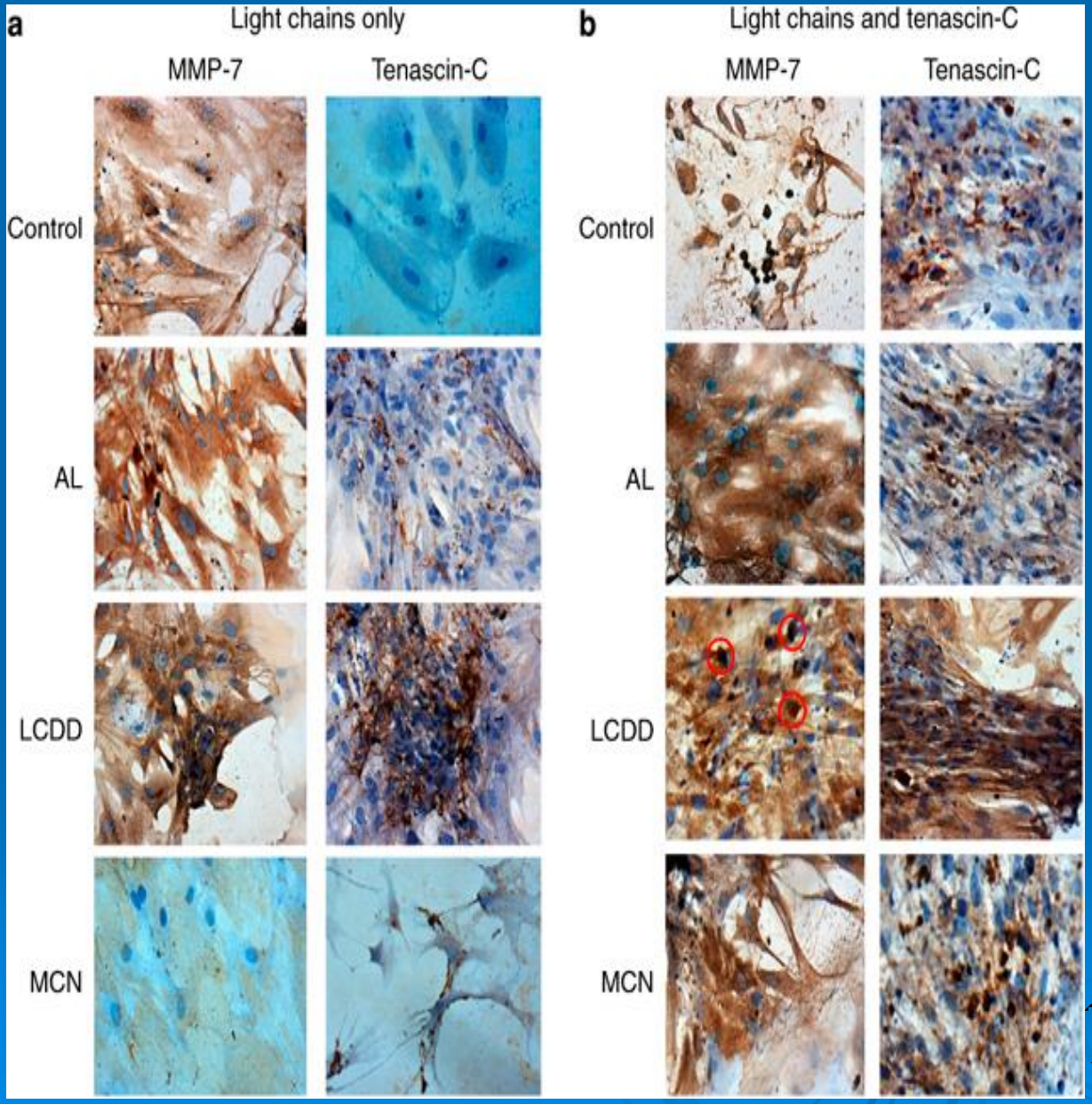




Collagen IV and tenascin-C expressions in the glomeruli obtained from renal biopsies of patient with glomerulopathies.

(a) Significantly higher expressions of tenascin-C expressions in the glomeruli from LCDD patients as compared with AL and controls,
(b) Analysis of the staining patterns showed significant differences in the expressions of both collagen IV and tenascin-C among the study groups.

