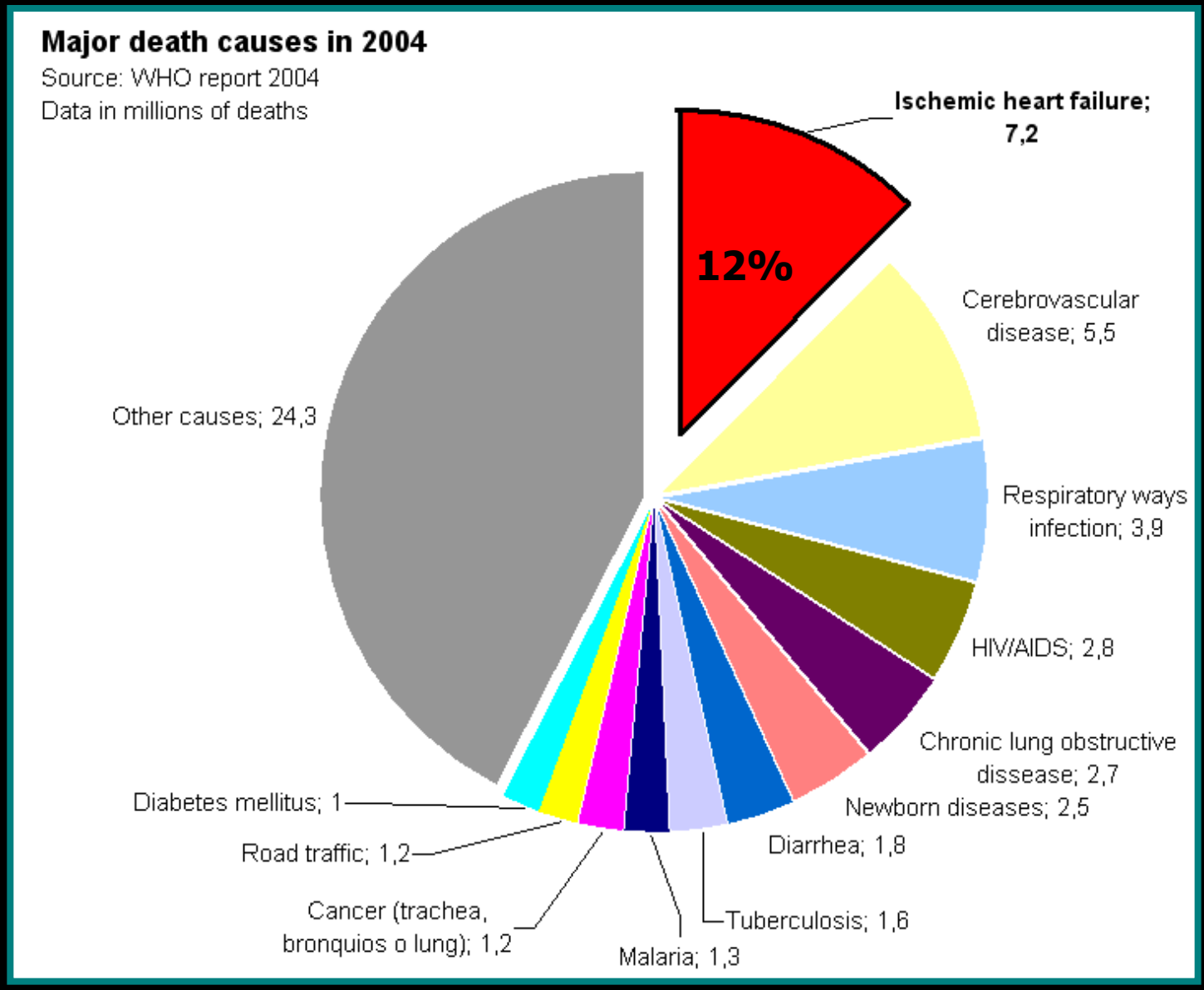
Joint BSPR/EBI Conference 2010
Proteomics: From Qualitative to Quantitative

PROTEOMIC ANALYSIS OF CARDIAC EXTRACELLULAR MATRIX IN A PORCINE MODEL OF ISCHEMIA-REPERFUSION INJURY

New roles for known proteins?

Javier Barallobre-Barreiro

PROTEOMICS ON ISCHEMIC HEART FAILURE: WHY?

