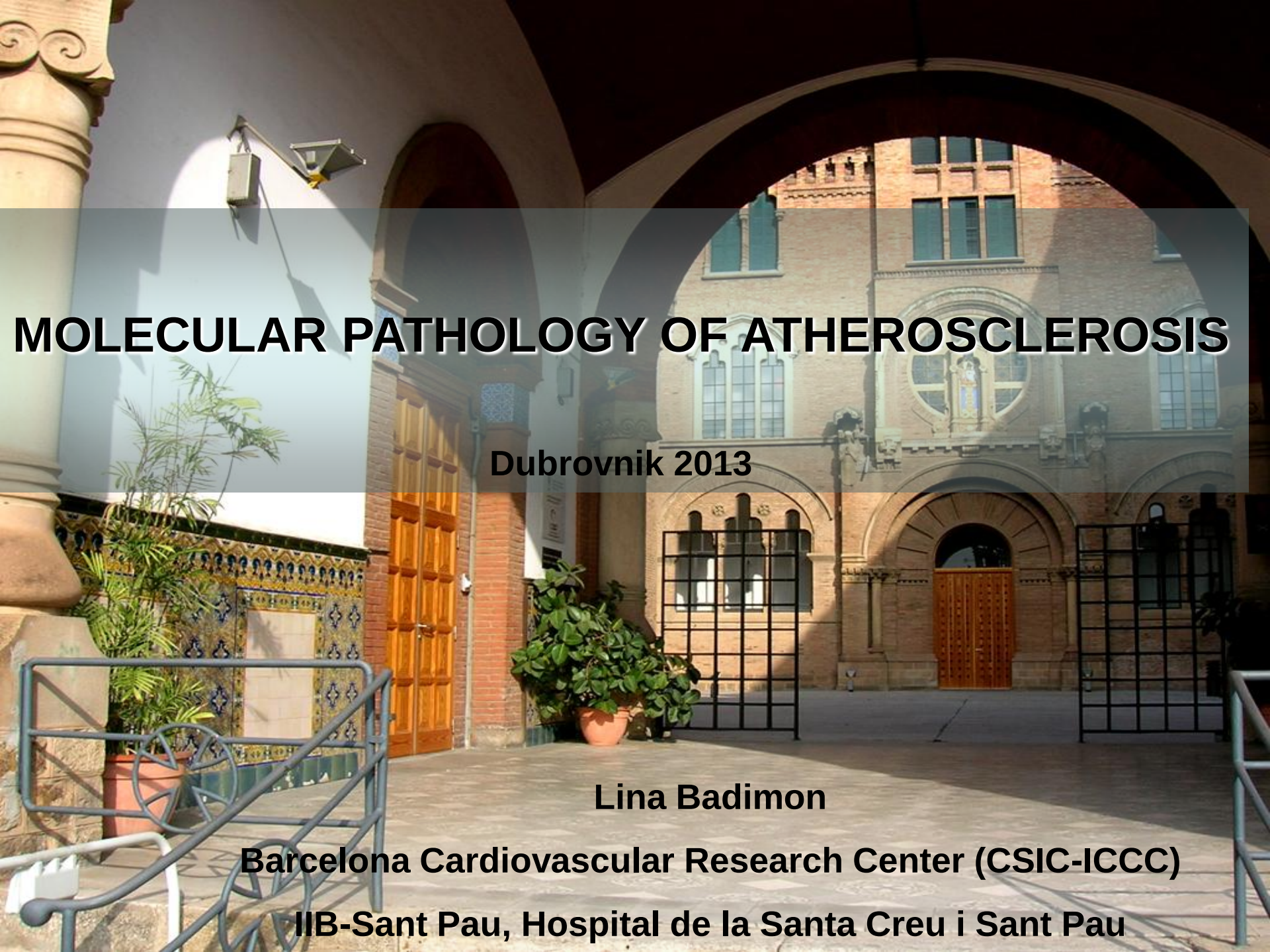


MOLECULAR PATHOLOGY OF ATHEROSCLEROSIS

Dubrovnik 2013

Lina Badimon

Barcelona Cardiovascular Research Center (CSIC-ICCC)

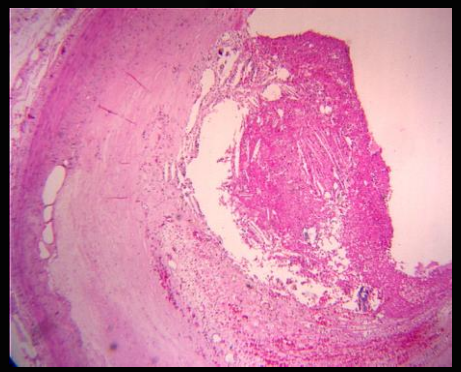
IIB-Sant Pau, Hospital de la Santa Creu i Sant Pau

ATHEROSCLEROSIS – ATHEROTHROMBOSIS – CLINICAL EVENTS

STROKE
TIAs

ACS

PAD



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CARDIOVASCULAR DISEASE - ATHEROTHROMBOSIS

Acute Ischemic Syndromes

- Contenders:
  - Platelets
  - White cells & RBCs
  - EPCs
  - Inflammation
  - Microparticles

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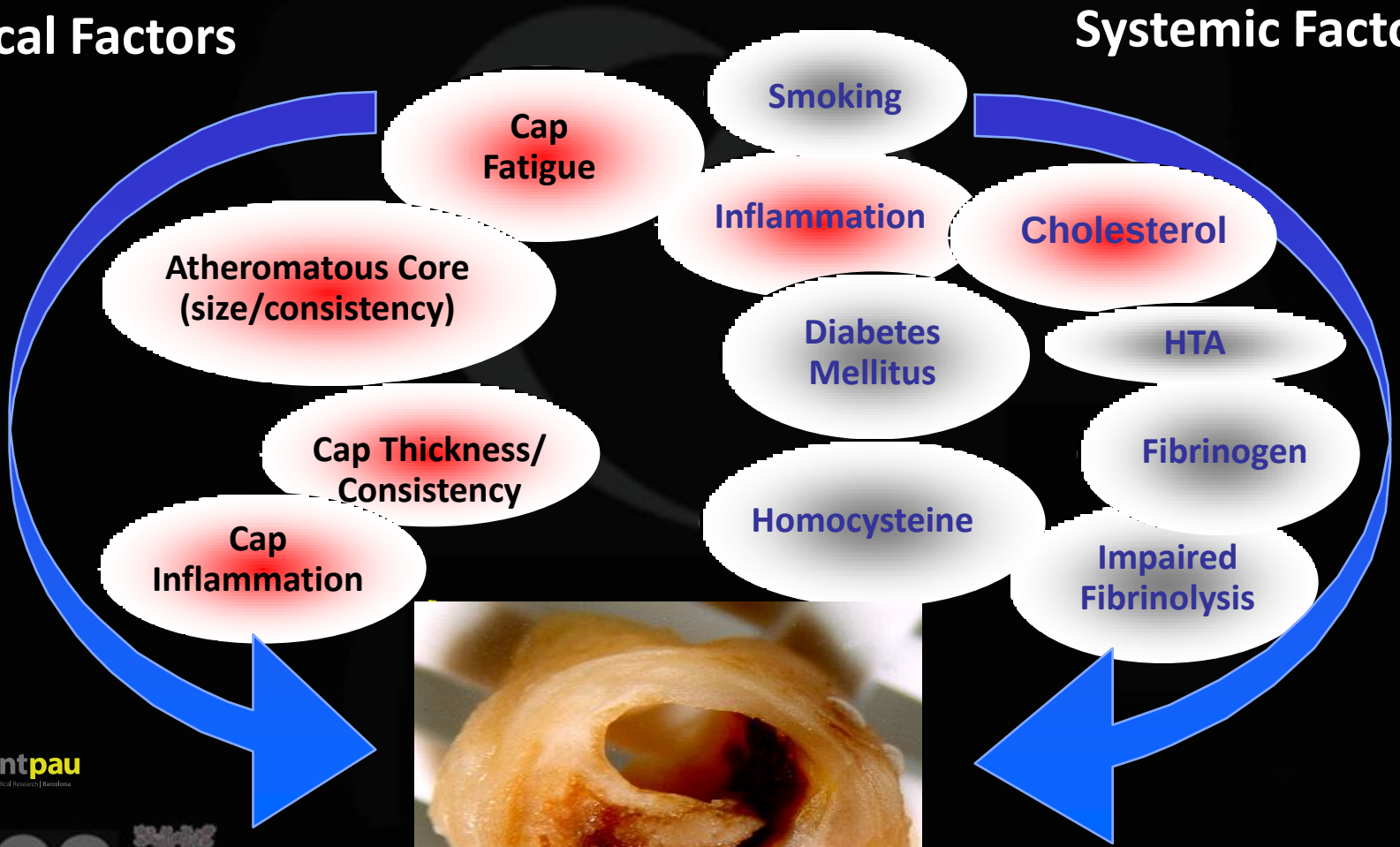
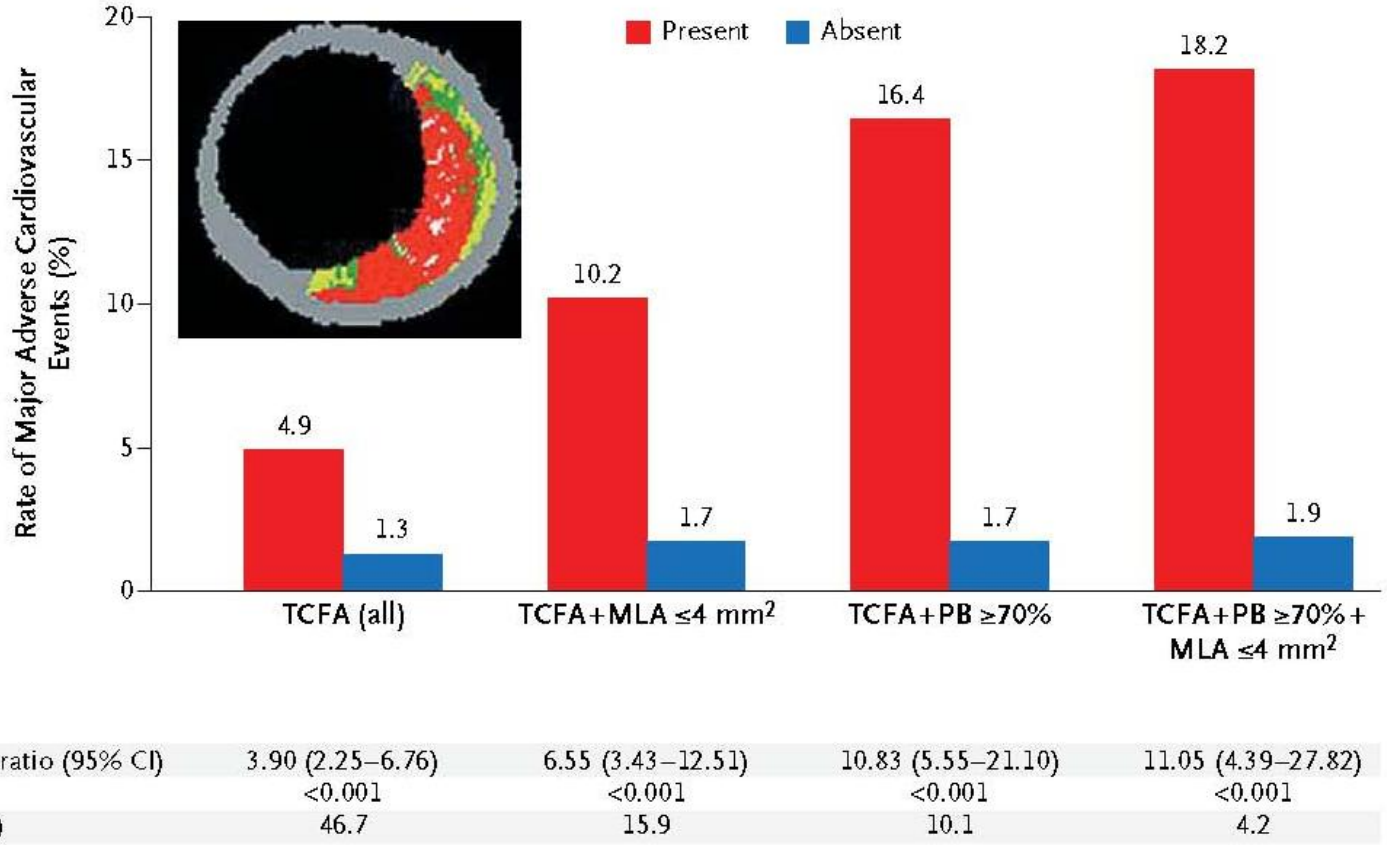
ICCC