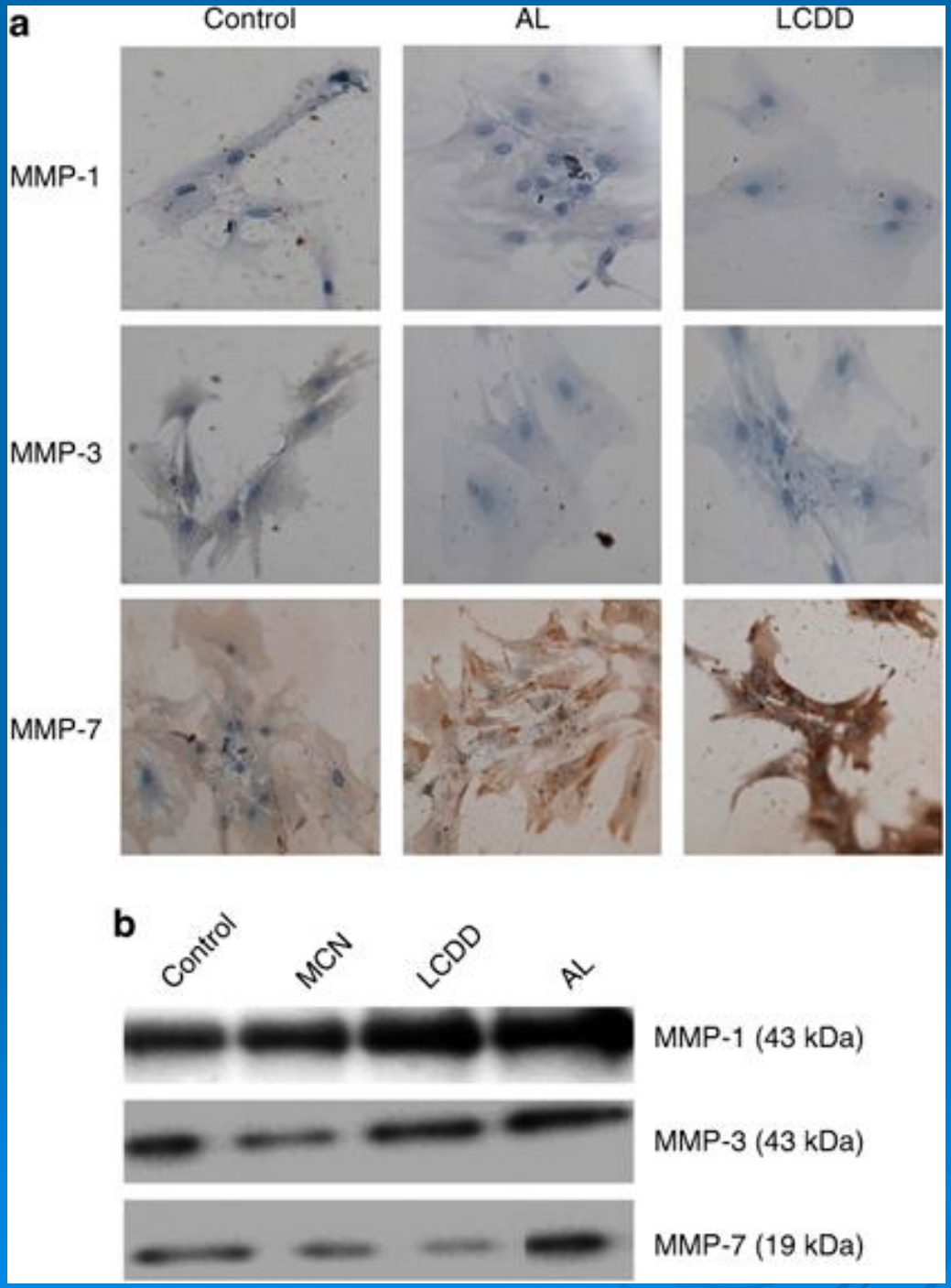
MMP-7 and tenascin-C expressions in HMCs treated with different light chains for 96 h.



