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The role of Calreticulin in renal fibrosis

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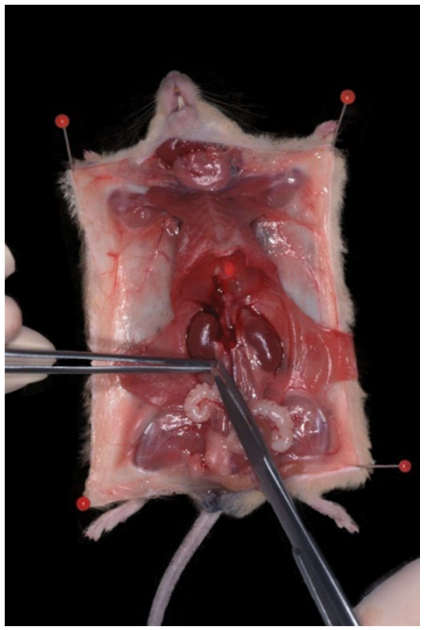
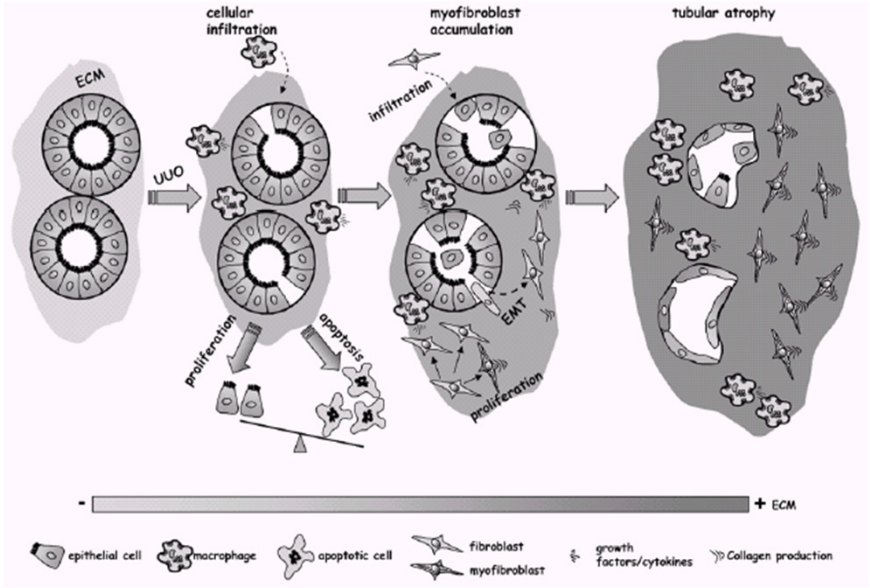
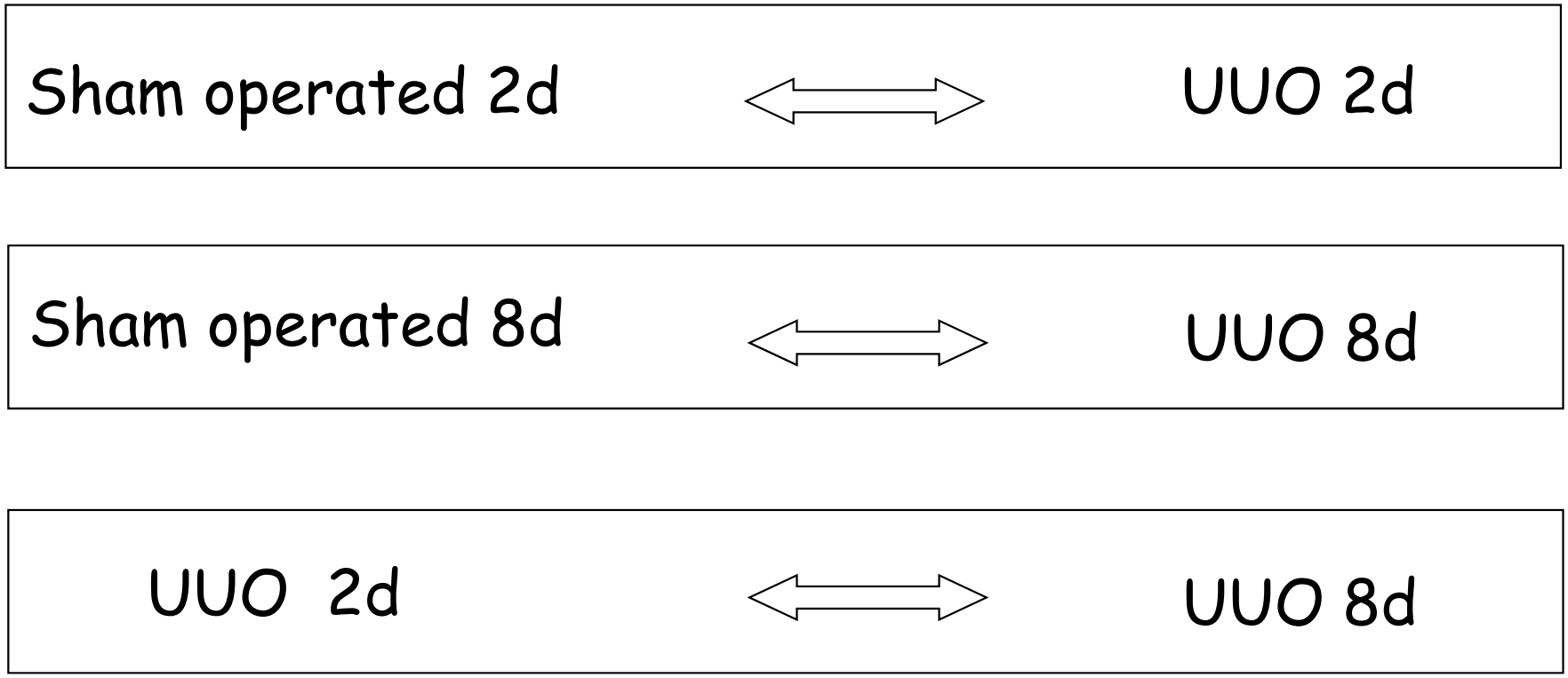
The Unilateral Ureteral Obstruction (UUO) model of kidney fibrosis



- in vivo model
- encompasses many aspects of other models of kidney fibrosis
- there are features that occur within 1 week
- mimics in a short time a situation that can take years in humans
- leaves one kidney intact
- there is evidence that animal models with UUO are reflective of the molecular changes in human situations