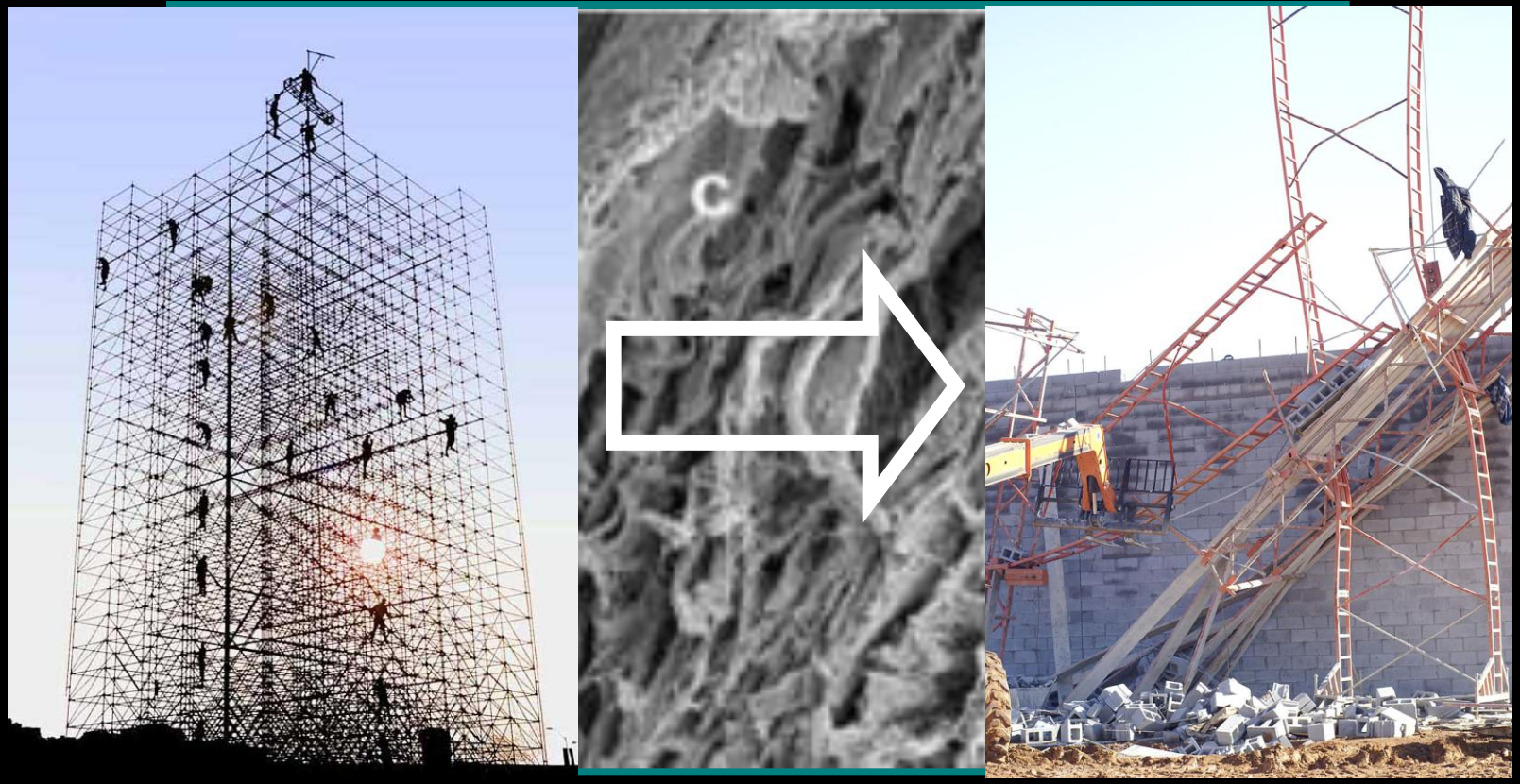


THE HEART FROM THE INSIDE

Heart is composed of **70% non-myocytes** and only 30% myocytes

but

most of the **studies have focused on myocytes**
with little emphasis on the other cell types and structures.



CARDIAC EXTRACELLULAR MATRIX

- Scaffold for the myoycte and nonmyocyte cells
- Surrounds and interconnects cellular structures
- Distributes mechanical forces
- Transmits mechanical signals to cells via surface ECM receptors
- Fluid movement in the extracellular environment

Proteoglycans & glycoproteins
Signaling and turnover of the ECM itself. Can bind factors that contribute to the concentration of inflammatory components forming chronic inflammation.

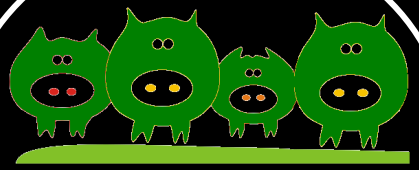
Interstitial collagens
The density of the ECM affects compliance, movement of cells and fluid within, availability of cell receptors and substrates and retention of ECM components, such as proteoglycans.

Proteases
Part of a biochemical cascade within the ECM. Essential for turnover of ECM components, activation of latent factors, and remodeling.

Cytokines
TGF-beta. Regulation of cell proliferation, migration, differentiation, apoptosis, and ECM production. Central role in fibrosis. Stimulates production of collagen while inhibiting its degradation.

Growth factors
Role in normal and pathological growth and so, in ECM changes

ANIMAL MODEL



CONTROLS

n=4, untreated

ISCHEMIA REPERFUSION

n=3, 2h of coronary oclussion

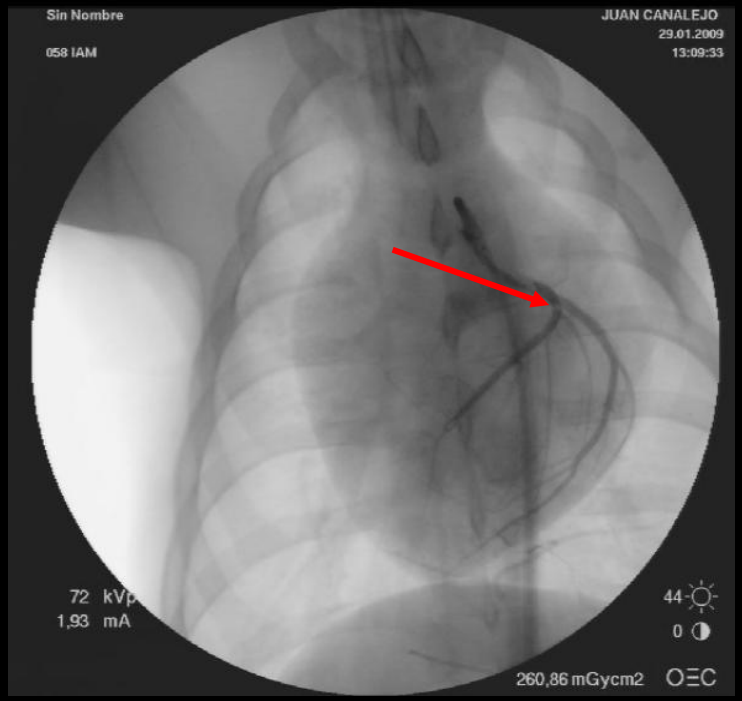
15 days

+

CHRONIC SAMPLE

2h coronary oclussion

2 months



VALIDATION OF OUR MODEL

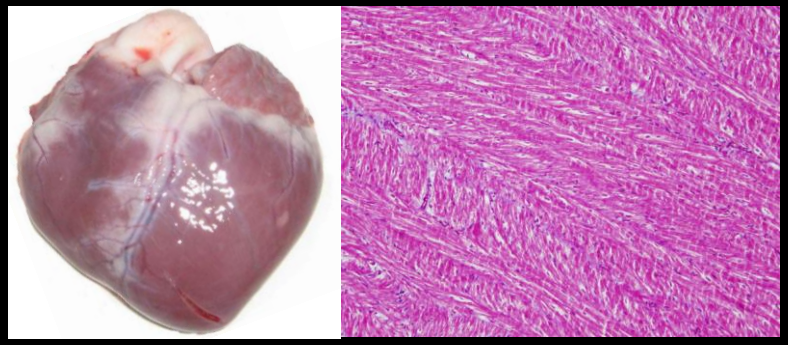
Basal

(Day 0)

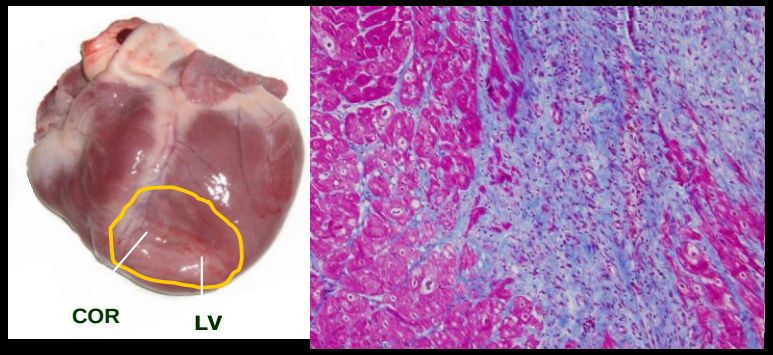
2 hours

Ischemia-reperfusion

(After 15 days)