Risk Factors for Plaque Progression and Clinical Complication





Lesion hazard ratio (95% CI)	3.90 (2.25–6.76)	6.55 (3.43–12.51)	10.83 (5.55–21.10)	11.05 (4.39–27.82)
P value	<0.001	<0.001	<0.001	<0.001
Prevalence (%)	46.7	15.9	10.1	4.2

Figure 2. Event Rates for Lesions That Were and Those That Were Not Thin-Cap Fibroatheromas, at a Median Follow-up of 3.4 Years.

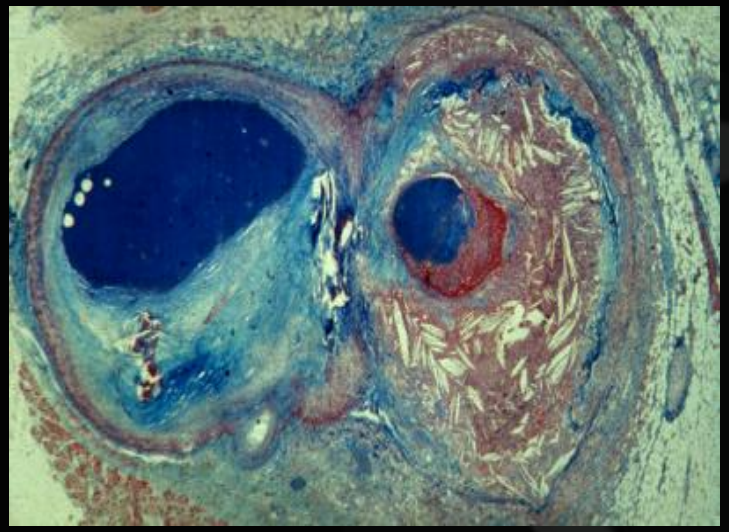
Event rates associated with 595 nonculprit lesions that were characterized as thin-cap fibroatheromas (TCFA) and 2114 that were not by means of radiofrequency intravascular ultrasonographic imaging are shown according to minimal luminal area (MLA) and plaque burden (PB) as detected on gray-scale intravascular ultrasonography. The inset shows an example of a thin-cap fibroatheroma imaged by radiofrequency ultrasonography. Data on prevalence are for one or more such lesions per patient. Lesions in patients with indeterminate events were excluded. (For additional details, see Table 6 in the Supplementary Appendix.) CI denotes confidence interval.

Badimon L et al 2008

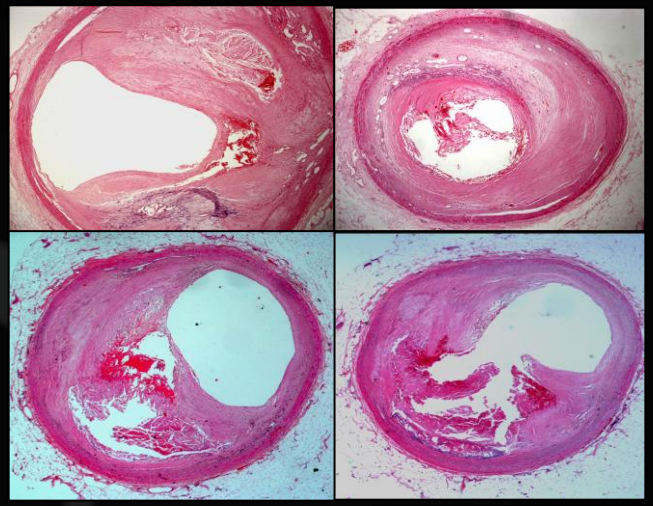
Barcelona CSIC

Badimon L et al 2008

HIGH RISK PLAQUES AND STRUCTURAL CHALLENGES

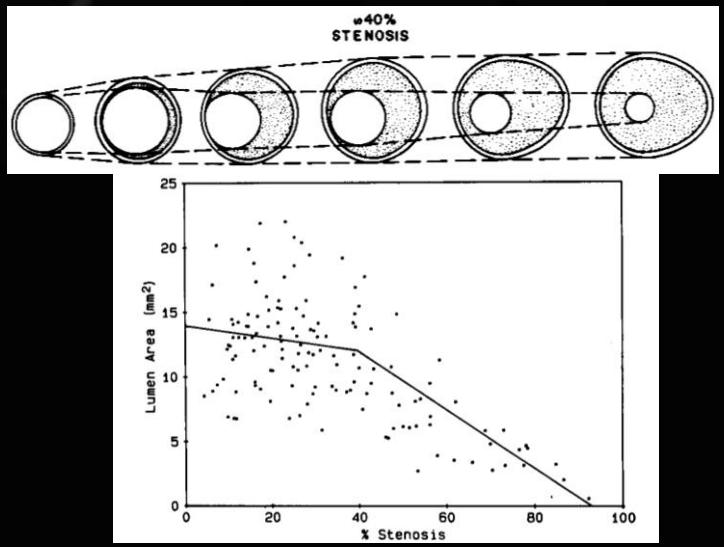


Falk E et al



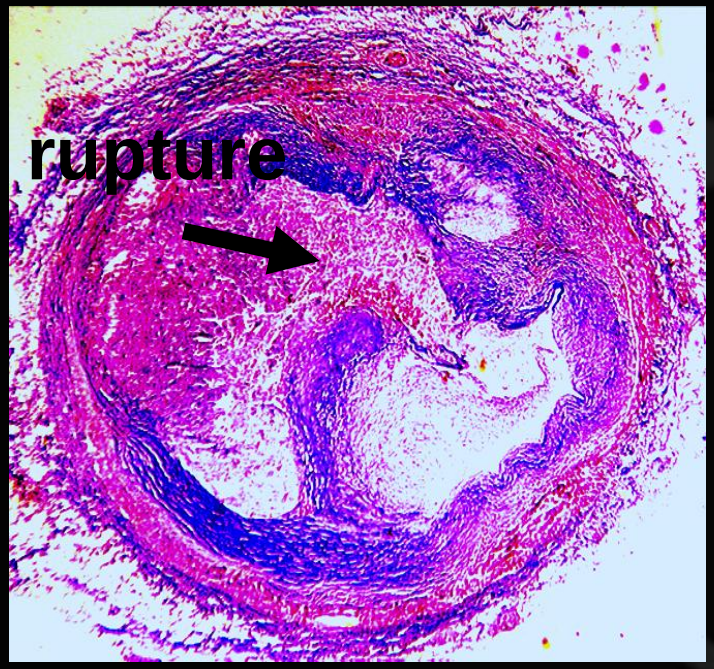
Badimon L, Juan O.

- **Unstable plaque**
  - **Low in collagen**
  - **Necrotic Core**
    (rich in lipids, inflammatory cells,linfocytes, microcalcifications)
  - **Thin fibrous cap (<65um)**



Glagov S, N Engl J Med 1987 May 28, 316(22)1371-5

HIGH RISK PLAQUES



- ↑ lipid core
- ↓ smooth muscle cells
- ↓ collagen fibers
- ↑ inflammatory cells
- ↑ necrotic core/thin fibrous plaque

CV RISK FACTORS

structural failure

↕

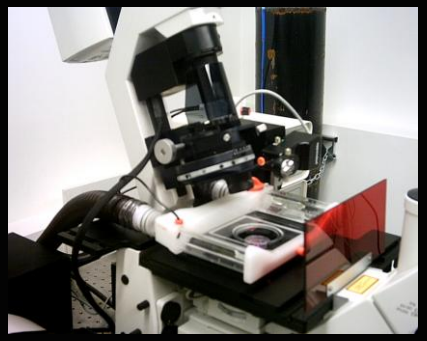
plaque EROSION / DISRUPTION

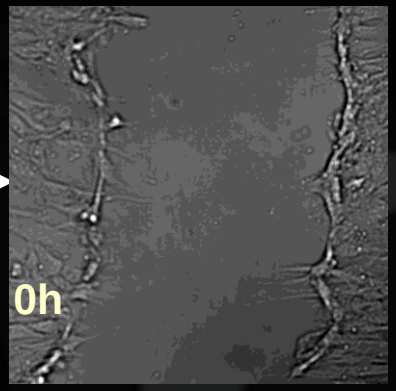
Badimon L 2011

Infiltrated lipids impair human coronary VSMC repair mechanisms

VSMC

+ /- agLDL
(16 hours)