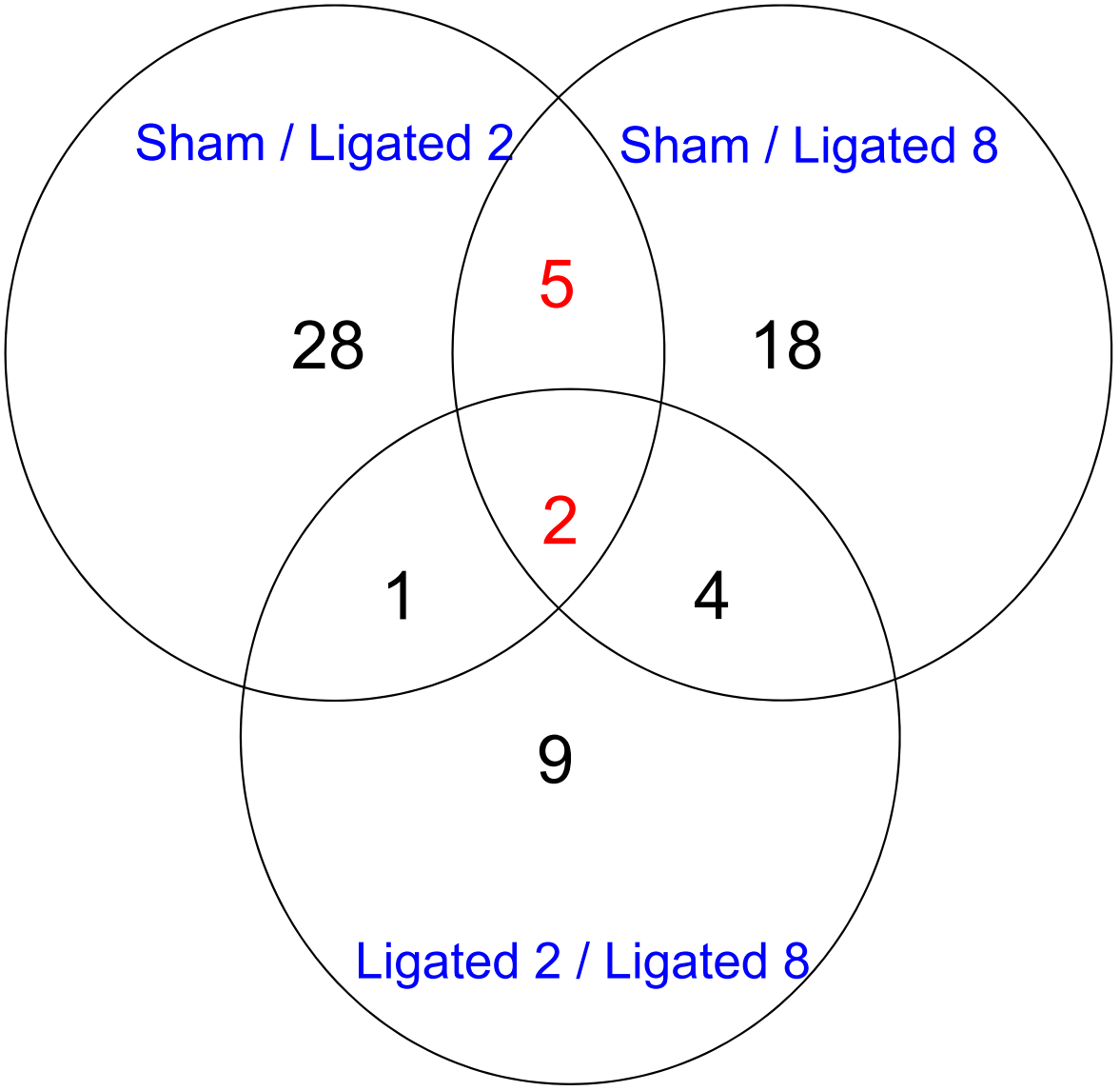


Proteomic analysis



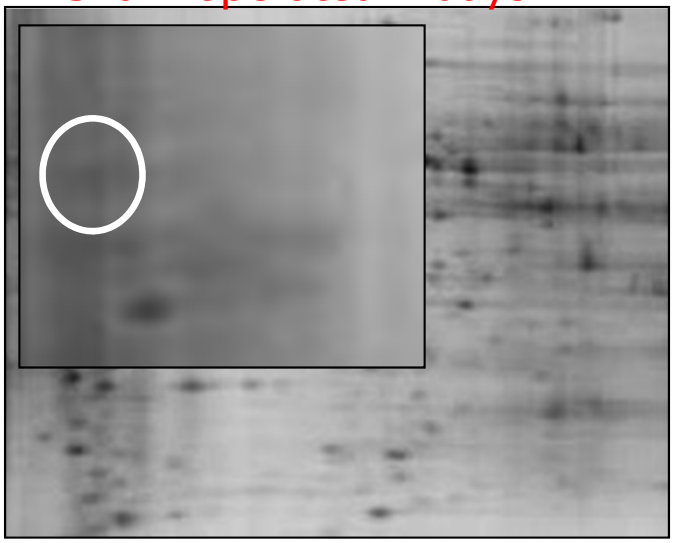
Kypreou K. *et al.*, *Proteomics*, 2008

Venn diagram of identified proteins

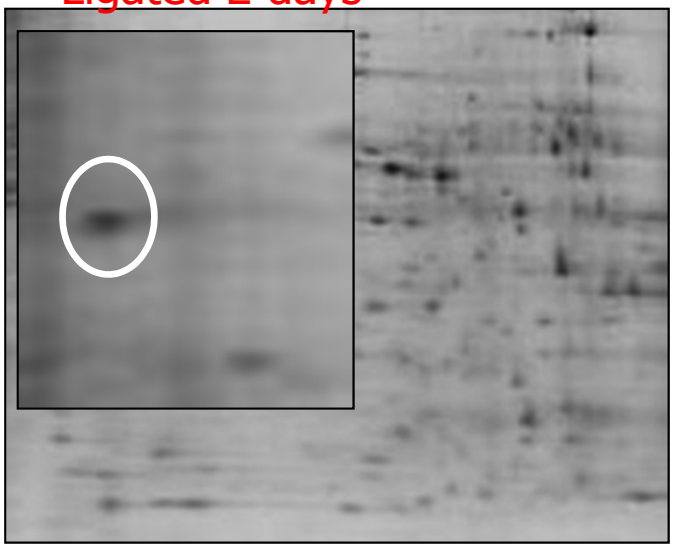


CALRETICULIN

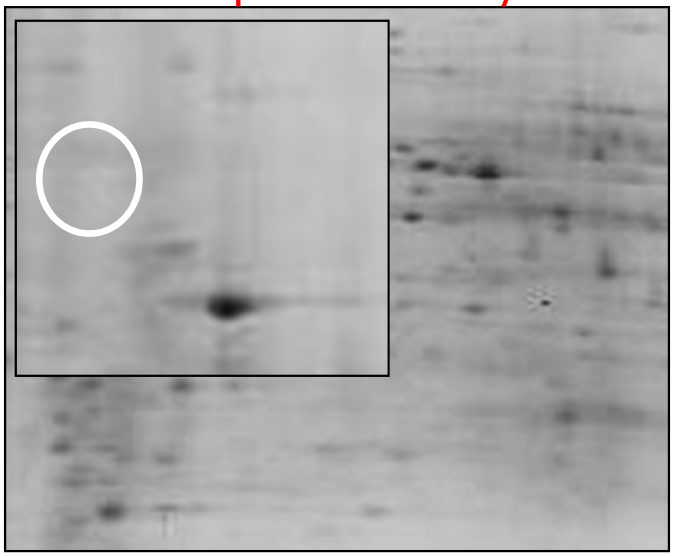
Sham operated 2 days



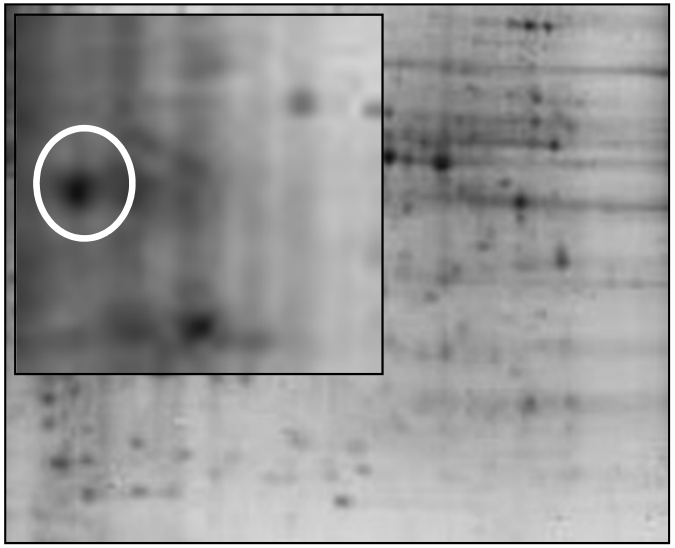
Ligated 2 days



Sham operated 8 days



Ligated 8 days



Calreticulin is a multifunctional protein

- First isolated in 1974 as a high-affinity Ca^{2+}-binding protein of the ER

Table 1. Effects of altered cellular expression of calreticulin[a]

CRT cellular expression level	Refs
Calreticulin upregulation	
Increased Ca^{2+} storage capacity of ER	14
Modulation of cell adhesiveness	19,36
Modulation of store-operated Ca^{2+} influx	14
Increased sensitivity to apoptosis	–[b]
Modulation of steroid-sensitive gene expression	18,19
Appearance of surface CRT	45
Modulation of SERCA2 function	32
Calreticulin deficiency	
Embryonic lethal at E14.5	28,29
Impaired cardiac development	28
Changes in cell adhesiveness	29,34
Increased resistance to apoptosis	76
Accumulation of misfolded proteins	–[b]
Modulation of Ca^{2+}-dependent gene transcription	28
Inhibition of agonist-dependent Ca^{2+} release from ER stores	28

[a]Abbreviations: CRT, calreticulin; E, embryonic day; ER, endoplasmic reticulum; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase.
[b]I. Ahsan, R. Knee and M. Michalak (unpublished).

Box 1. Selected cellular functions attributed to calreticulin

Adhesion
- Acrosome function and sperm motility
- Activates integrins
- Affects cell migration
- Control of cellular adhesiveness
- Inhibits angiogenesis
- Initiates cell spreading
- Regulates expression of vinculin
- Upregulates expression of N-CAM
- Wound healing

Blood function
- Anti-thrombotic activity
- Autoantigen
- Binds to complement C1q (C1q receptor?)
- Component of lytic granules in CTLs and NKs
- Component of tick saliva
- Inhibits perforin-dependent killing
- Interacts with perforin
- Modulates platelet activation

Development
- Affects cardiac development
- Affects neuronal development
- Essential for mouse embryogenesis
- Induces complete cardiac block
- Oocyte fertilization
- Regulates bone cell function

ER functions
- 'Ca^{2+} sensor' in the ER lumen
- Binds to Mg^{2+}-ATP
- Ca^{2+} binding and storage
- ER chaperone
- Essential for glycoprotein maturation
- Important for MHC class I assembly
- Modulates inositol-(1,4,5)-trisphosphate-dependent Ca^{2+} release
- Modulates SERCA2b function
- Regulation of store-operated Ca^{2+} influx
- Zn^{2+} binding and storage

Gene expression
- Androgen-sensitive gene in prostate cancer
- Control of Rubella virus replication
- Control of steroid-sensitive gene expression
- Marker of viral infection
- Modulates vitamin D3 signal transduction
- Participates in host response to tumor

Others
- Affects phosphotyrosine level
- Important for cellular proliferation
- Increases sensitivity to apoptosis
- Induces NO formation in endothelial cells
- Intracellular iron transport
- Longterm memory molecule in *Aplysia*
- Mediates mitogenic effects of fibrinogen
- Stress protein

Abbreviations: CTL, cytotoxic T-lymphocyte; ER endoplasmic reticulum; MHC, major histo-compatibility complex; N-CAM, neural cell-adhesion molecule; NK, natural killer cell; NO, nitric oxide; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase.

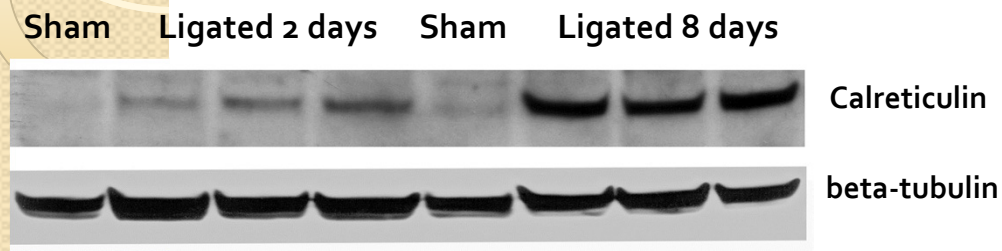
For further details, see review articles in Refs a–d and references therein.