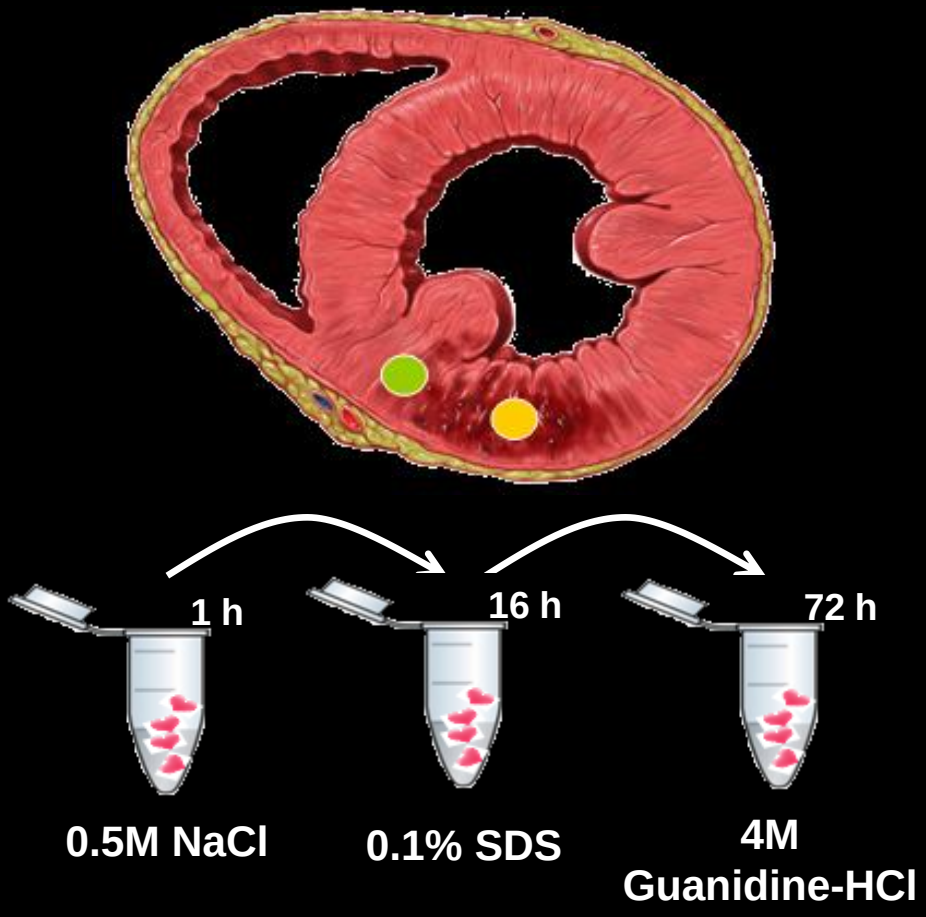
Control

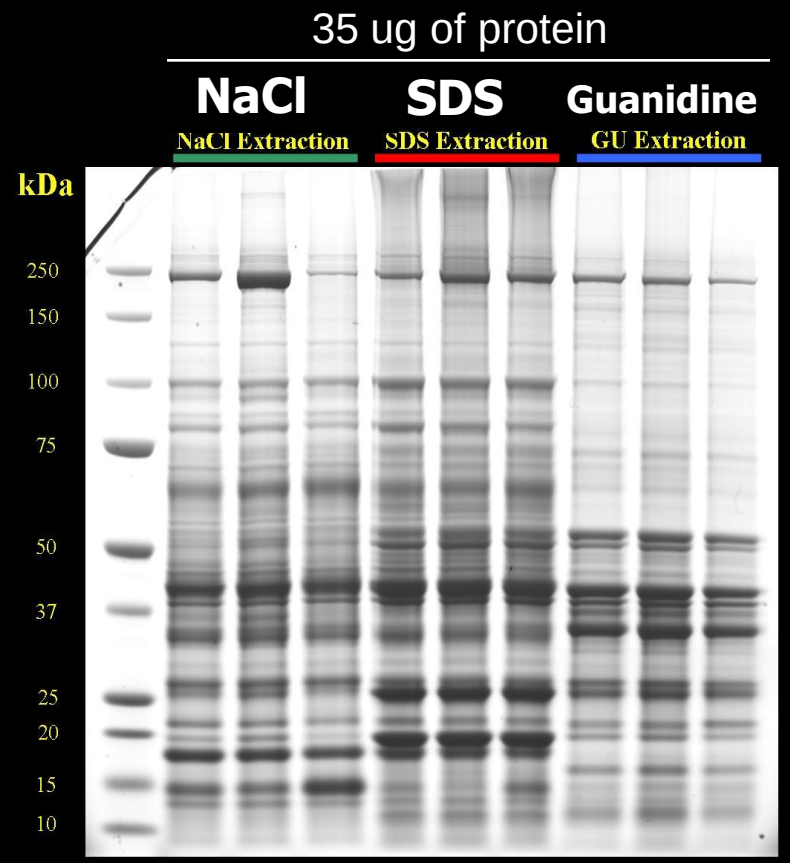


15 days after ischemia-reperfusion

SEQUENTIAL EXTRACTION

Didangelos A, Yin X, Mandal K, Baumert M, Jahangiri M, Mayr M. ***Proteomic characterization of extracellular space components in the human aorta***. MCP in Press. June 15, 2010.





PROTEIN IDENTIFICATION

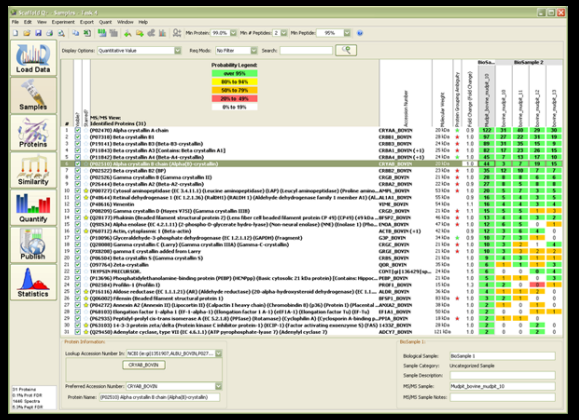
Silver stain → Digestion (trypsin) → Peptide separation

Dionex Ultimate 3000 HPLC

LQT Orbitrap XL

Hybrid Pig / Human DB → X! Tandem, SeQuest

Scaffold



[Spectral count]