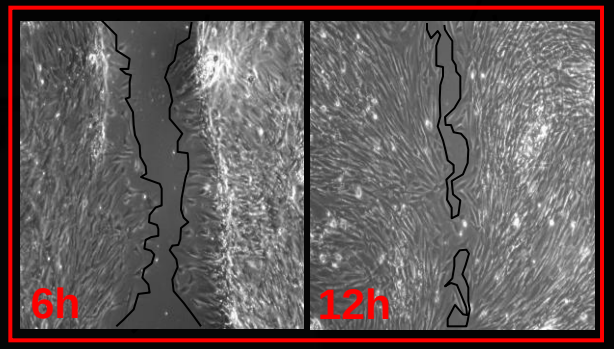


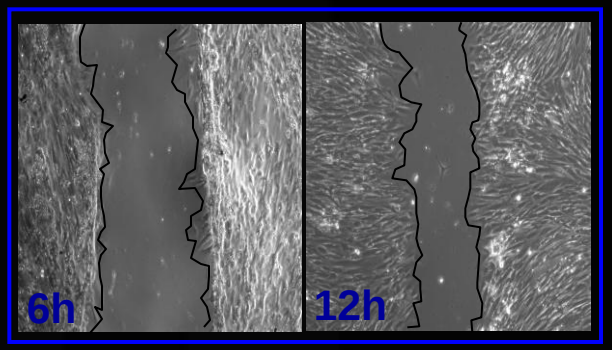


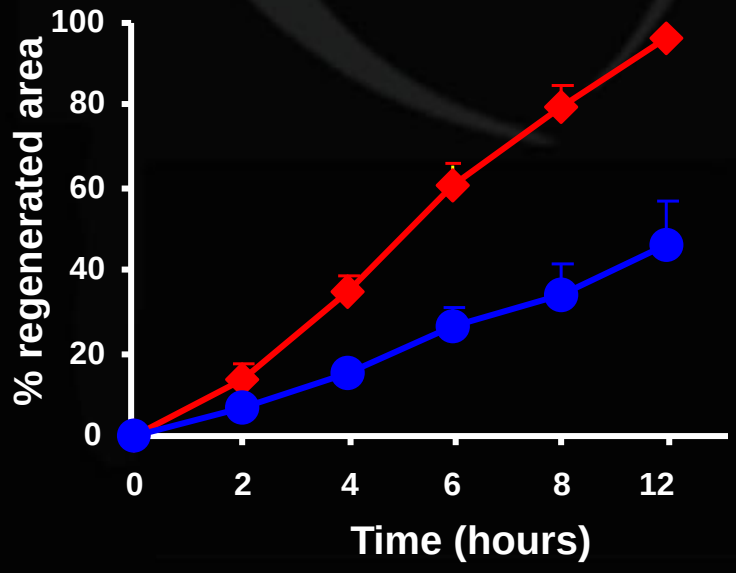
double sided
scrape-wound

control

agLDL



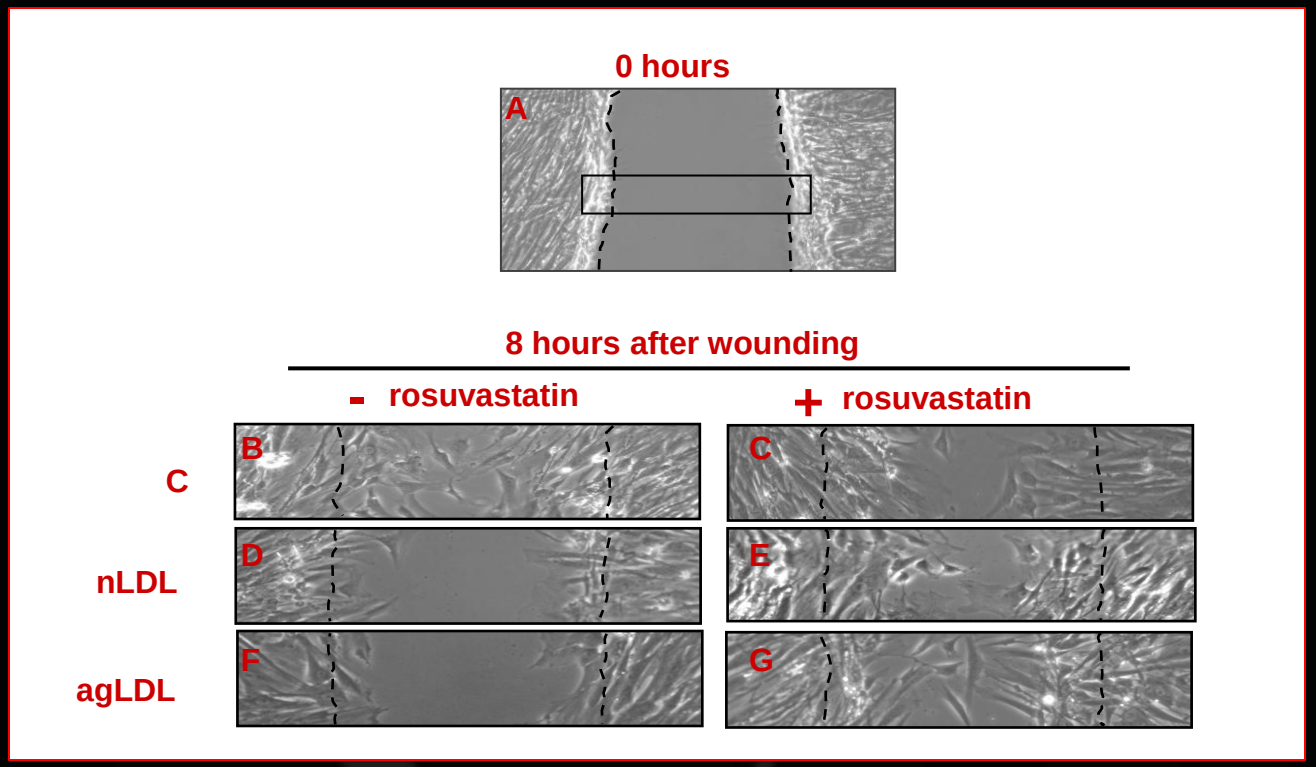


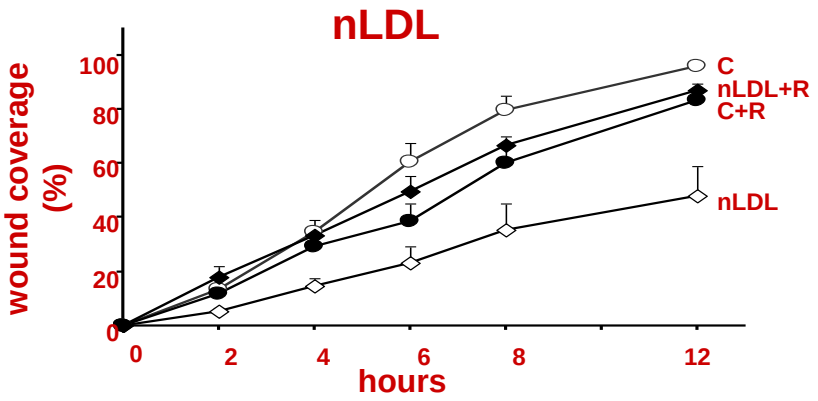


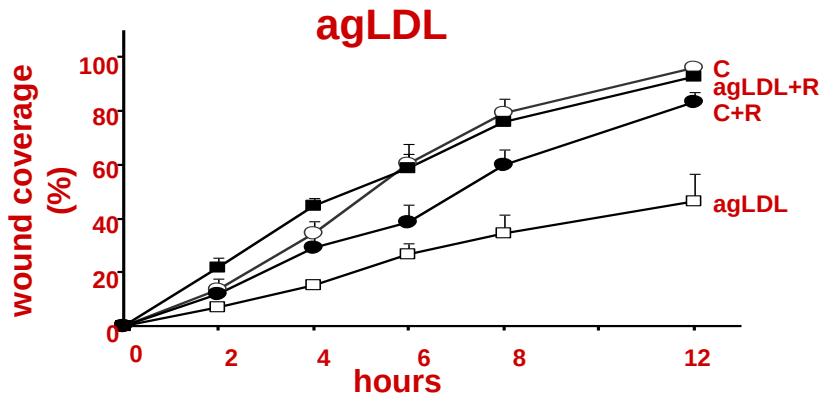
control

agLDL

Padro et al. Cardiovasc Res 2007



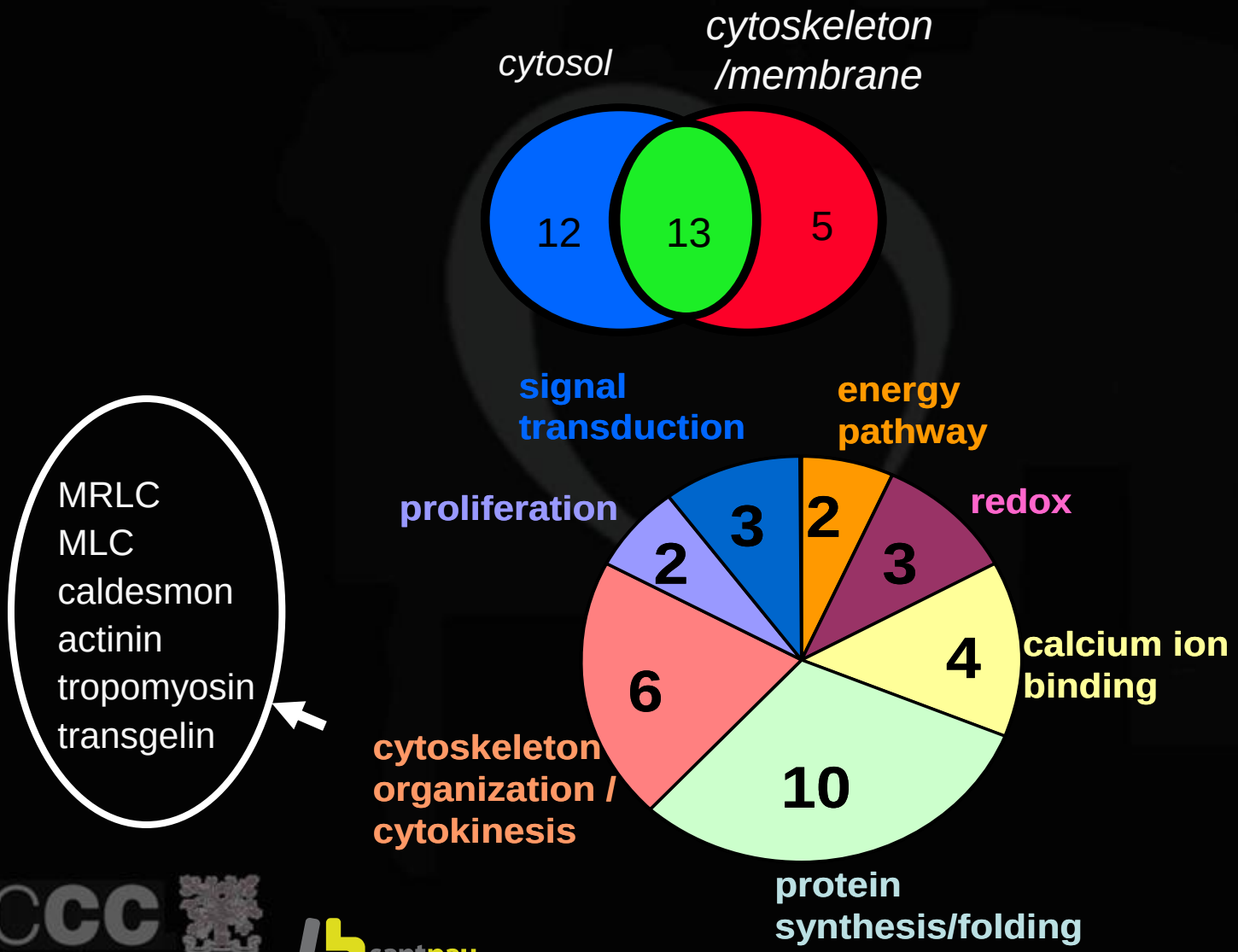




Barcelona CSIC

Padró T, Lugano R, García-Arguinzonis M, Badimon L.PLoS One. 2012

Infiltrated lipids impair human coronary VSMC repair mechanisms

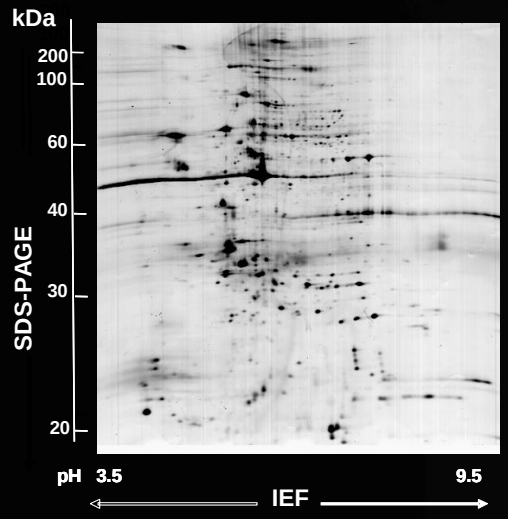


Padró T, Peña E, García-Arguinzonis M, Llorente-Cortes V, Badimon L. Cardiovasc Res. 2008