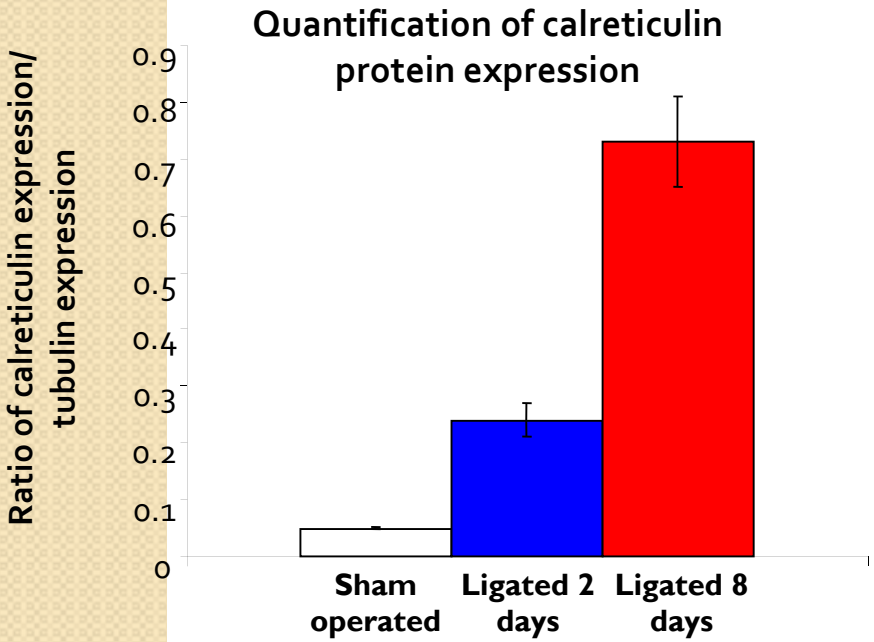
References
a Eggleton, P. *et al.* (1997) Clinical relevance of calreticulin in systemic lupus erythematosus. *Lupus* 6, 564–571
b Crofts, A.J. and Denecke, J. (1998) Calreticulin and calnexin in plants. *Trends Plant Sci.* 3, 396–399
c Nakhasi, H.L. *et al.* (1998) Implications of calreticulin function in parasite biology. *Parasitol. Today* 14, 157–160
d Michalak, M. *et al.* (1999) Calreticulin: one protein, one gene, many functions. *Biochem. J.* 344, 281–292

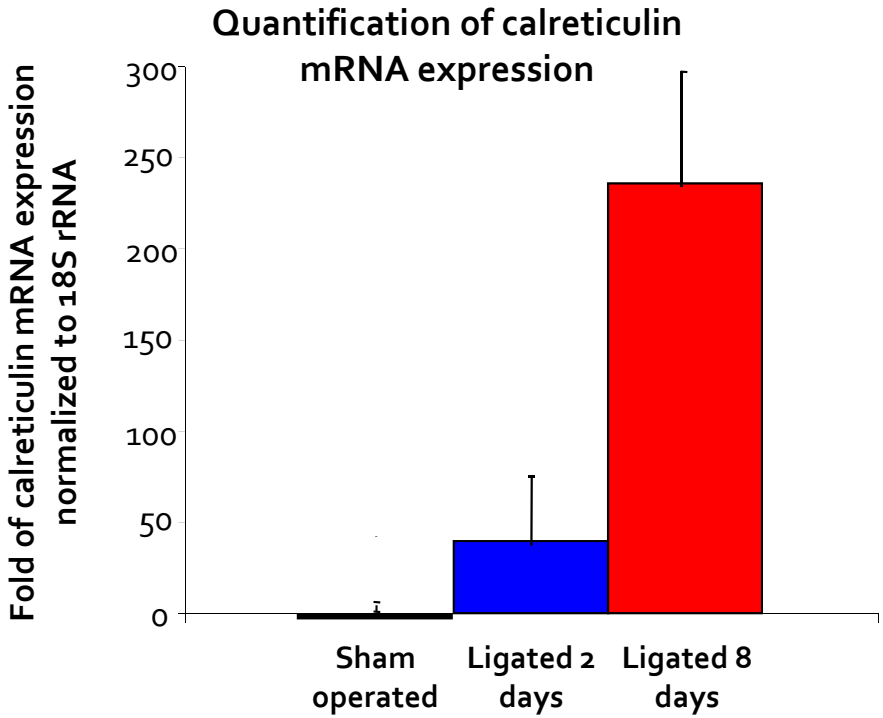
TRENDS in Cell Biology, Vol.11, 2001

There is no correlation with fibrotic processes yet !

Confirmation of Calreticulin upregulation in fibrotic samples







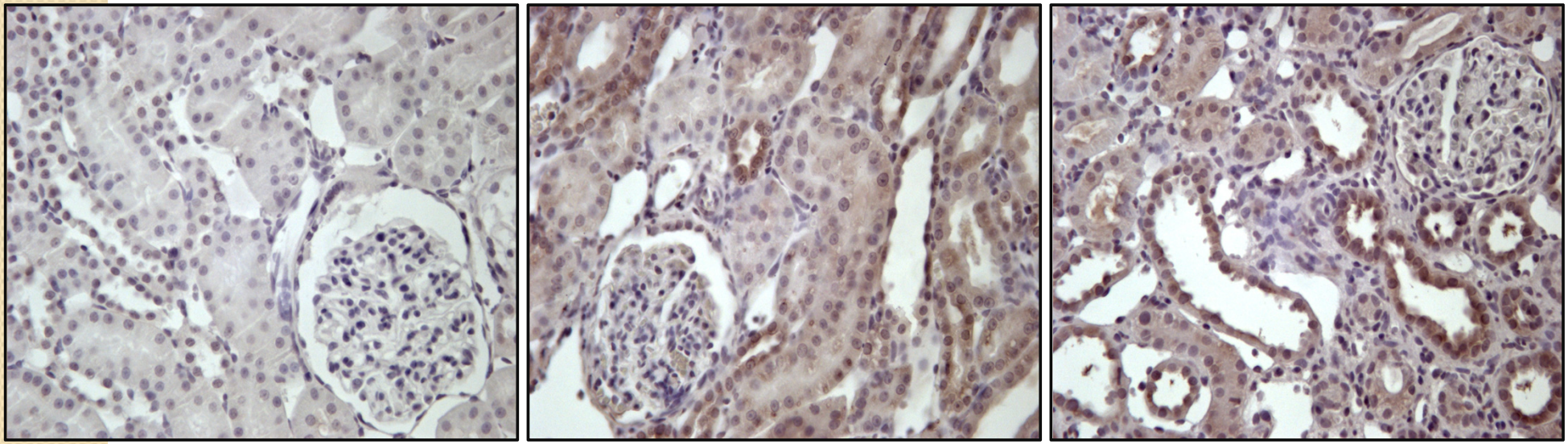
Kypreou K. *et al.*, *Proteomics*, 2008

Calreticulin is upregulated in the tubular cells of the kidney

Sham operated

Ligated 2 days

Ligated 8 days

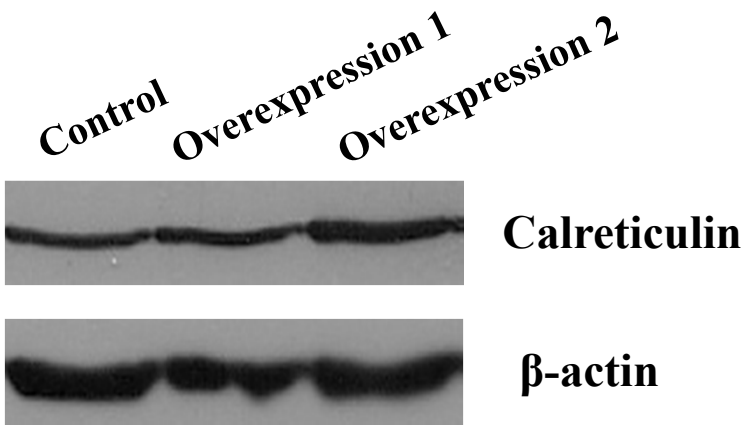


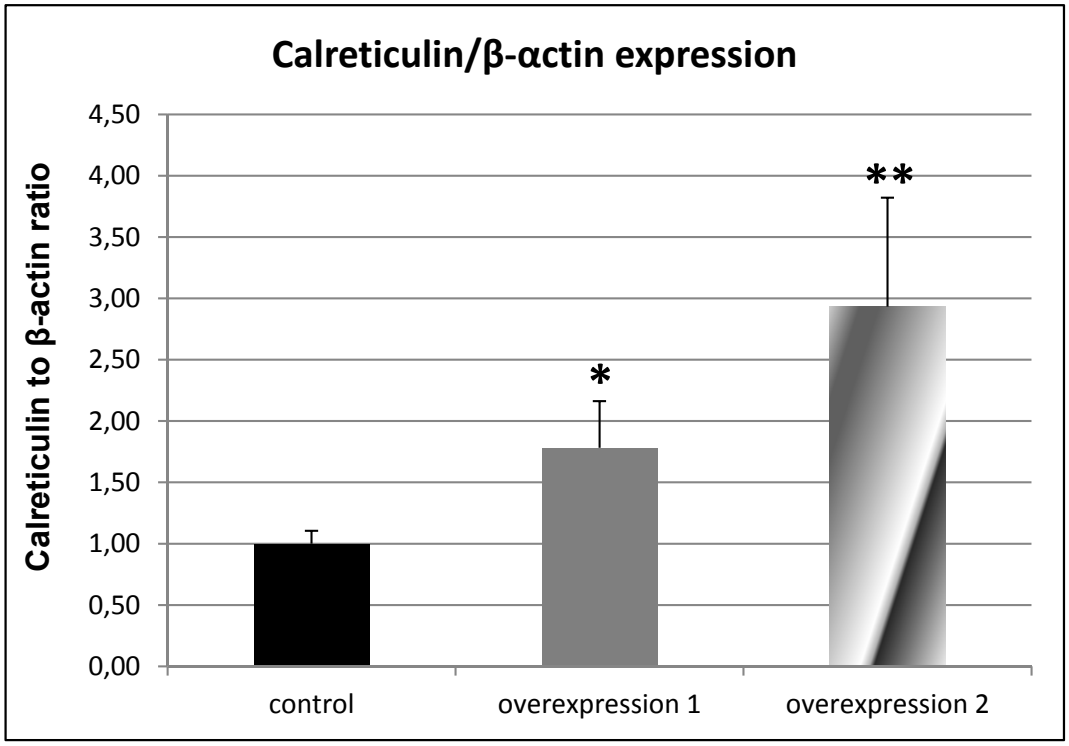
WHAT IS THE ROLE OF CALRETICULIN IN FIBROSIS?