LCDD-LC significantly increased extracellular Tenascin-C and intracellular MMP-7

a) The addition of exogenous tenascin-C with light chains enhanced expression of MMP-7 in HMC.

b) LCDD-LC increased intracellular MMP-7 and the extracellularly tenascin-C, in which appear as accentuated globules within the cytoplasm (red circles).

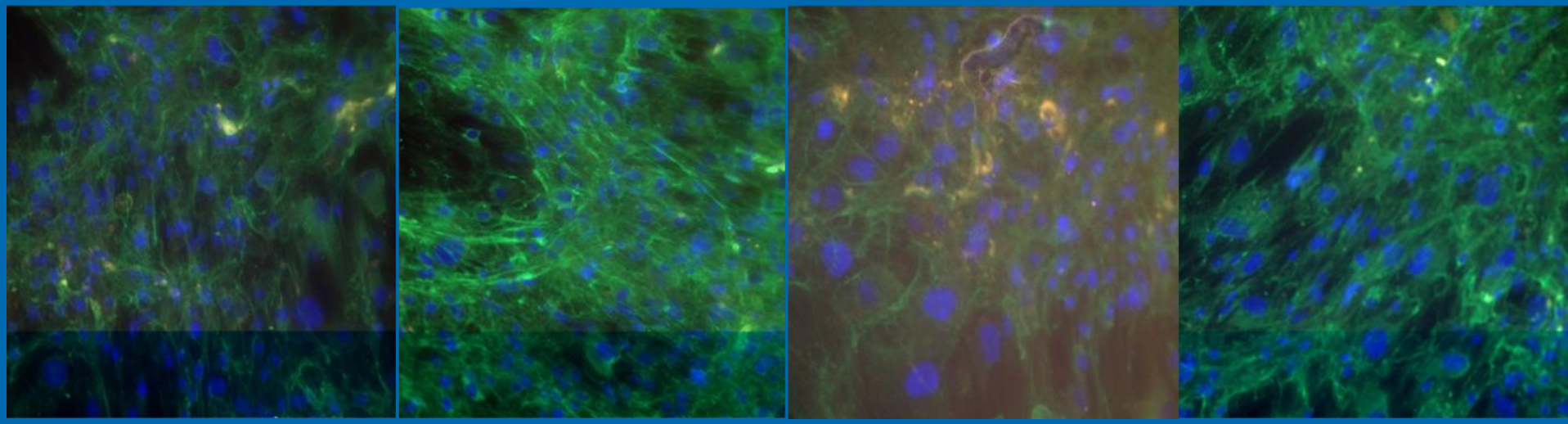


MMP-1, -3, and -7 expressions in the groups of G-LC treated HMCs

(a) LCDD and ALLC induced intracellular expressions of MMP-7 but not that of MMP-1 and MMP-3

(b) MMP-1 and MMP-3 expressed stronger band in G-LC treated HMC, but MMP-7 did not.

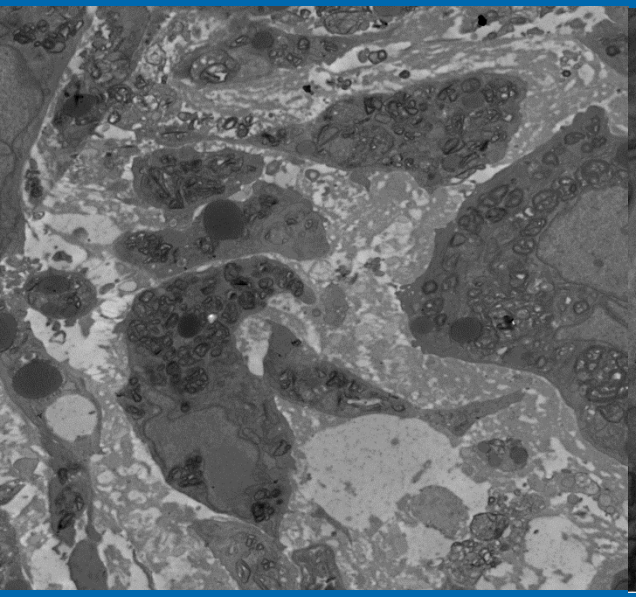
Collagen IV decreased and Tenascin increased in LCDD-LC treated Rat MCs while MMP7 recovered the changes