Proteome of human coronary SMC

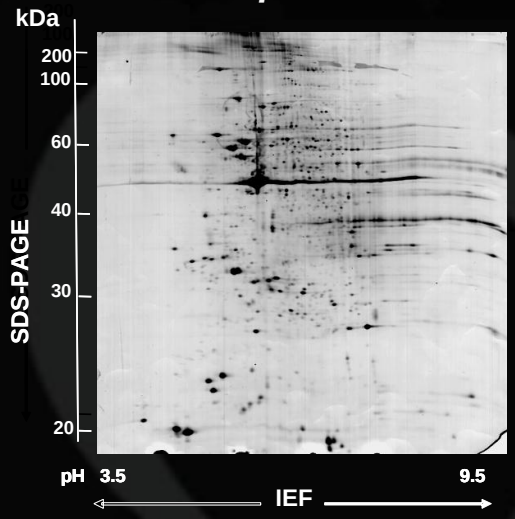
Cytosol

Tris Fraction

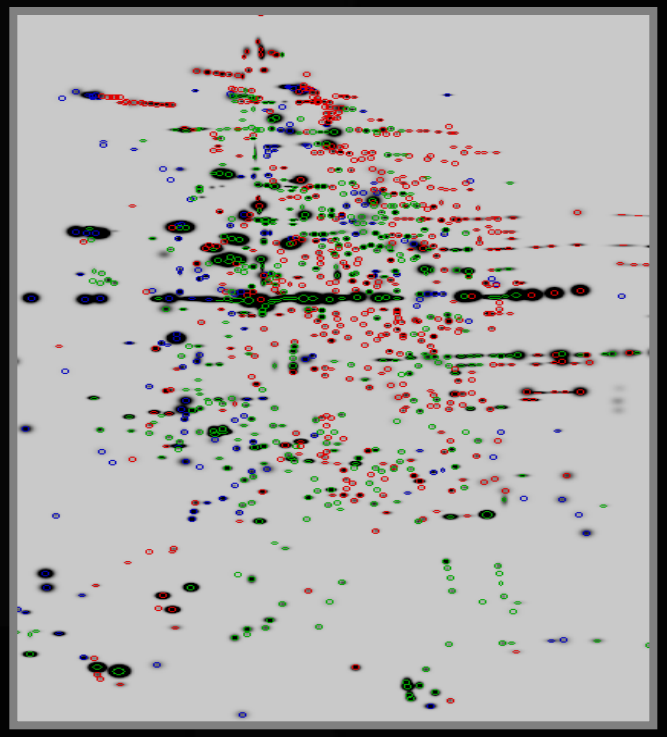


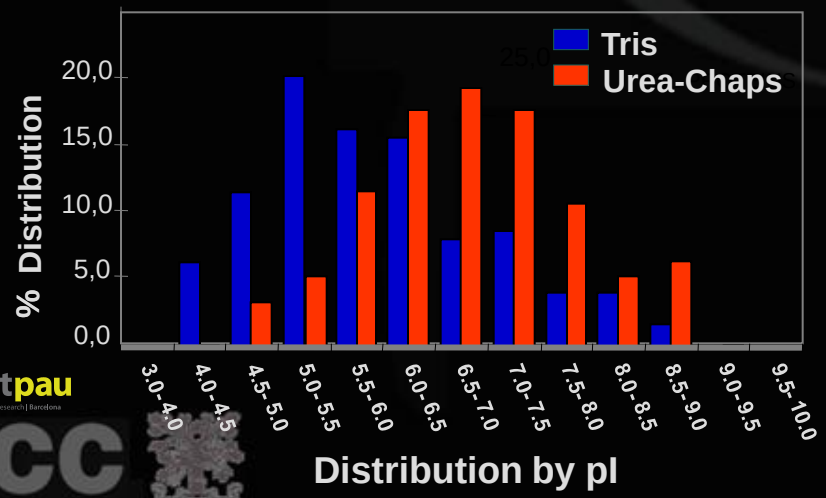
Cytosk./membrane

Urea-Chaps Fraction



Total spots 880 ± 176





Fraccion Tris (16%)

Fracción Urea-Chaps (48%)

Both Fractions (36%)

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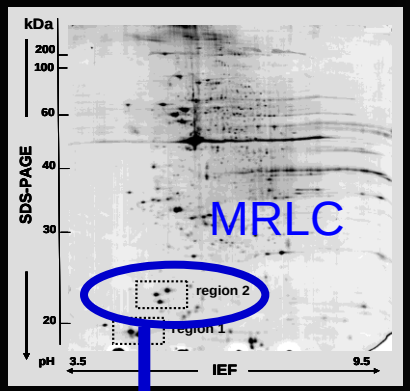
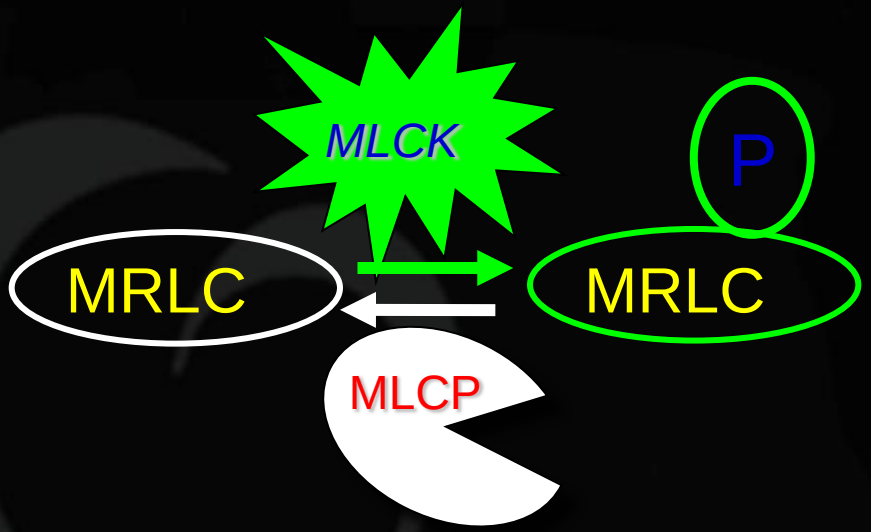
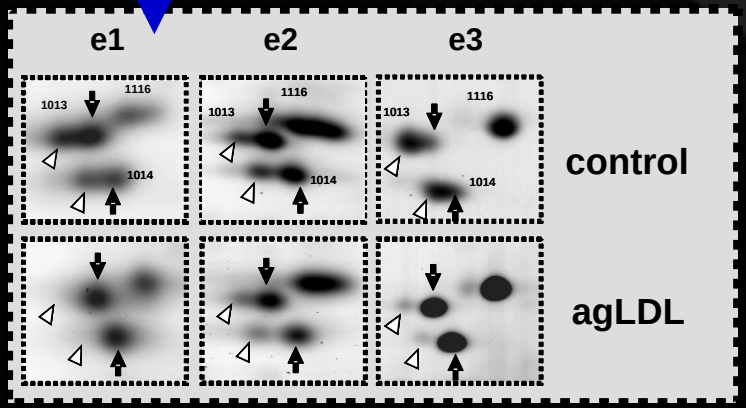
agLDL induce changes in the proteomic profile of myosin regulatory light-chain (MRLC) in SMC