- ***Phenotypic observations in a tubular cell line after Calreticulin overexpression***

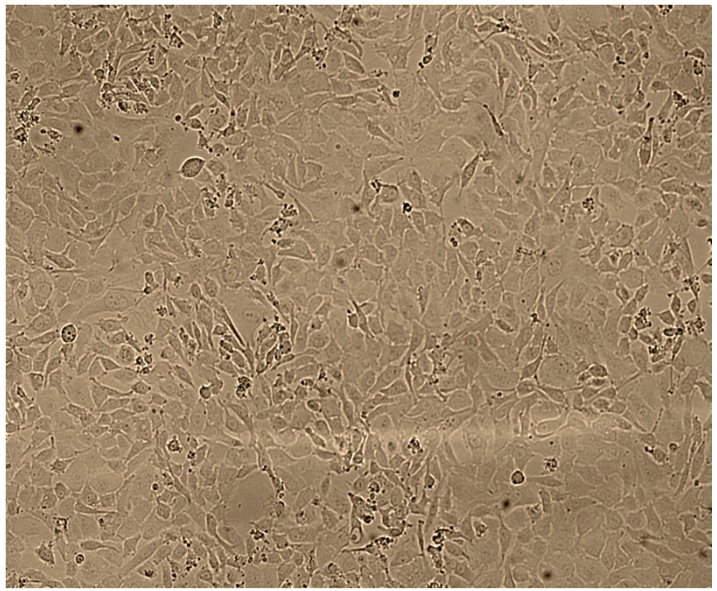
Calreticulin overexpression in HK-2 cells

- HK-2 cell lines, which stably express the mouse Calreticulin cDNA.

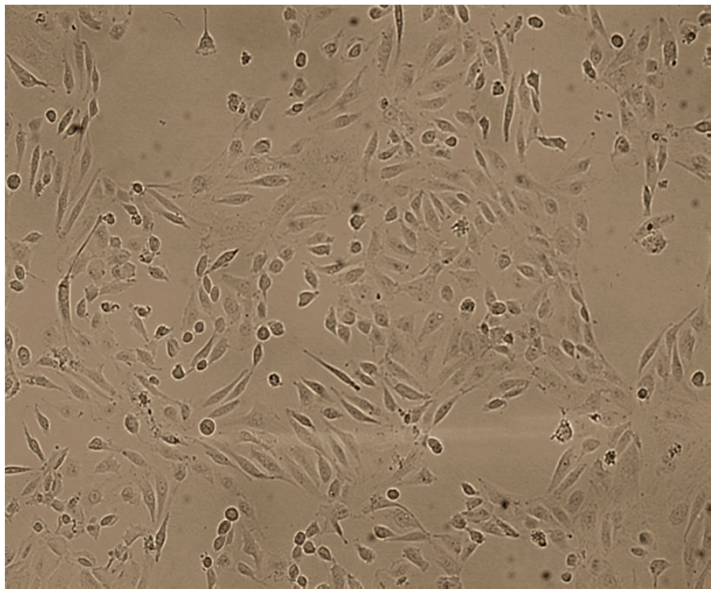




Calreticulin-overexpressing cells acquire an altered morphology

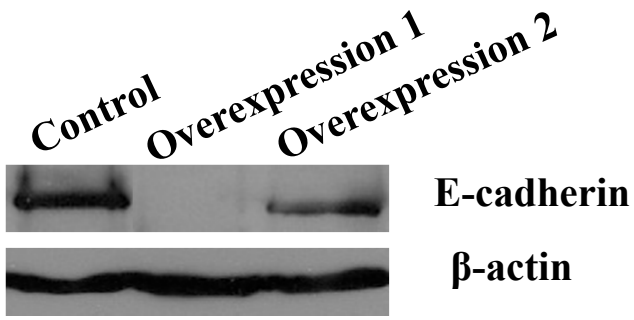


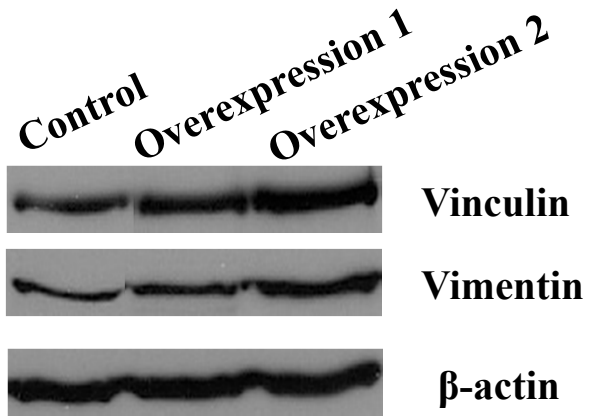
Control

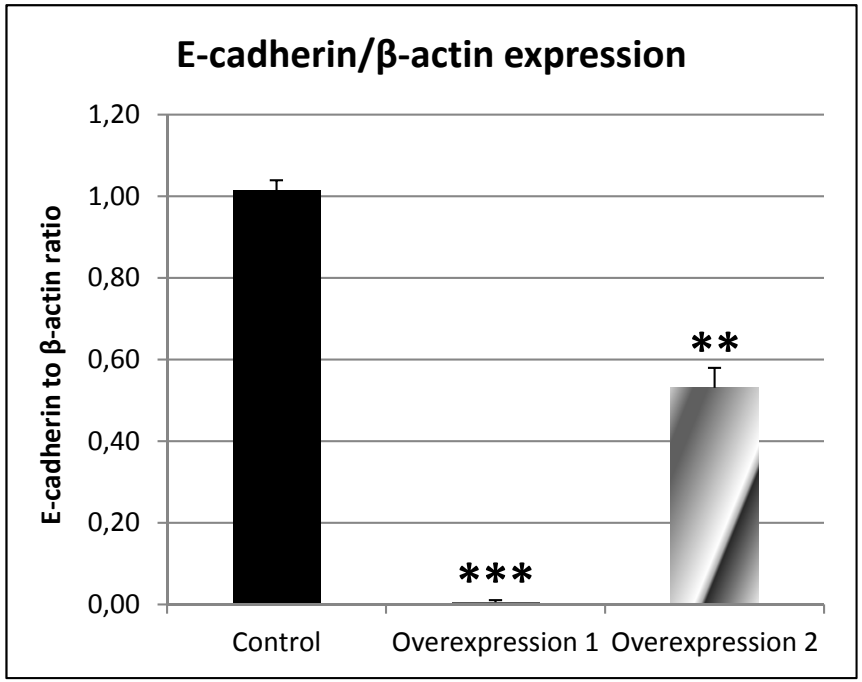


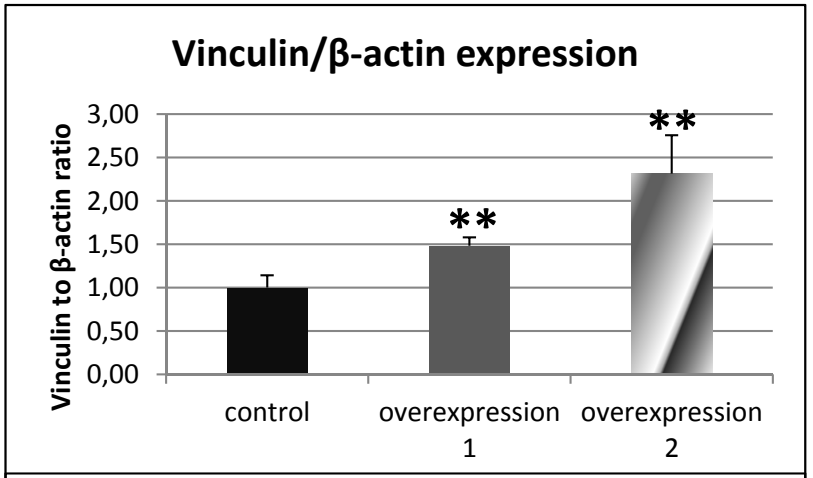
Overexpression 2

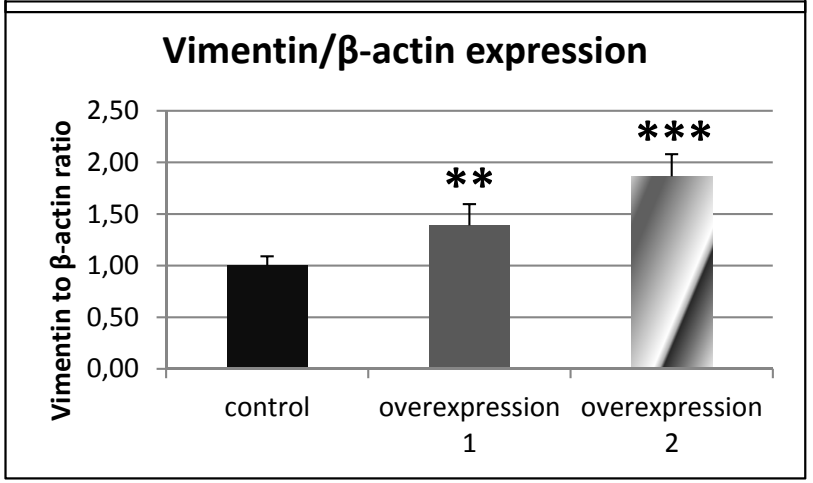
Differentially expressed proteins in Calreticulin-overexpressing HK-2 cells