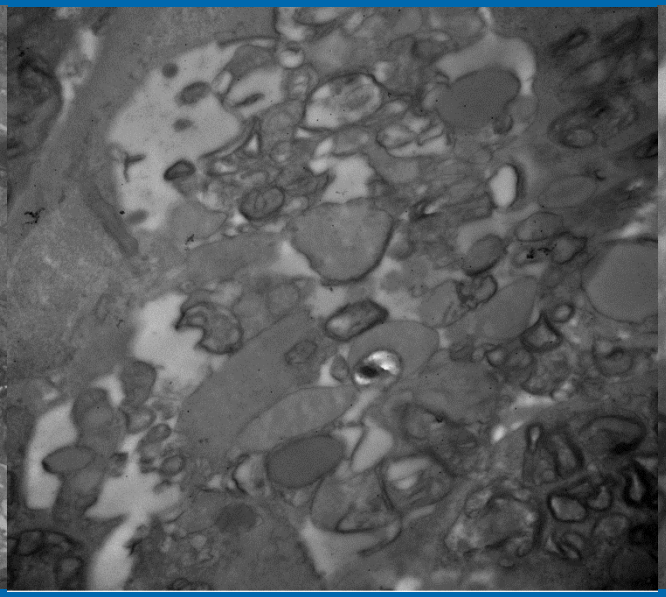


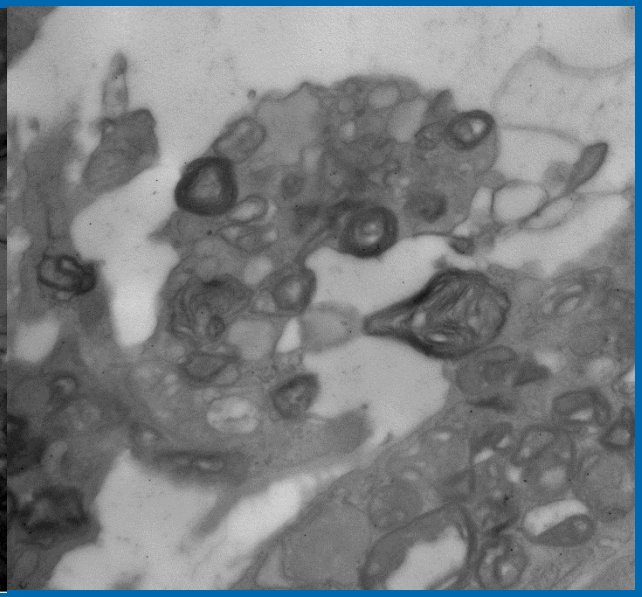
Medium control | MMP7 treated | LCDD-LC treated | LCDD+MMP7 treated

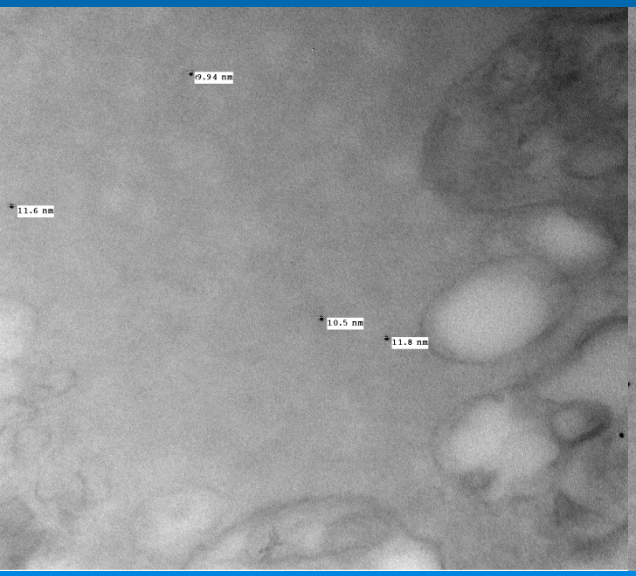
Immunofluorescent double-staining of Collagen IV and Tenascin