cytoskeleton

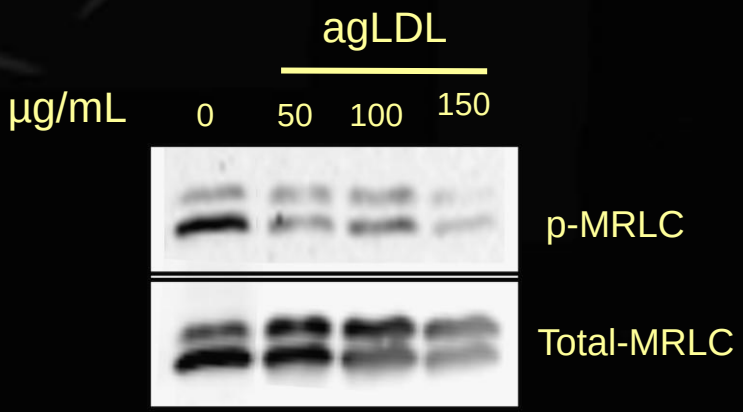




dephosphorylation



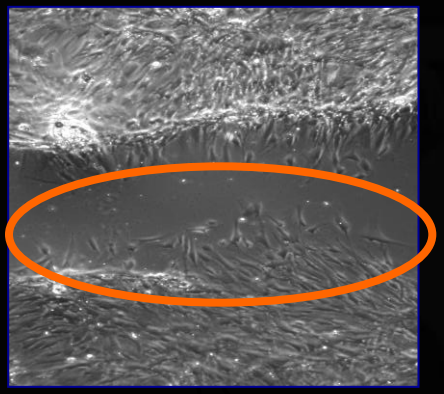
Western blot analysis



ICCC Barcelona CSIC santpau

Padró et al. Cardiovasc Res 2008; Padro et al JTH 2010; Lugano et al CVR 2013

Localization of MRLC in VSMC at the migrating front



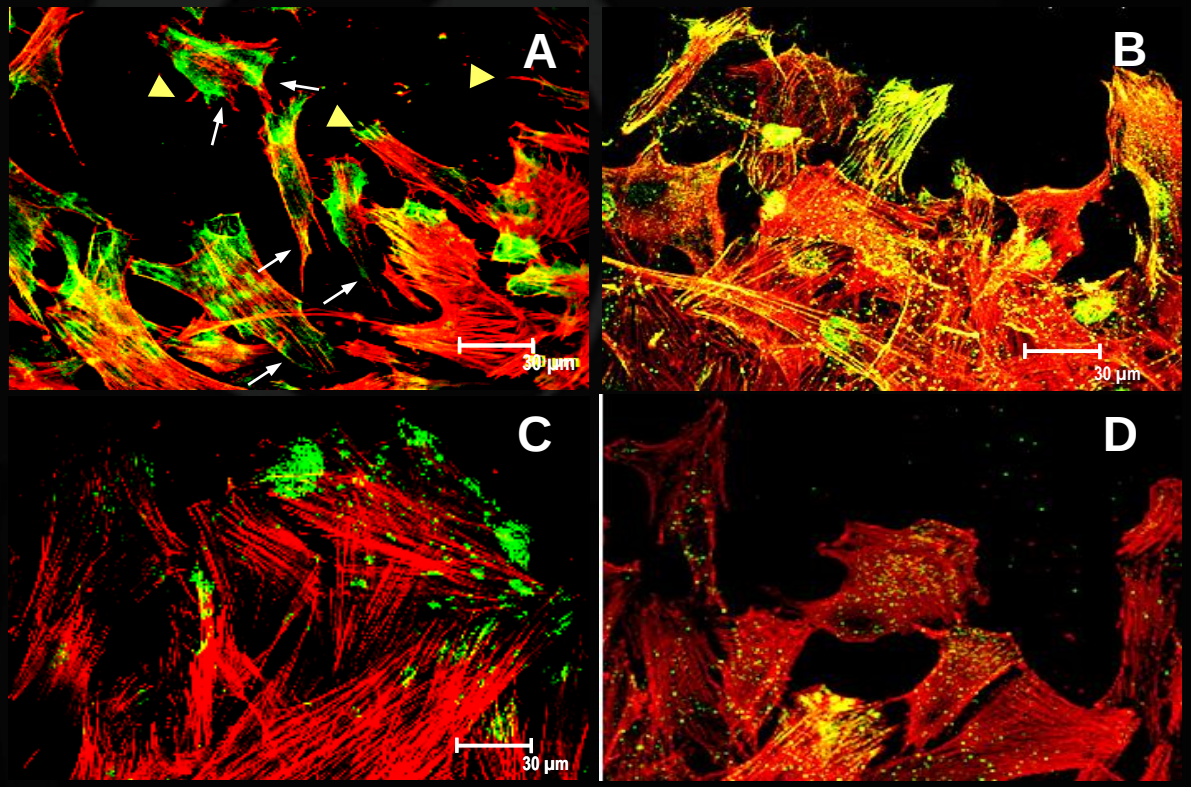
Confocal microscopy

Total MRLC

P-MRLC

control

agLDL



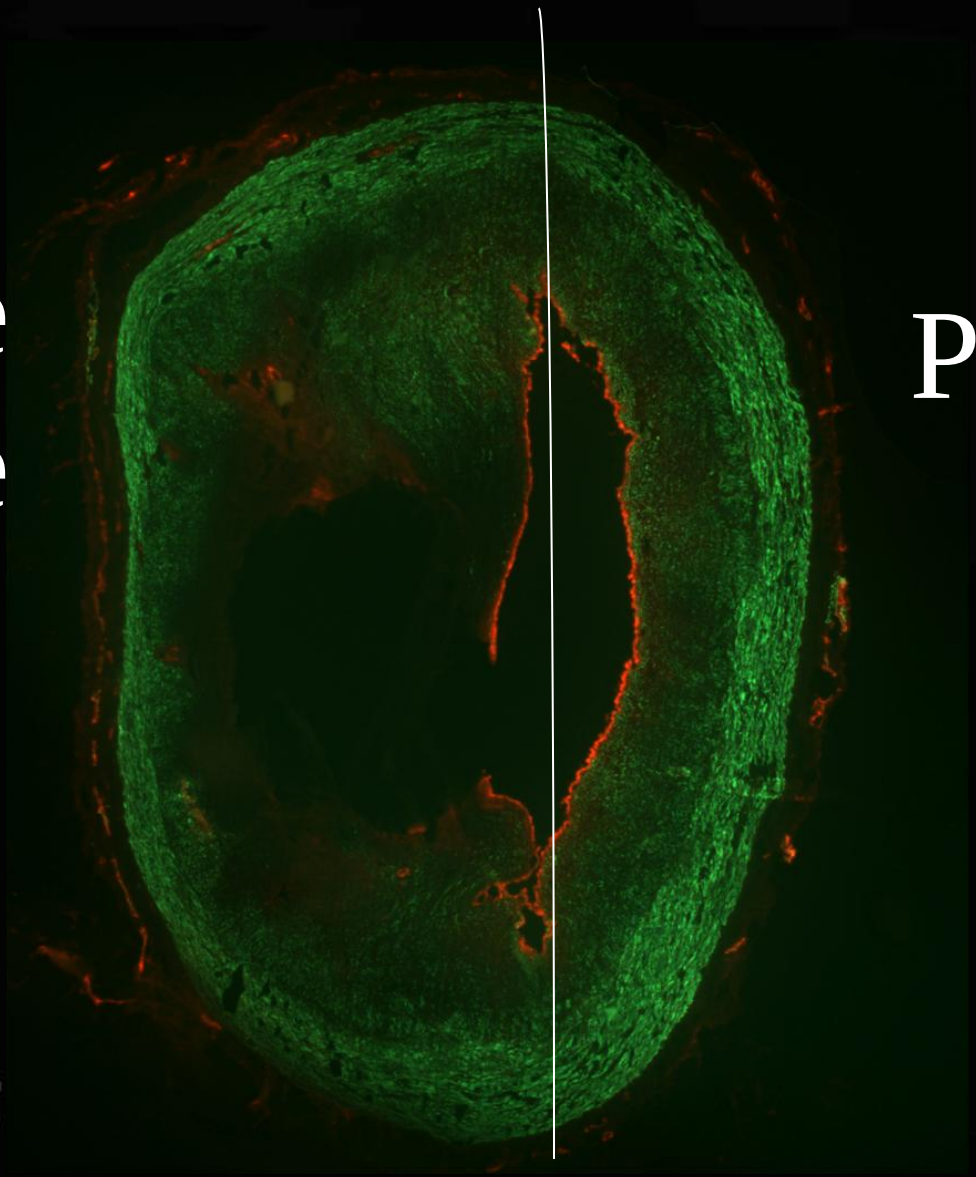
F-Actin (red): Allexa -594 Phalloidin

MRLC ; P-MRLC (green): FITC

Padro et al, Cardiovasc Res, 2008

TRANSCRIPTOMICS – HUMAN CORONARY ARTERIES

Severe Plaque



Plaque-IT

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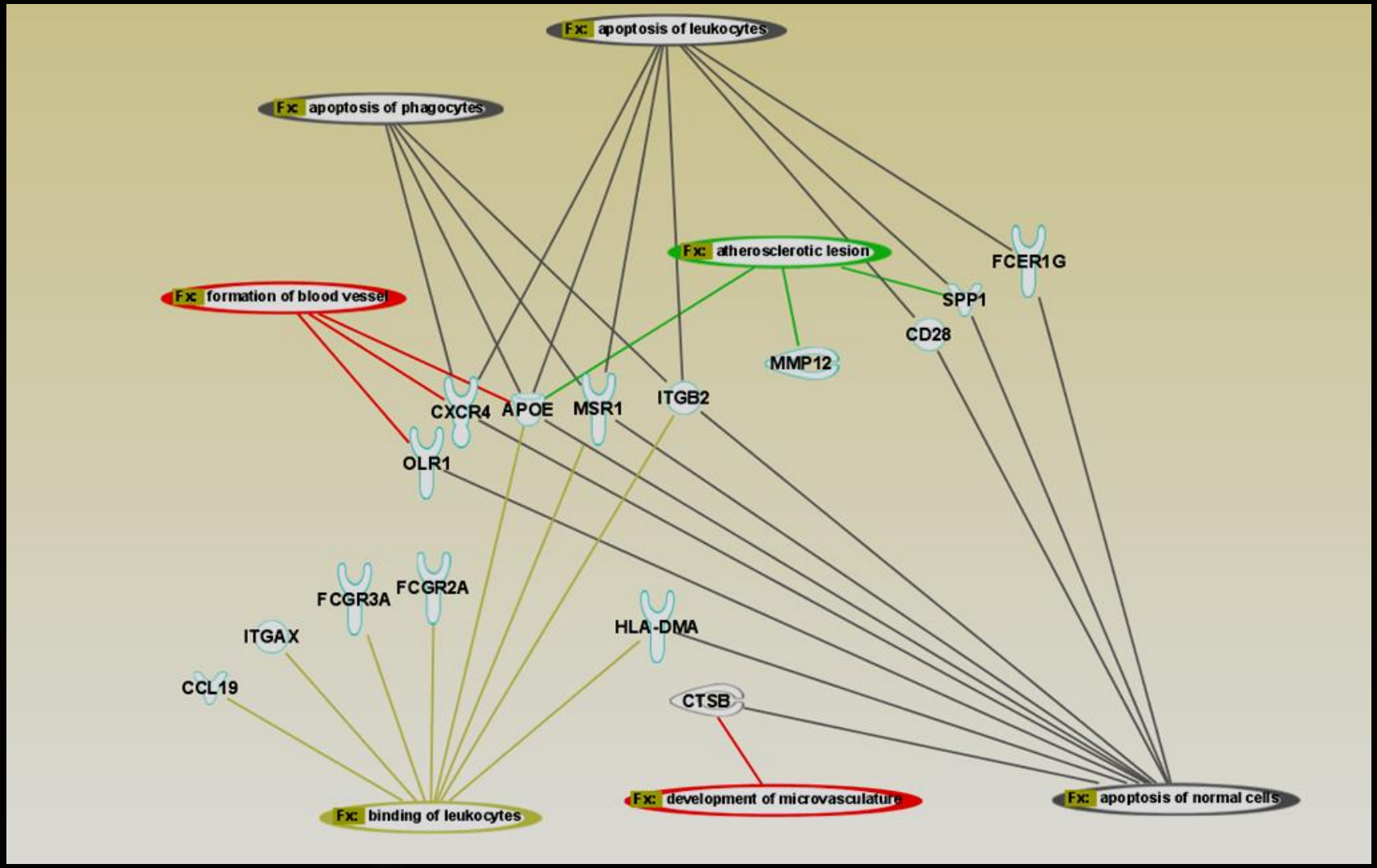
PO functions



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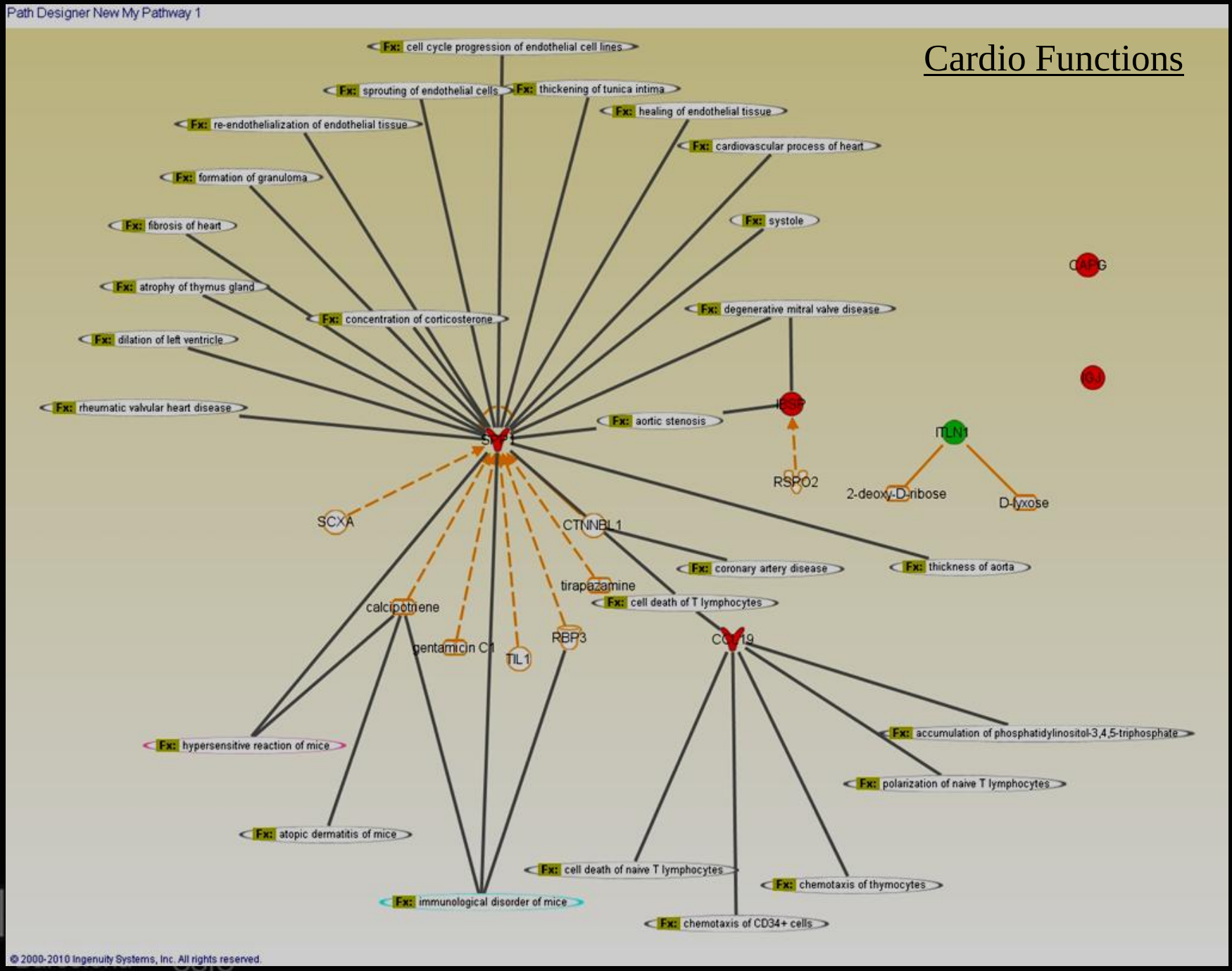
Path Designer New My Pathway 1
Cardio Functions
Fx: cell cycle progression of endothelial cell lines
Fx: sprouting of endothelial cells
Fx: thickening of tunica intima