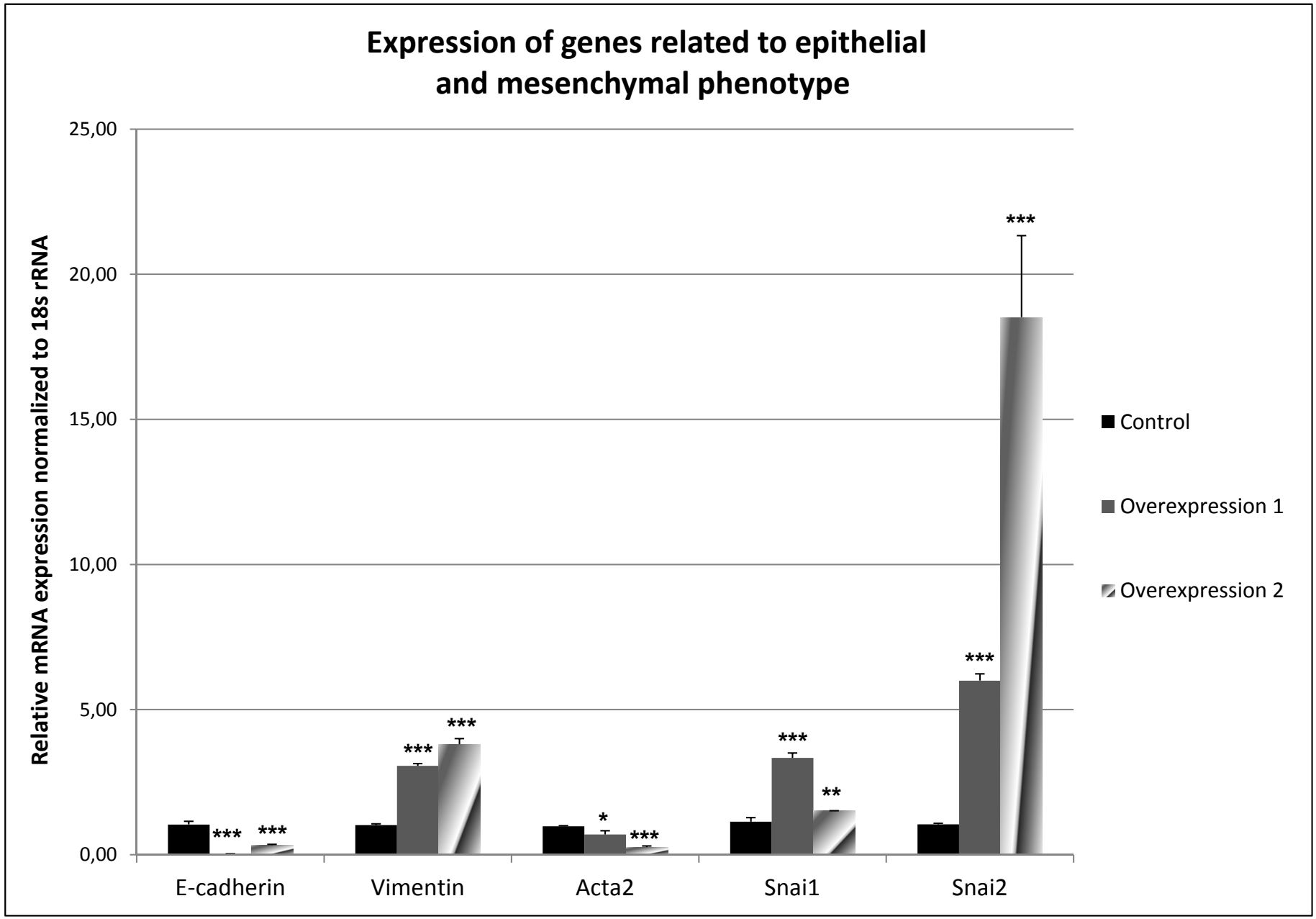




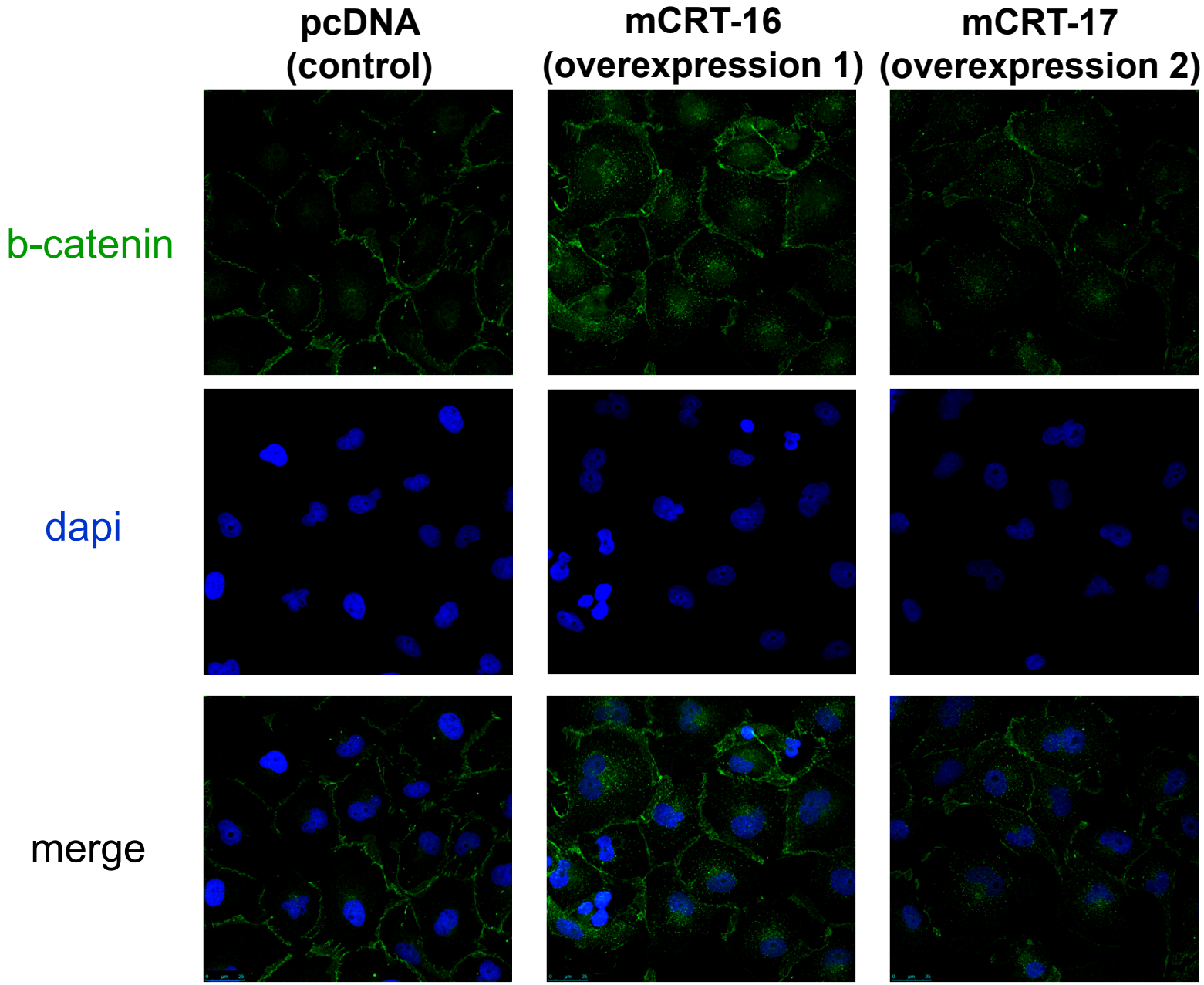




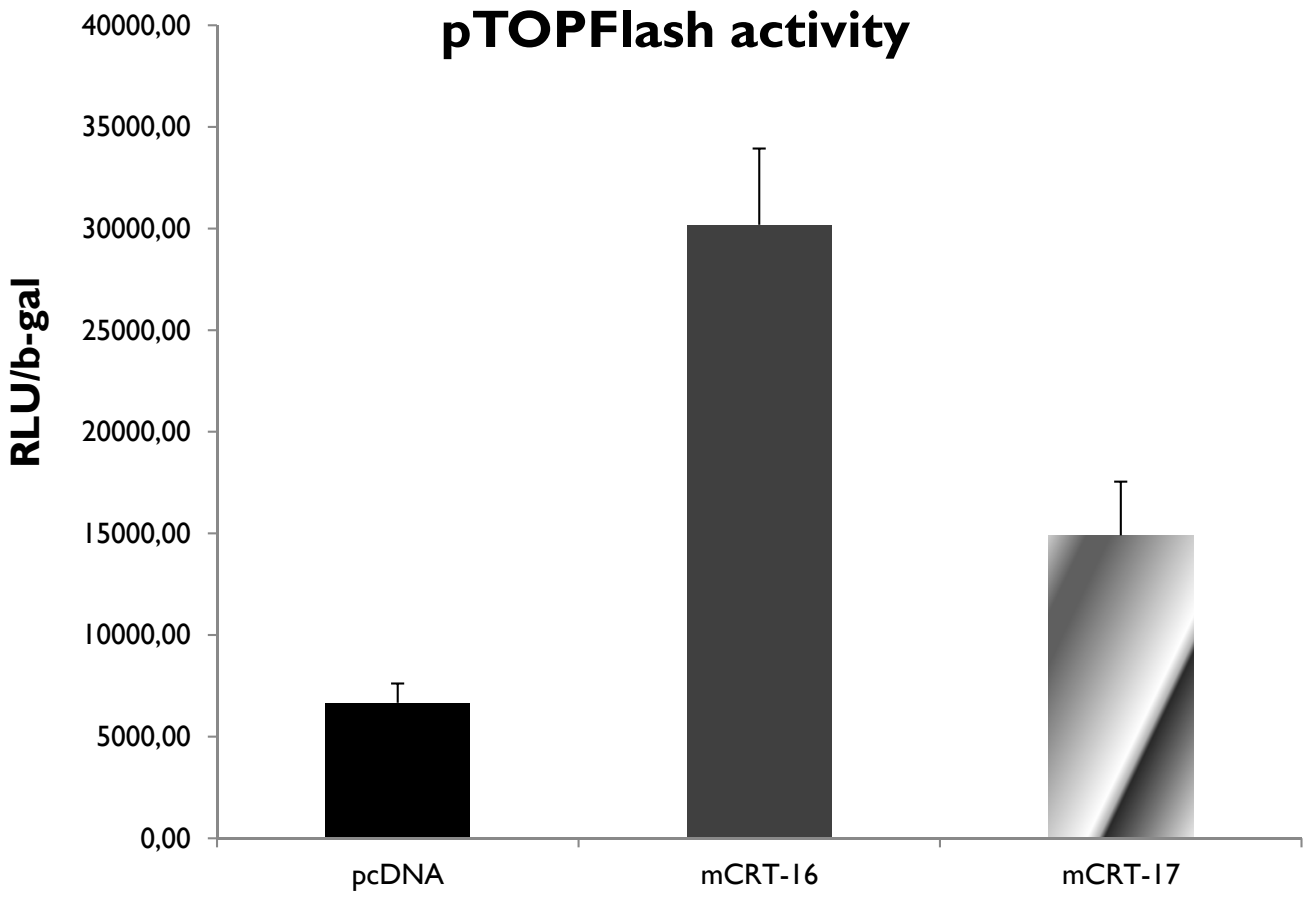
Differentially expressed genes in Calreticulin-overexpressing cells