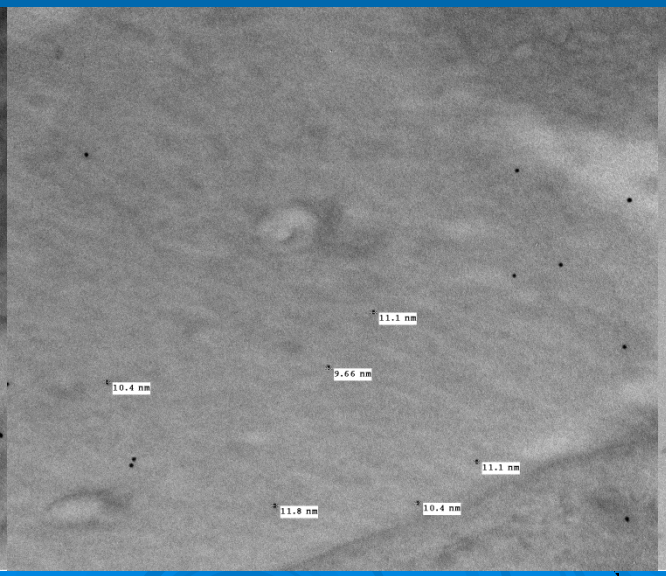
LCDD-LC induced apoptotic HMC and Tenascin Rich ECM