Fx: healing of endothelial tissue
Fx: re-endothelialization of endothelial tissue
Fx: cardiovascular process of heart
Fx: formation of granuloma
Fx: fibrosis of heart
Fx: systole
Fx: atrophy of thymus gland
Fx: concentration of corticosterone
Fx: degenerative mitral valve disease
Fx: dilation of left ventricle
Fx: rheumatic valvular heart disease
Fx: aortic stenosis
RSPO2
ITLN1
2-deoxy-D-ribose
D-lyxose
SCXA
CTNNBL1
Fx: coronary artery disease
Fx: thickness of aorta
tirapazamine
calcipotriene
Fx: cell death of T lymphocytes
gentamicin C1
RBP3
TIL1
Fx: hypersensitive reaction of mice
Fx: accumulation of phosphatidylinositol-3,4,5-triphosphate
Fx: polarization of naive T lymphocytes
Fx: atopic dermatitis of mice
Fx: cell death of naive T lymphocytes
Fx: chemotaxis of thymocytes
Fx: immunological disorder of mice
Fx: chemotaxis of CD34+ cells
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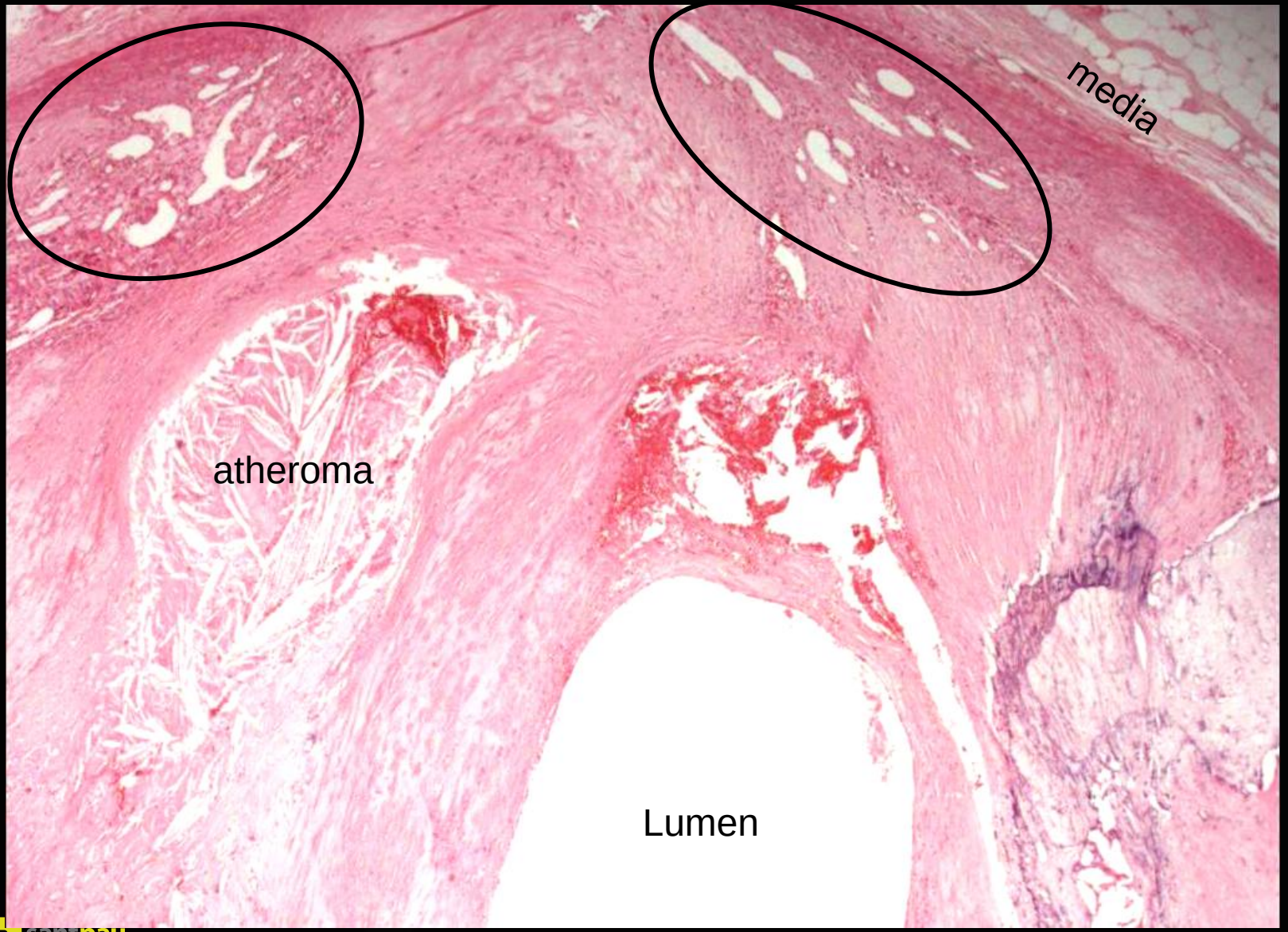
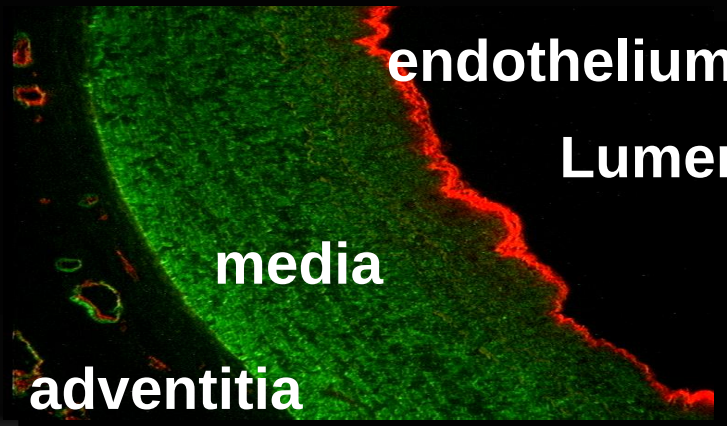
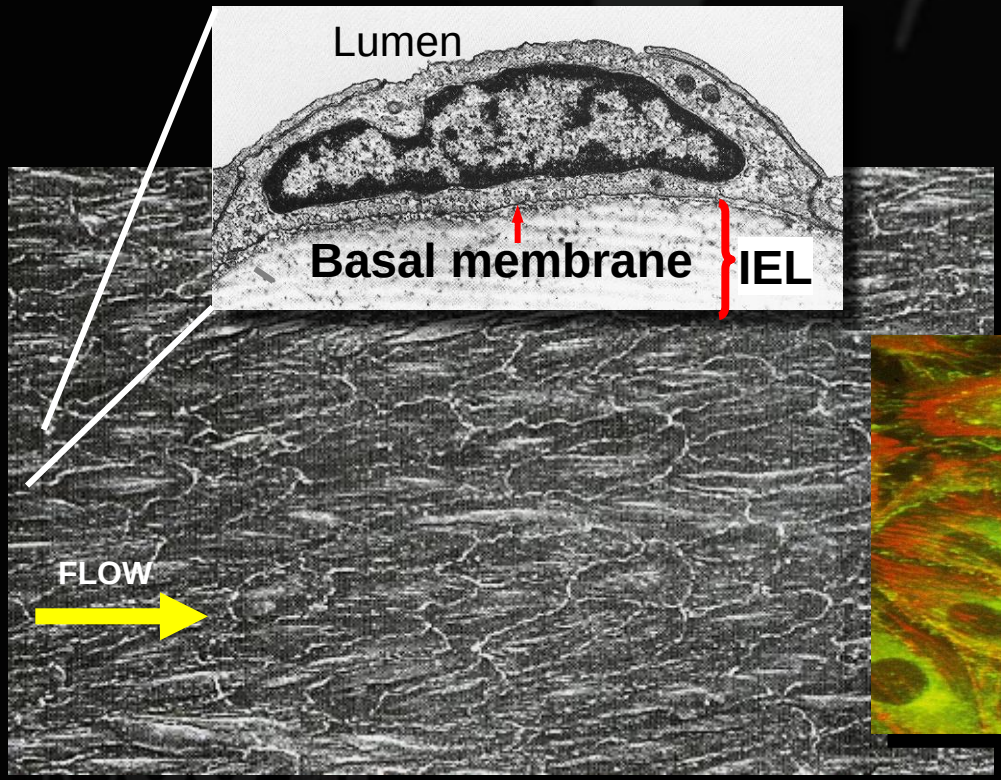