VERIFICATION OF EXTRACTION PROCEDURE

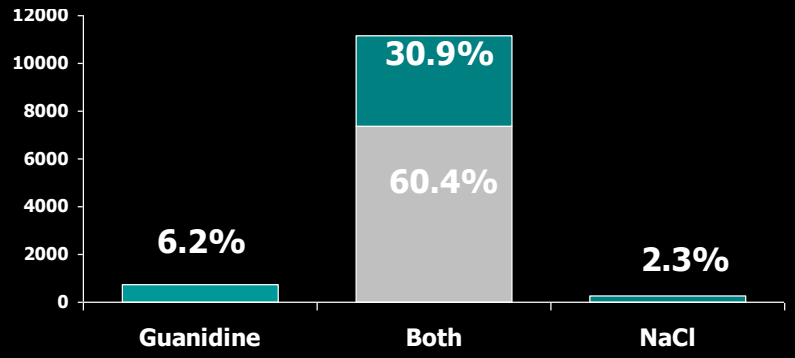
Proteoglycans (60)

Guanidine

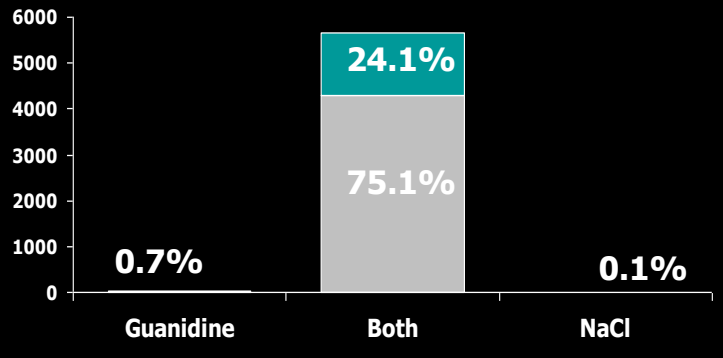
NaCl

Collagens (28)

Proteoglycans: spectra distribution

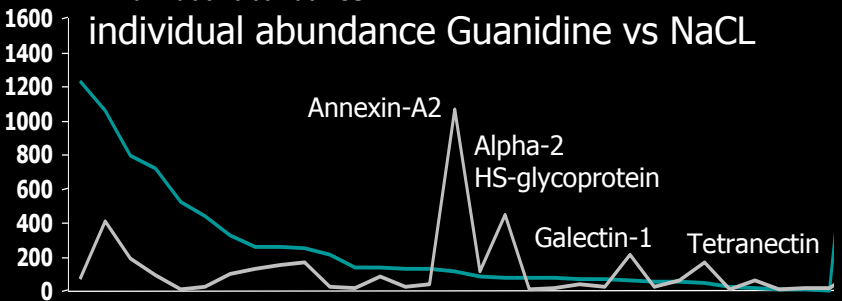


Collagens: spectra distribution



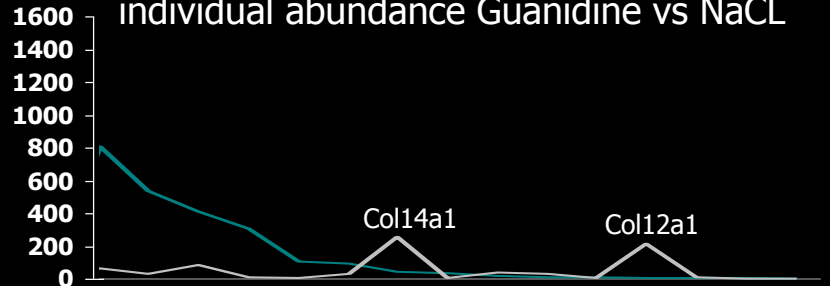
Proteoglycans:

individual abundance Guanidine vs NaCL



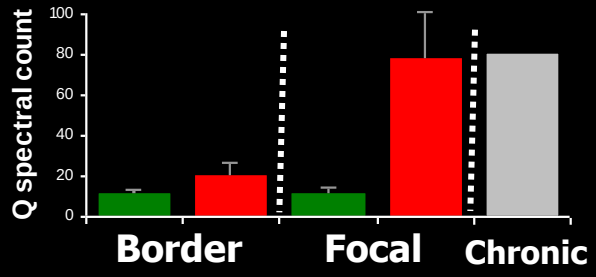
Collagens:

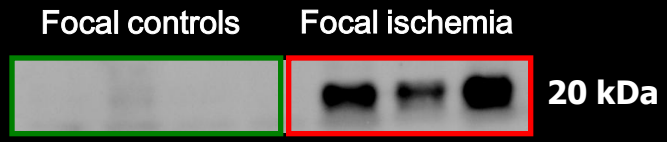
individual abundance Guanidine vs NaCL



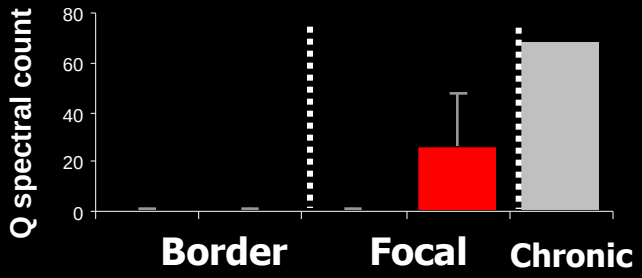
HIGHLIGHTS OF PARTICULAR PROTEINS

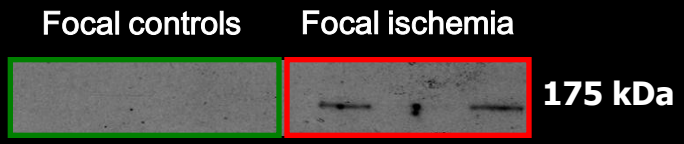
DERMATOPONTIN



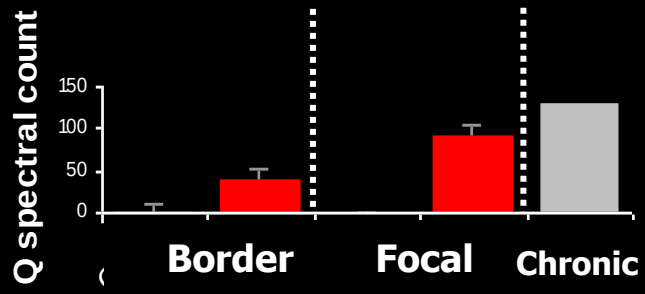


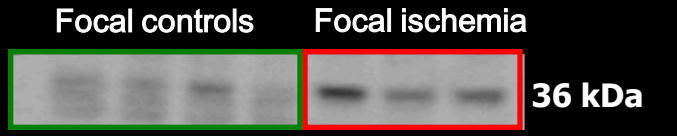
ACLP

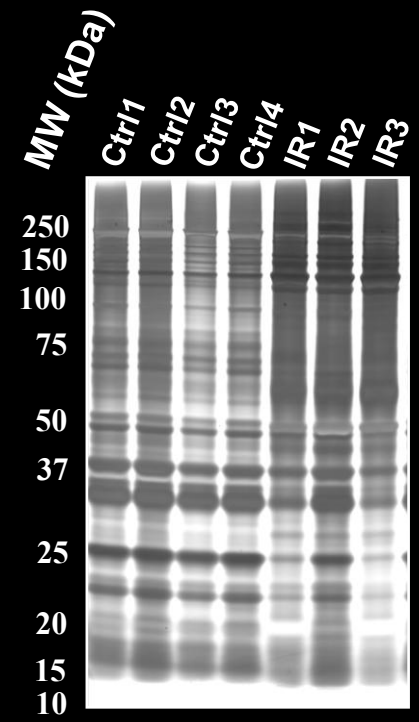




BIGLYCAN

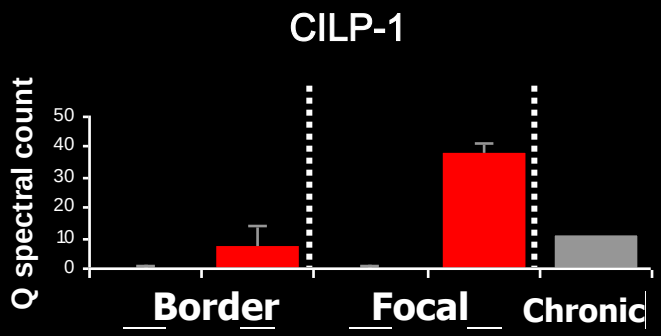


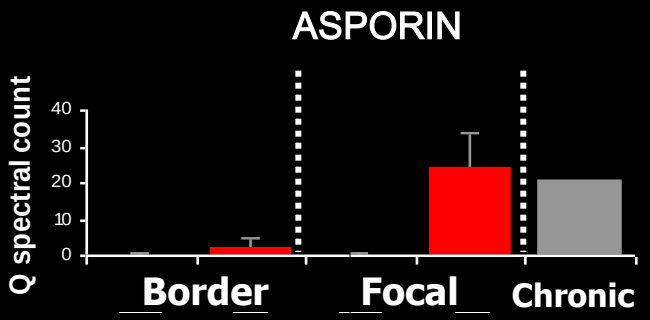


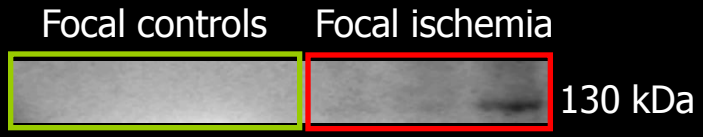


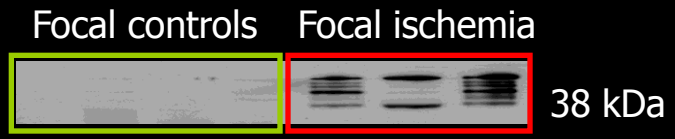
Guanidine Focal

HIGHLIGHTS OF PARTICULAR PROTEINS

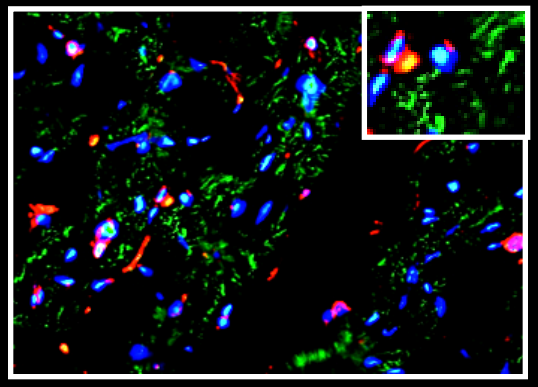




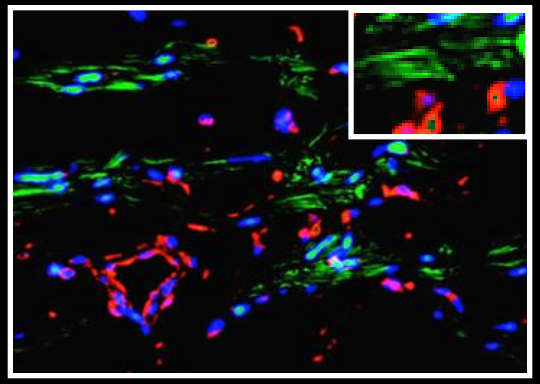




CILP-1 + Vimentin + DAPI

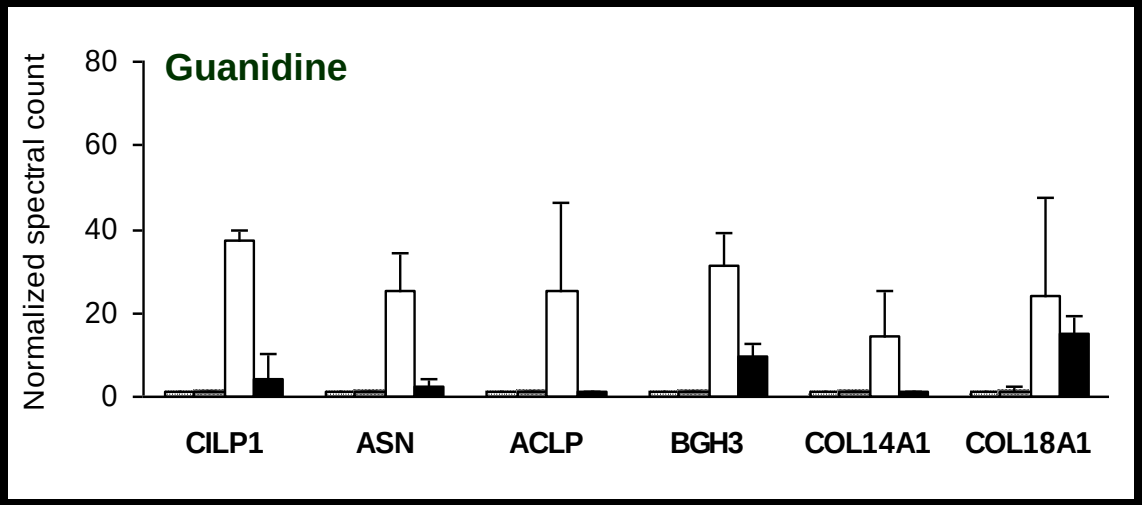


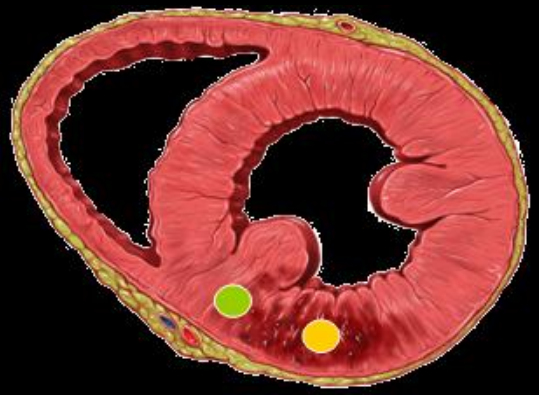
Asporin + Vimentin + DAPI

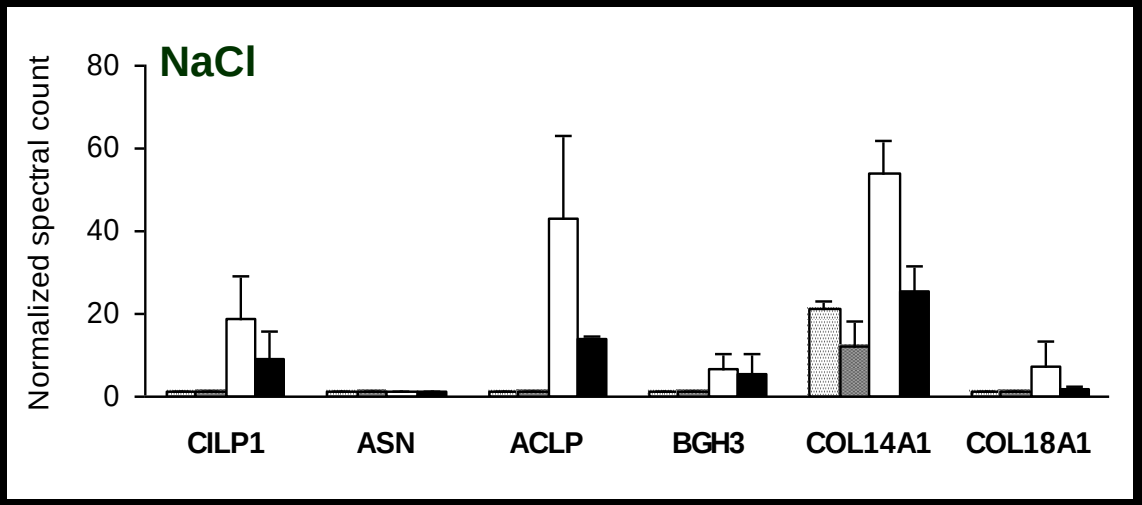


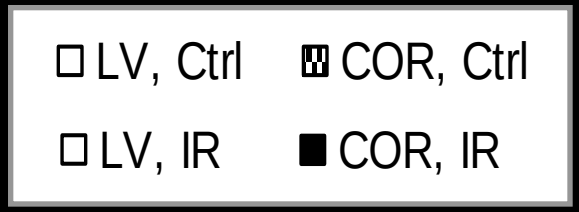
FOCAL INJURY VS BORDER REGIONS

Differences on spectral count