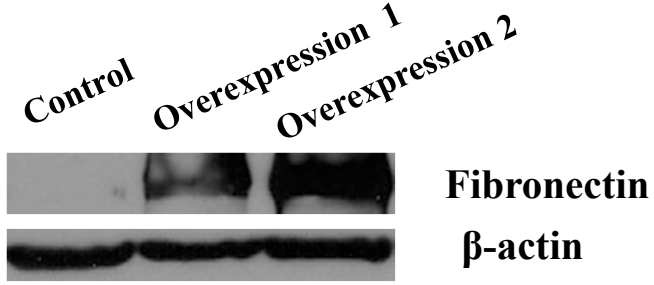
β-catenin expression/localization in the Calreticulin-overexpressing cells

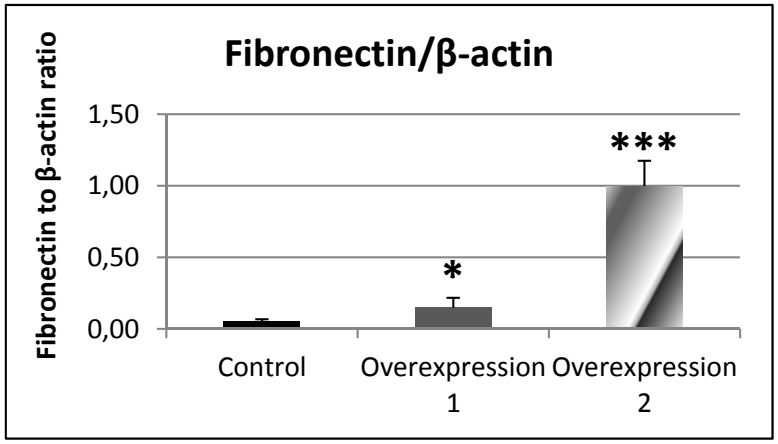


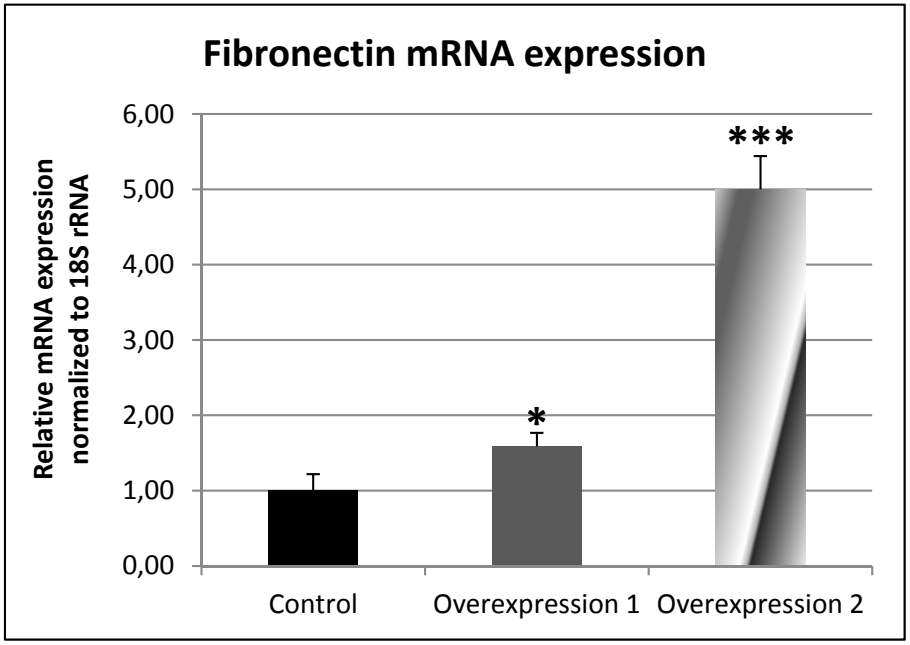
Calreticulin overexpression induces the activity of the TCF-luc-reporter pTOPFlash

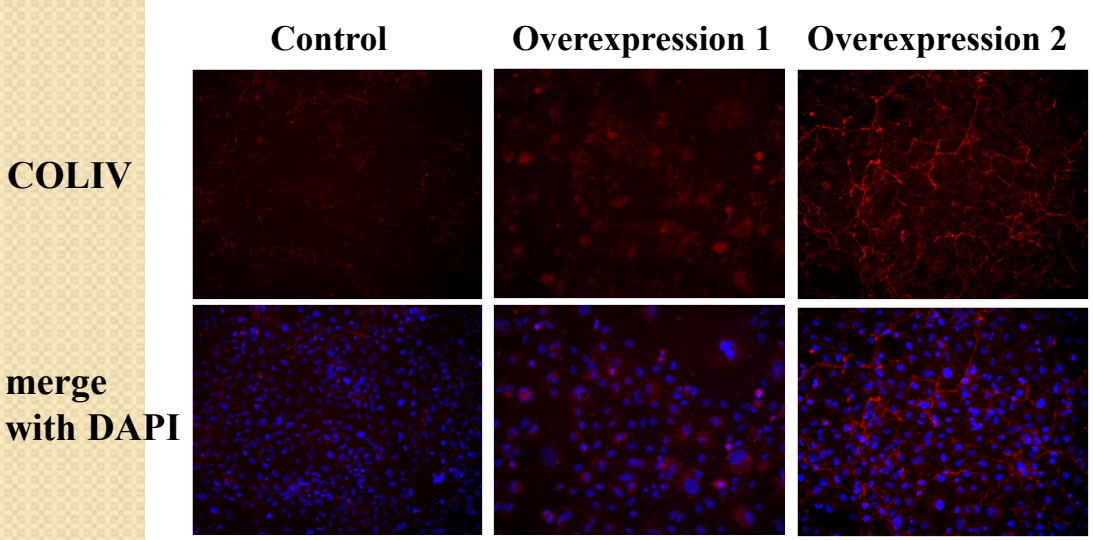


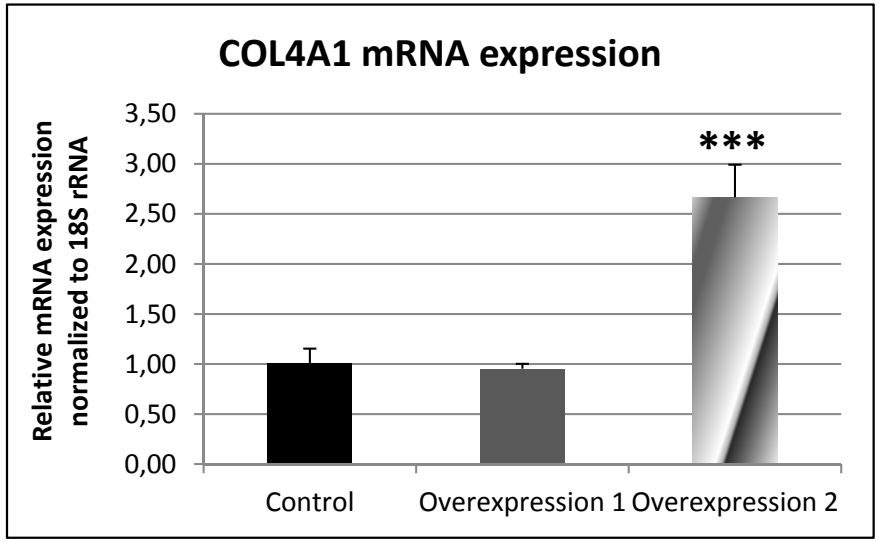
Calreticulin overexpressing cells have an increased secretory profile





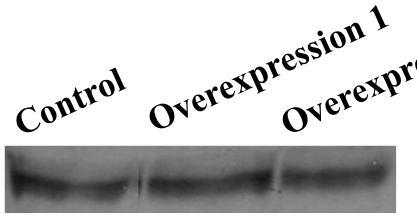






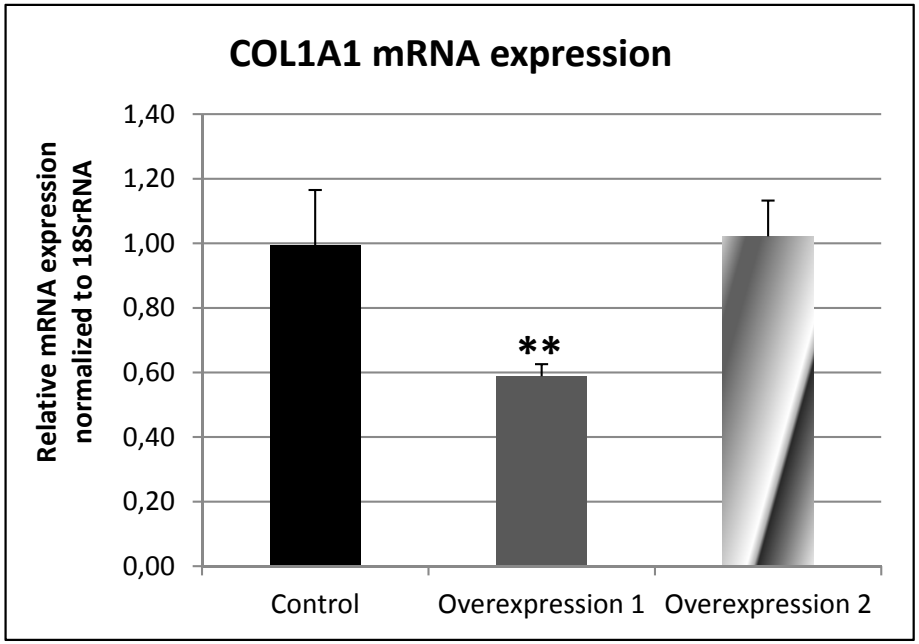
Collagen I expression levels are not increased by Calreticulin overexpression