Angiogenesis in Human Coronary Atherosclerotic Plaques
Juan O. & Badimon L. ICCC Database.

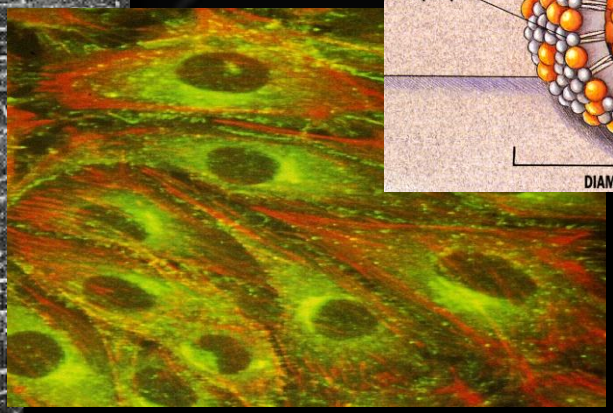
THE VASCULAR WALL AND THE ENDOTHELIUM



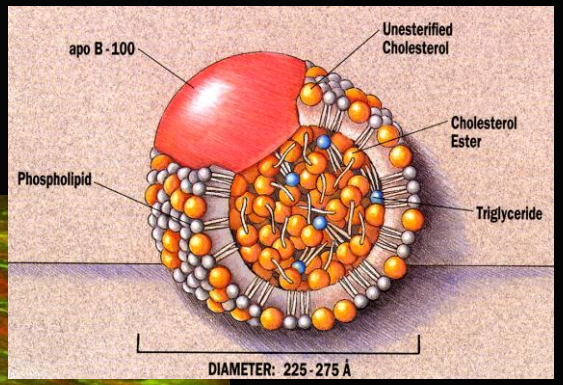
- Endothelium (vWF)
- VSMCs (alfa actin)