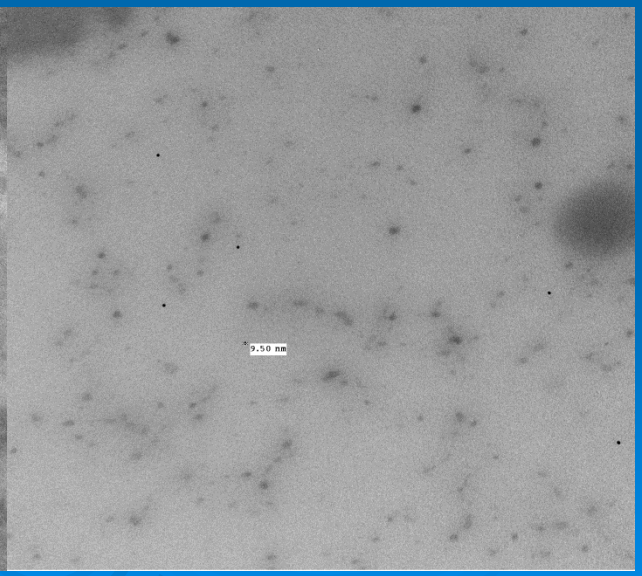




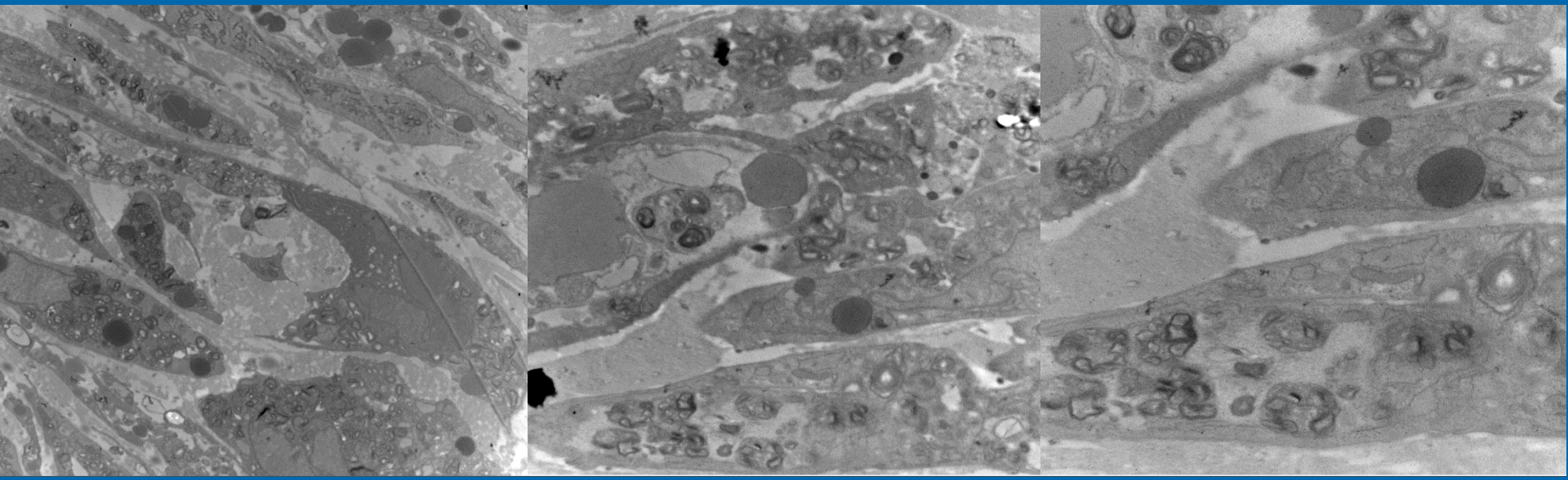


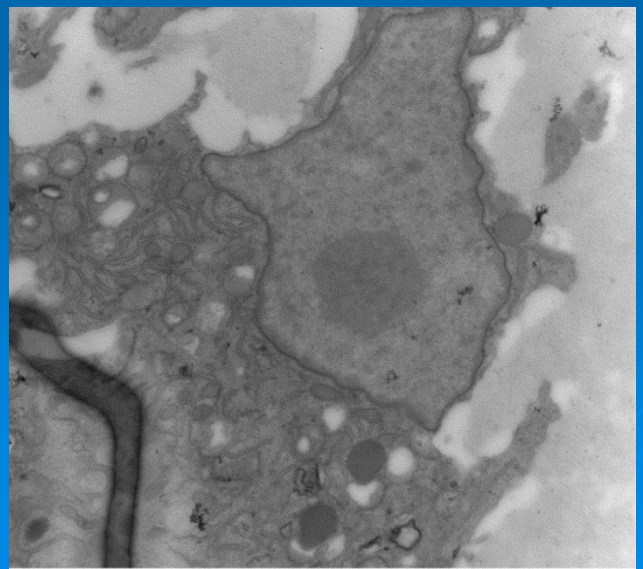


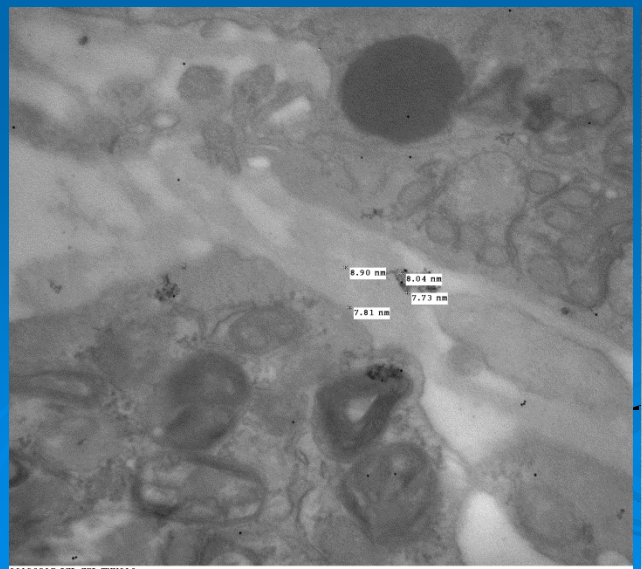




HMSCs restore the LCDD-LC damaged HMCs and restore the Tenascin Rich ECM back to Collagen IV dominated ECM









- These experimental platforms have allowed us to correlate in-vitro data with animal results and have shown that the sequence of events participating in the pathological processes are identical.
- Each platform offers specific advantages and disadvantages to answer different questions.

- Combining the data that has been obtained has resulted in a full appreciation of what is involved in the genesis of the mesangial damage.

- The use of stem cells to repair and restore the damaged mesangium has provided insights into how mesenchymal stem cells do so offering a promising new therapeutic avenue to treat conditions such as diabetic nephropathy and thrombotic microangiopathies among others.

Our long-term objective

- Development of therapeutic modalities to prevent, ameliorate or slow down irreversible renal damage
- To make the present apparently irreversible renal damage reversible in the future.

Thank you!