Collagen I secretion in conditioned medium

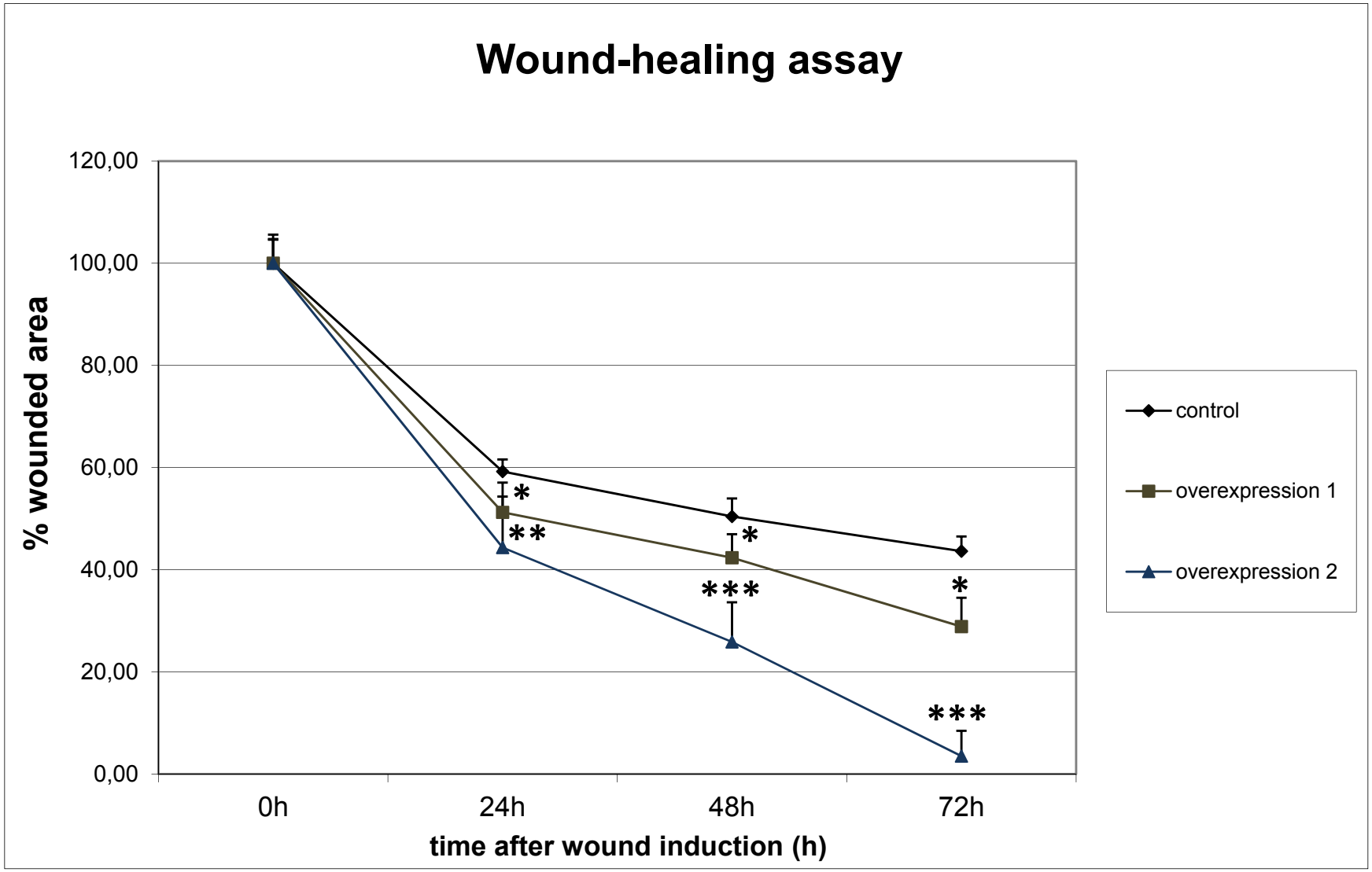


COLIA1

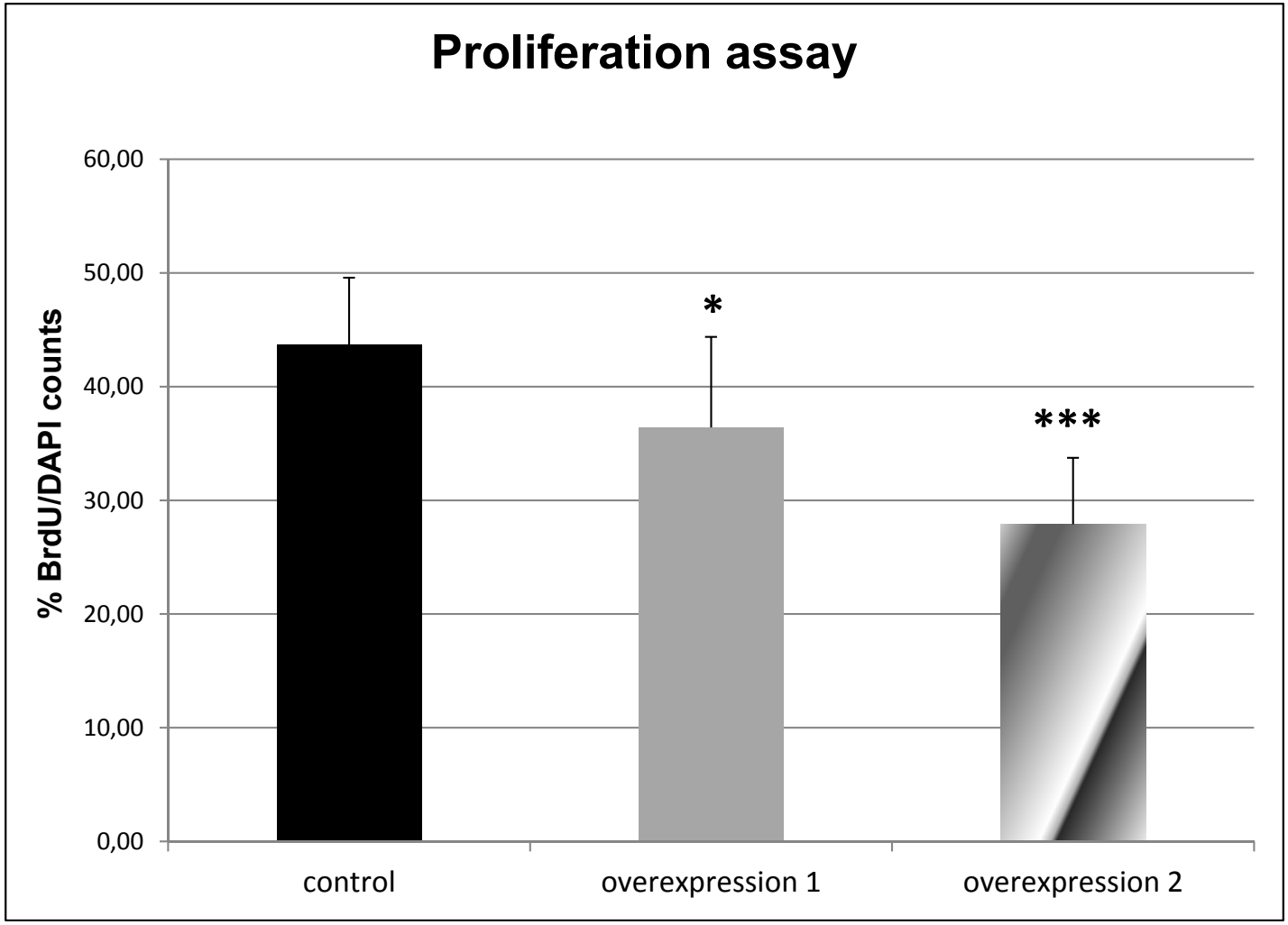
Ponceau S



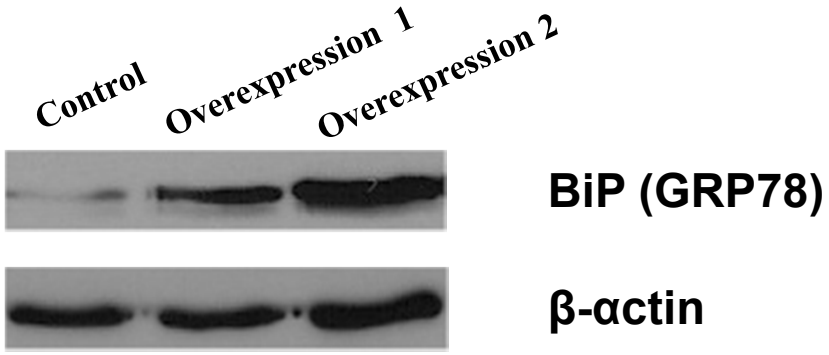
Calreticulin overexpression enhances the migratory capacity of HK-2 cells