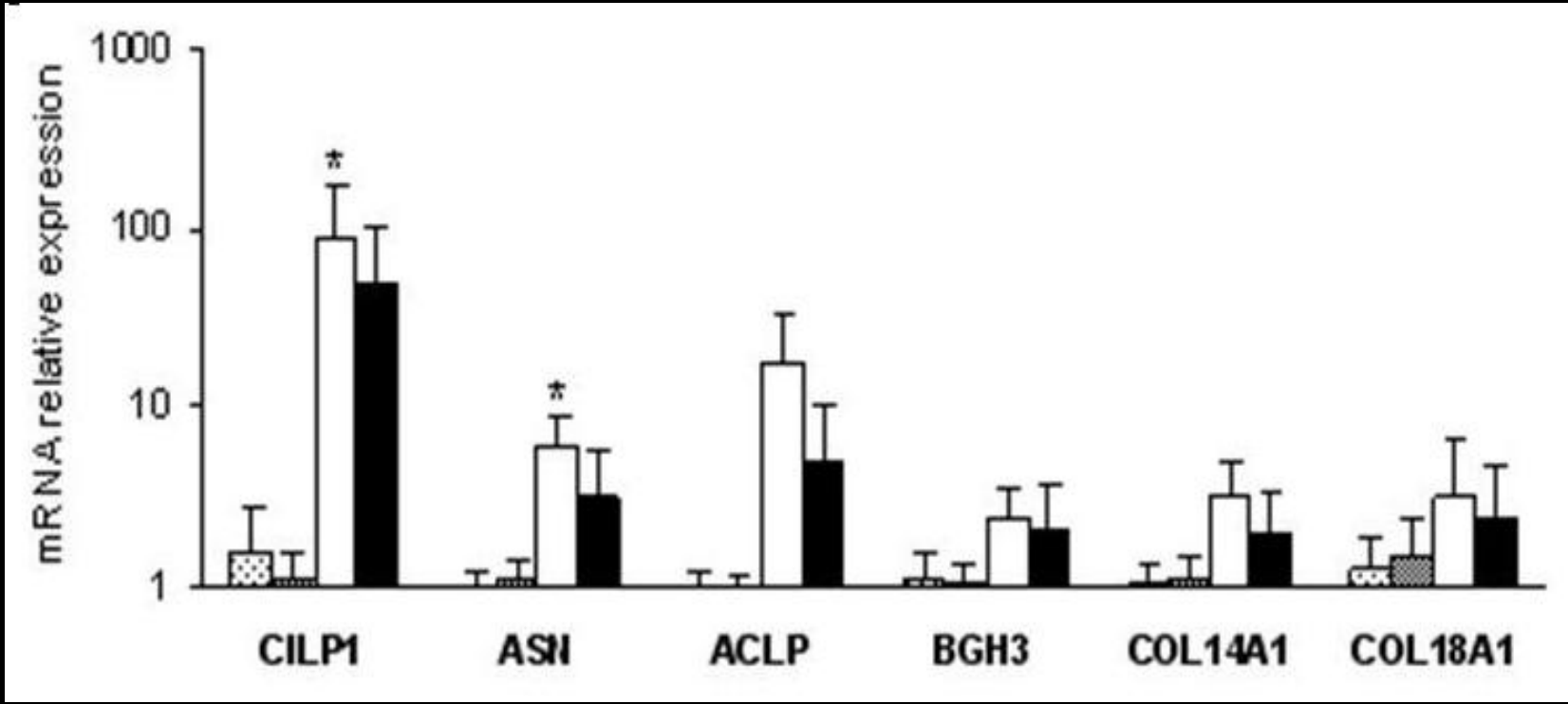






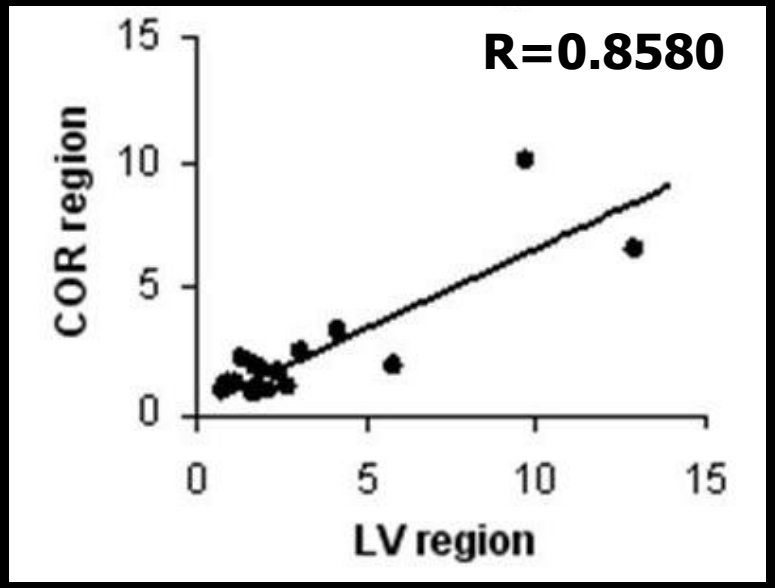
FOCAL INJURY VS BORDER REGIONS

Differences on mRNA expression

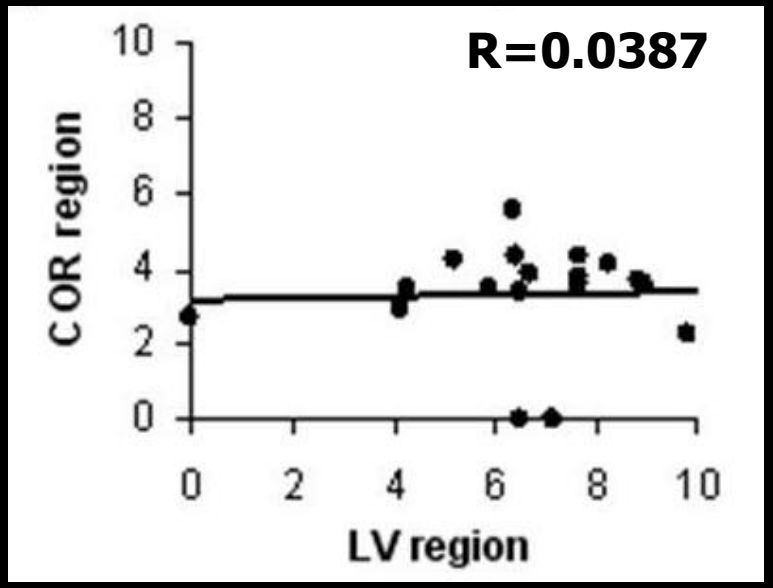


FOCAL INJURY VS BORDER REGIONS

qRT-PCR

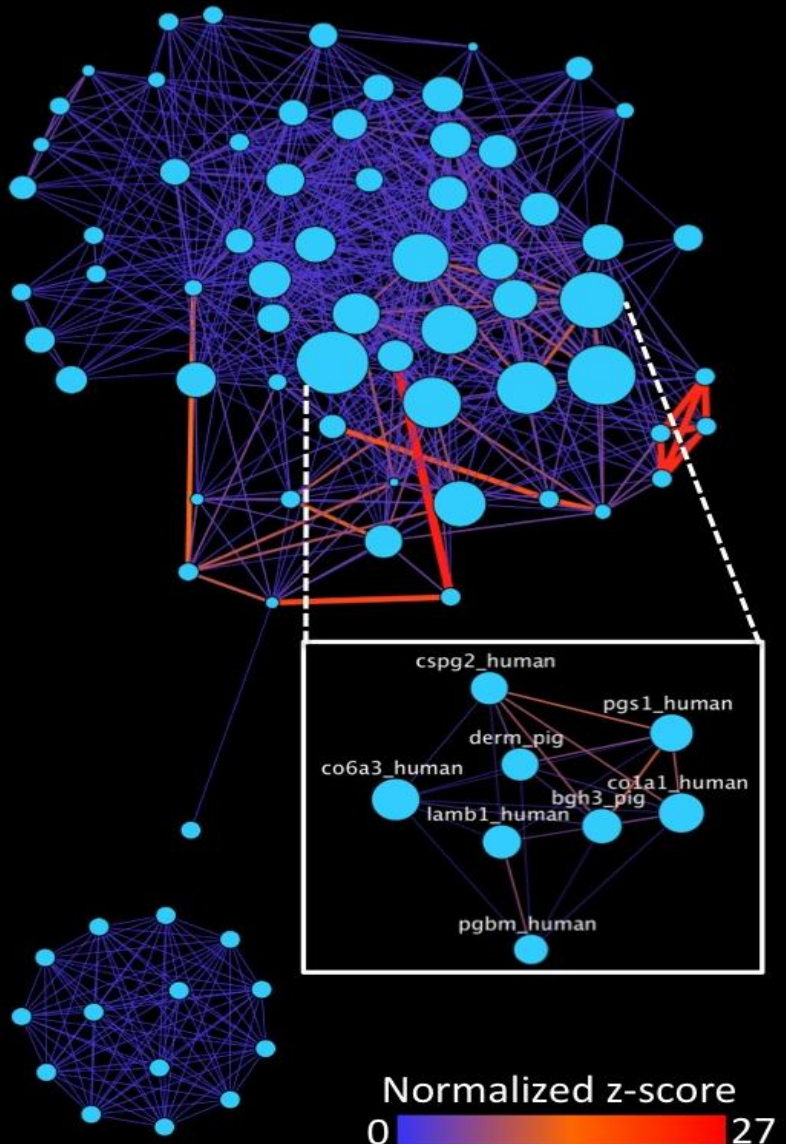


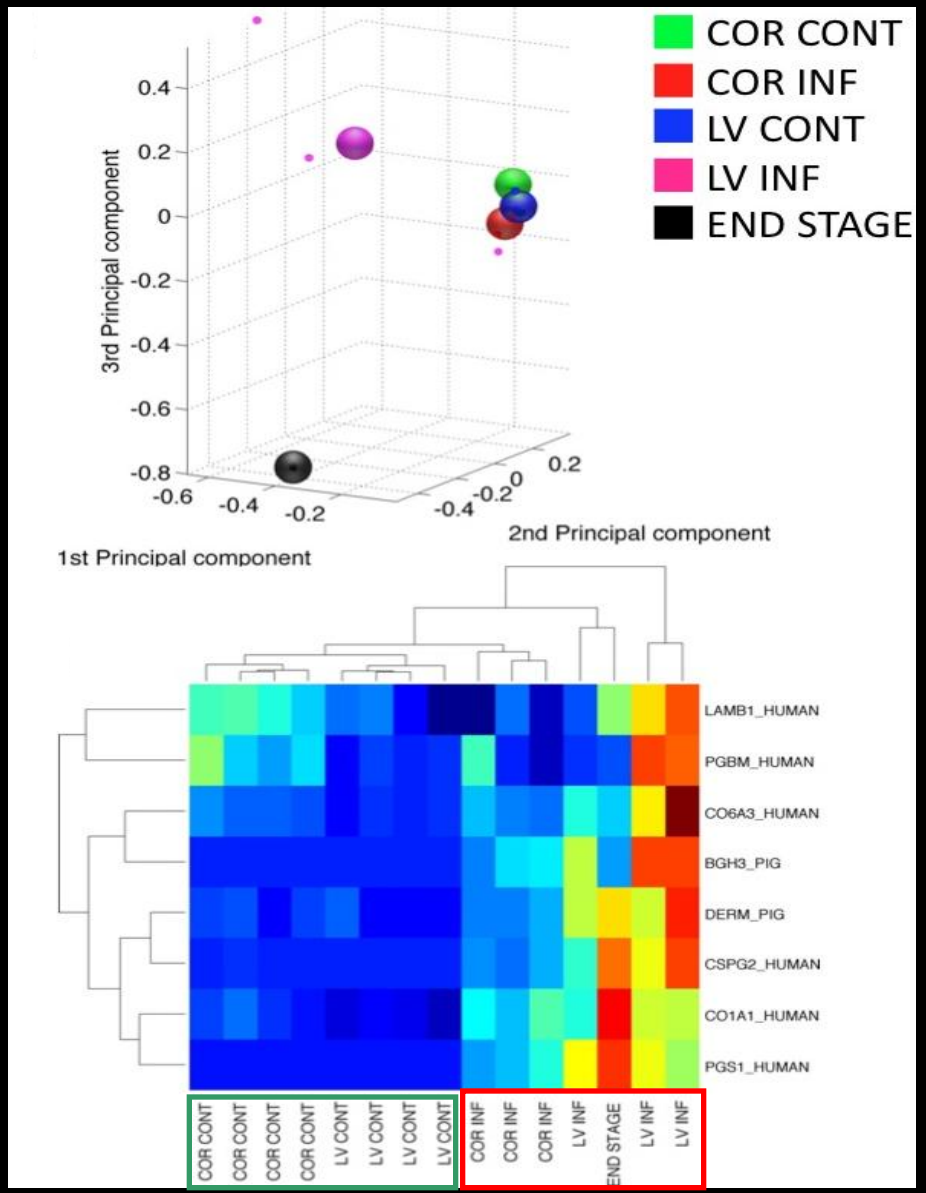
Spectral count



mRNA picks the current trend on both regions, but do not discriminate actual differences due to **ECM accumulation.**

A FURTHER ANALYSIS





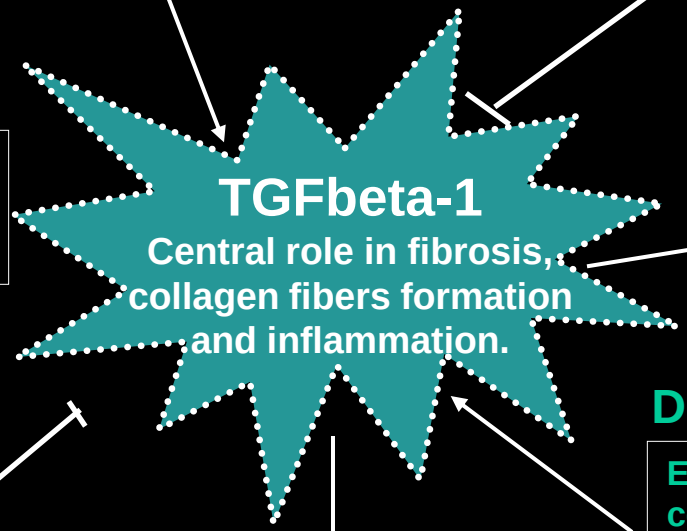
HOW MUCH IS KNOWN ABOUT THIS?

	Total entries	+ Matrix	+ Heart/Cardiac	+ Heart Remodelling
CILP (1)	26 (1)	19	0	0
Asporin	40	36	0	0
Dermatopontin	30	27	1	0
ACLP	35	8	1*	0
Mimecan	39	14	2	0
Nidogen 2	38	25	2	0
Versican	822	574	<15	3
Vitronectin	4226	1728	<30	6
Perlecan	760	398	4**	0
Decorin	1680	113	58	14
Agrin	773	173	5	0
Collagen I	3053	1580	191	52
Collagen III	721	309	95	27
Collagen XIV	31	21	0	0

HEART REMODELING: "X" AND heart AND remodeling NOT arterial NOT aorta NOT valve NOT aortic
HEART/CARDIAC : "X" AND heart OR cardiac NOT arterial NOT aorta NOT valve NOT aortic

**Identification*
***Very specific papers*

IS THERE A COMMON LINK ?



Mimecan

Bone specific protein. Induces **bone** formation in conjunction with **TGF-beta-1** or TGF-beta-2.

Asporin

2001."Identification and characterization of asporin. A novel member of the **SLRPs** closely related to decorin and biglycan."

Matrilin-4

Major component of the **ECM of cartilage**. Present in embryonic kidney, lung and placenta.

?

BGH3

TGFbeta-induced protein g-h3

Binds to type I, II, and IV collagens. **Inducted by TGFbeta**.

Dermatopontin

Enhances TGFB1 activity. Accelerates **collagen fibril formation**, and stabilizes collagen fibrils against low-temperature dissociation.

CILP-1

Cartilage Intermediate Layer Protein 1

Cartilage specific. Role in cartilage scaffolding. May act by antagonizing TGFB1 and IGF1. Suppresses sulfated proteoglycan synthesis. May **inhibit TGFB1-mediated induction of cartilage matrix genes.**

ACLP *Aortic Carboxipeptidase*

Promotes macrophage **inflammatory responsiveness** by up-regulating NF-kappaB via Ikappa-B-alpha negative regulation.

CONCLUSIONS AND FUTURE DIRECTIONS

- This is the first comparative proteomic study focusing on cardiac ECM after ischemia.

- qRT-PCR correlation experiments showed proteomics to pick differences not measurable by other techniques.

- We have discovered a set of ECM proteins that might have critical roles on wound healing and scar formation after ischemic heart failure, so this may offer invaluable clues leading to new therapeutic approaches

- The actual role of highlighted proteins here should be figure of further study.

- Quantitative proteomics in tissues will be essential for the application of proteomics in clinical research.

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Thank you!