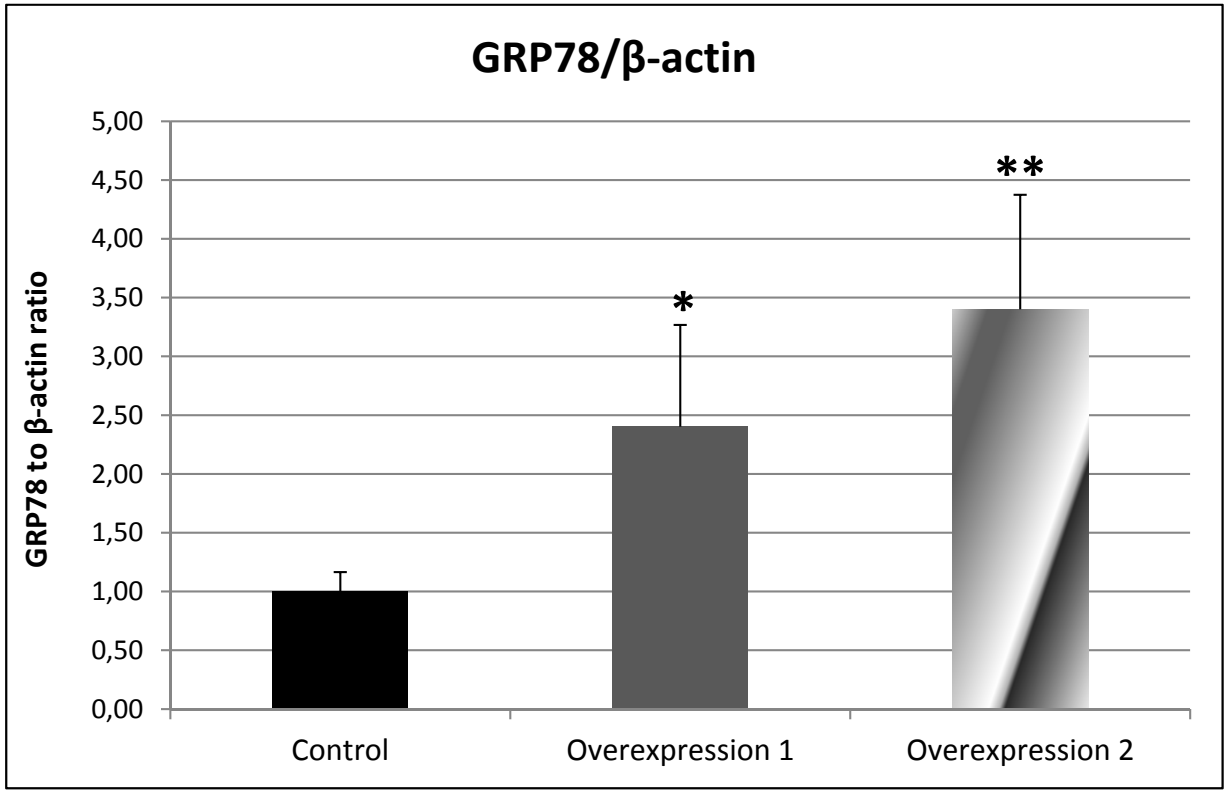


Calreticulin-overexpressing cells have lower proliferation capacity than the control cells

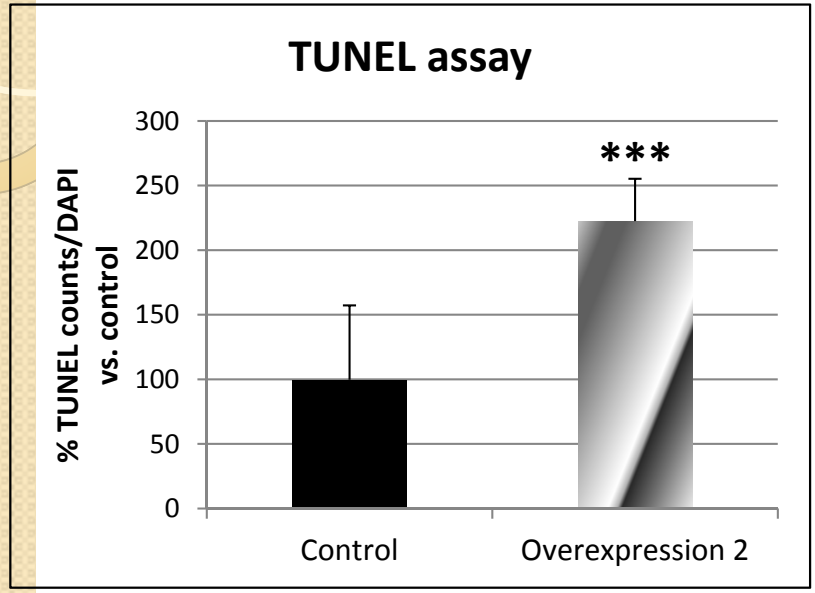


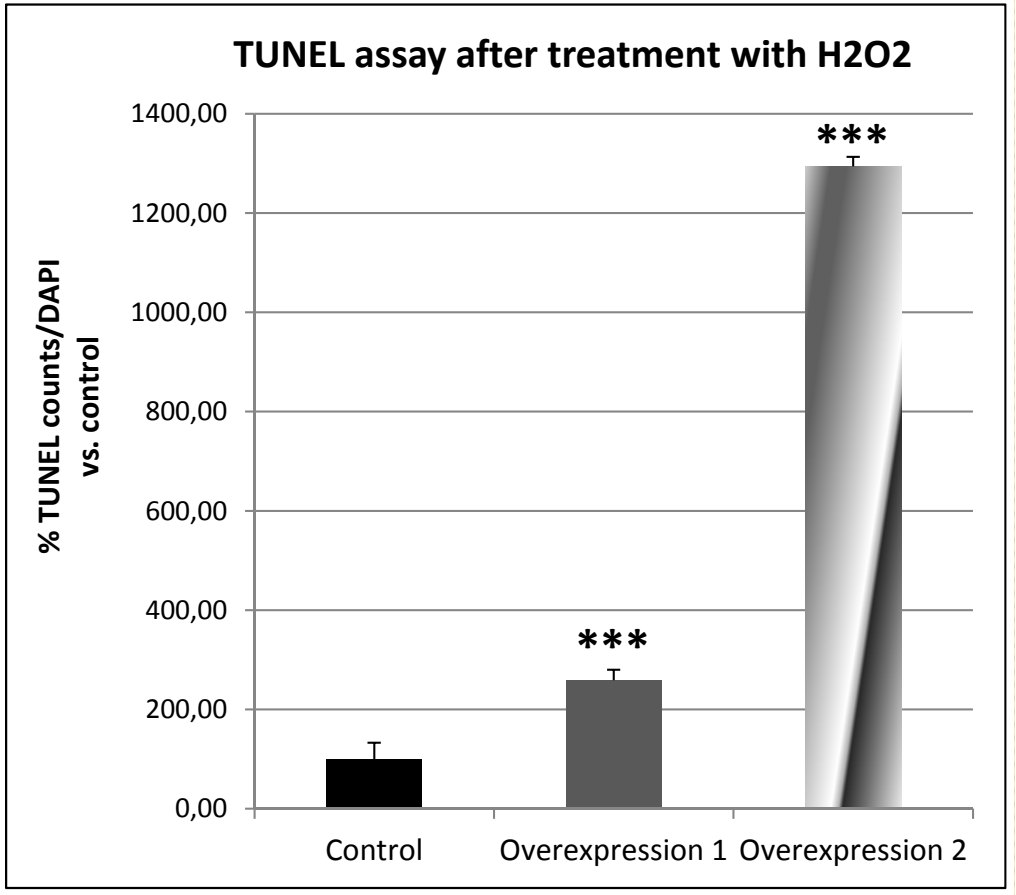
Calreticulin-overexpressing cells exhibit increased cellular stress

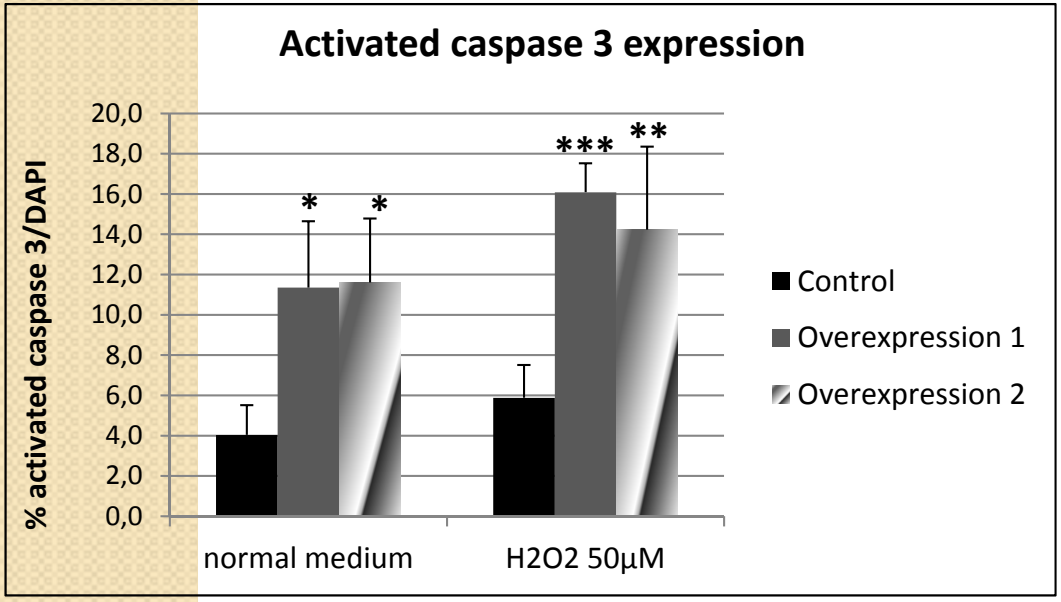




Calreticulin overexpression enhances dramatically cell sensitivity to apoptosis







Conclusions

- Calreticulin overexpression in HK-2 cells:
  - -reduces E-cadherin expression
  - -increases vimentin and vinculin expression
  - -induces Snail1/2 expression and β-catenin activation
  - -reduces aSMA expression while not affecting ColI production

thus resulting in a decline of the epithelial cell phenotype, without inducing complete EMT.

- Calreticulin overexpression in HK-2 cells:
  - -increases the fibronectin and collagen IV production
  - -increases significantly the ER stress and the migration capacity
  - -reduces the proliferation rate while increases the cell sensitivity to apoptosis,

all of which represent characteristics of fibrosis.

All the above points introduce Calreticulin as a novel potential central molecule in the fibrotic processes.

Acknowledgements

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