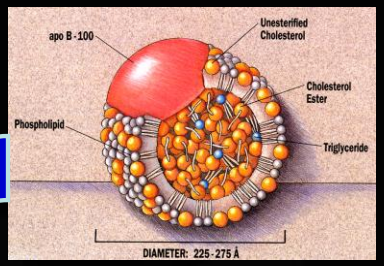
Endothelium: SEM

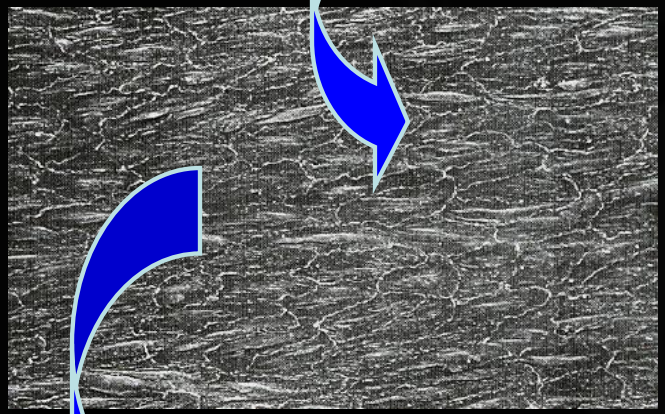


Faloidin

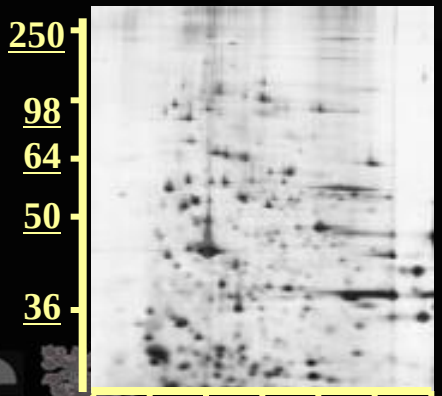


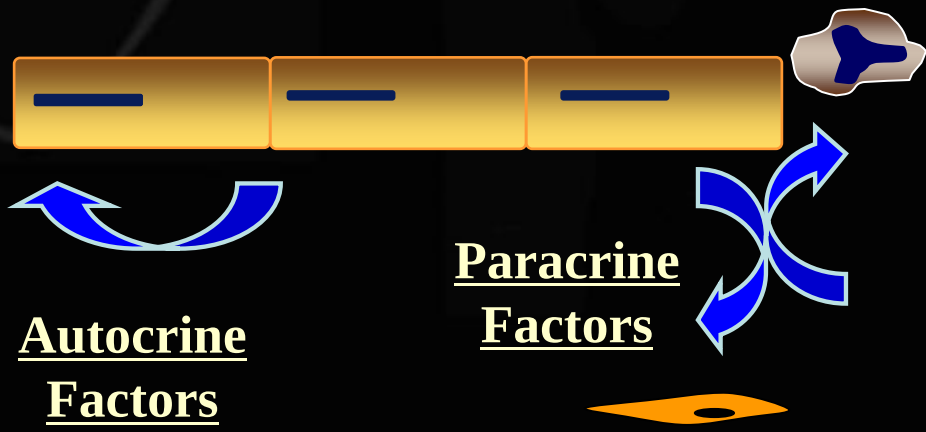
Modified from Badimon L, 2005





2D- gel electroforesis



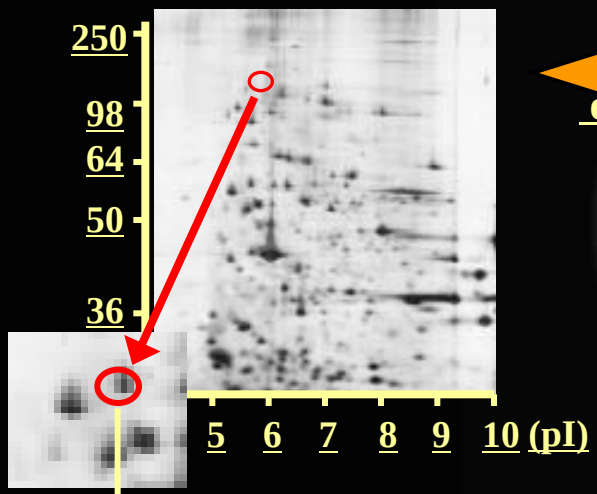


Autocrine Factors

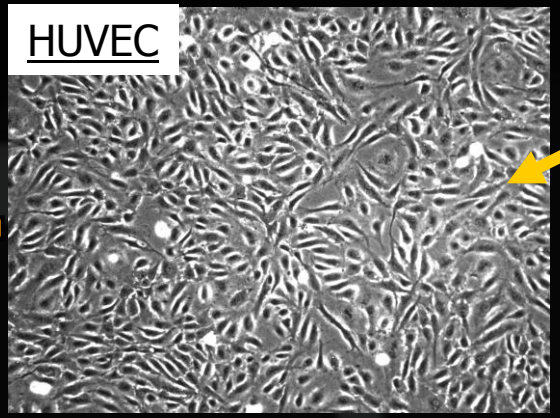
Paracrine Factors

PROTEOMICS

2D- gel electroforesis

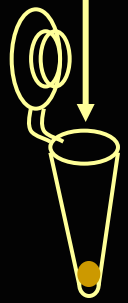


Sequential protein extraction



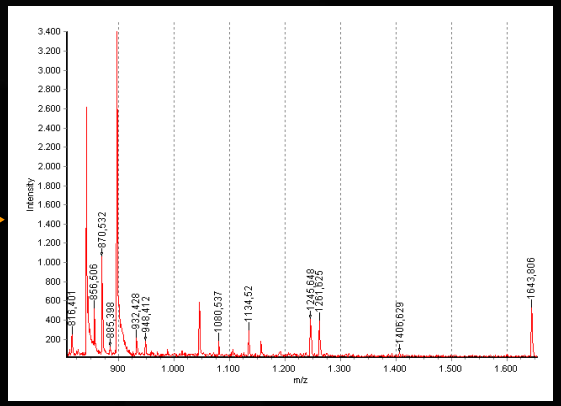
-/+LDL

180 mg/dl



1. Tryipsin digestion
2. Peptide extraction

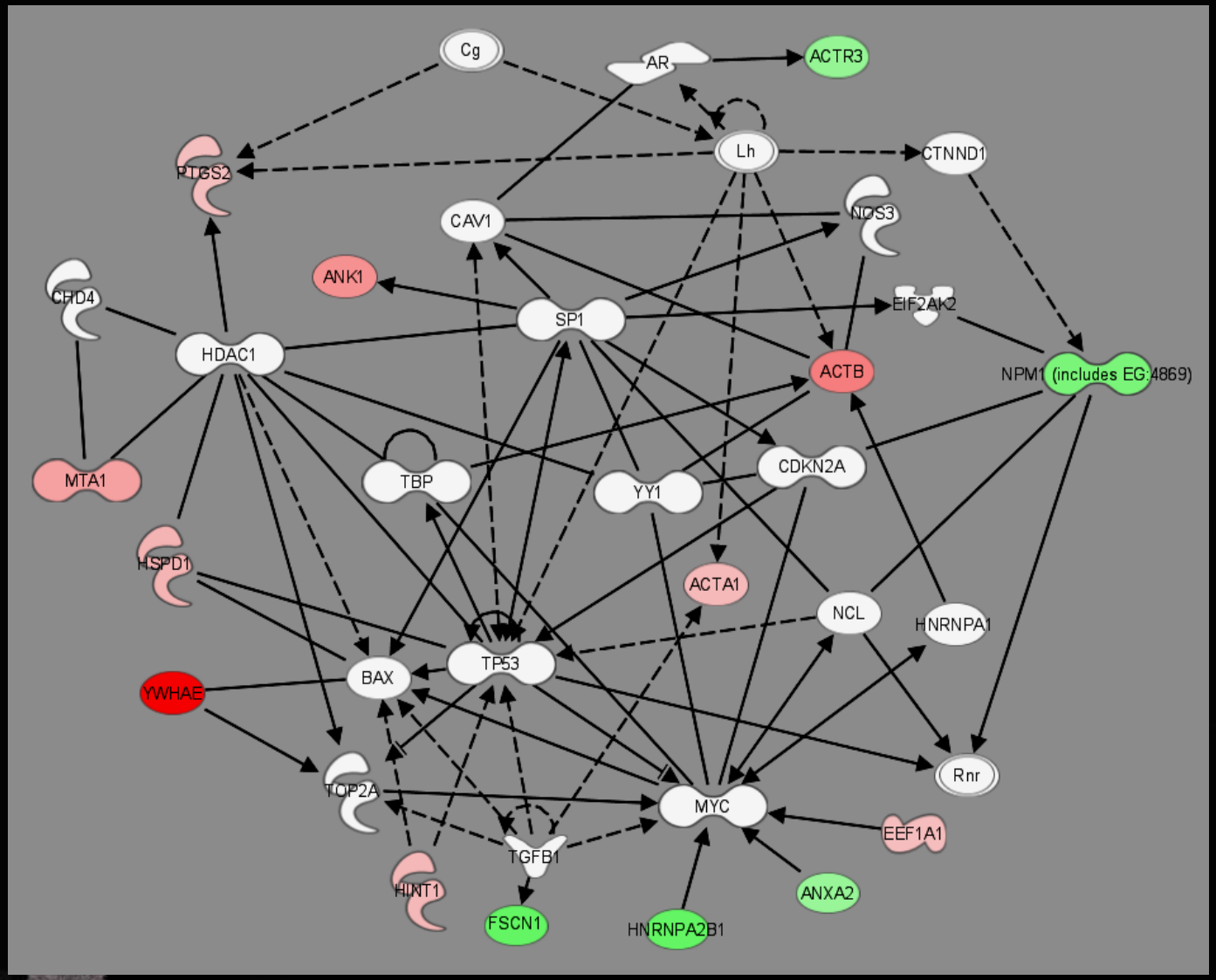
MALDI - ToF



Adquisition and spectral analysis

CSIC ICCC HOSPITAL DE LA SANTA CREU I SANT PAU UNIVERSITAT AUTÒNOMA DE BARCELONA

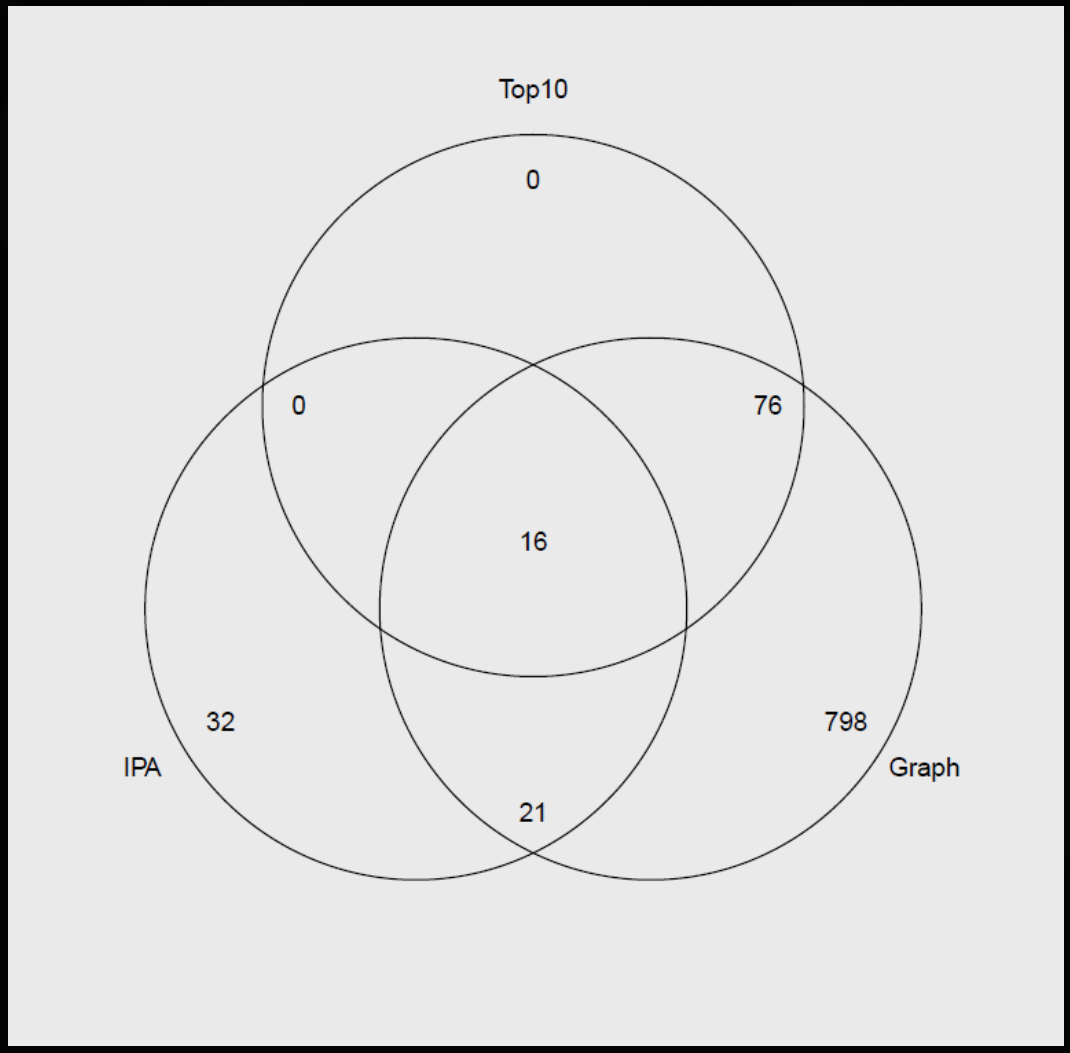
INDUCED ENDOTHELIAL CELL CYTOSOLIC PROTEOME



RED, upregulated proteins; GREEN, dowregulated proteins; WHITE, IPA-generated protein
Color intensity, level of regulation

BADIMON L, ALARCON JL, CARDUS A, PADRO T. UNPUBLISHED OBSER

INDUCED ENDOTHELIAL CELL CYTOSOLIC PROTEOME



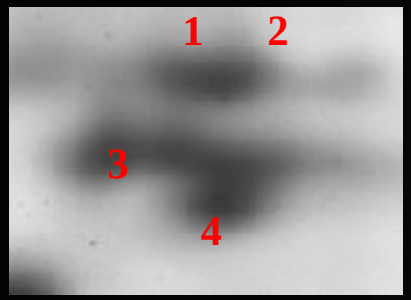
Badimon L, Alarcon JL, Cardus A, Padro T. Unpublished observations

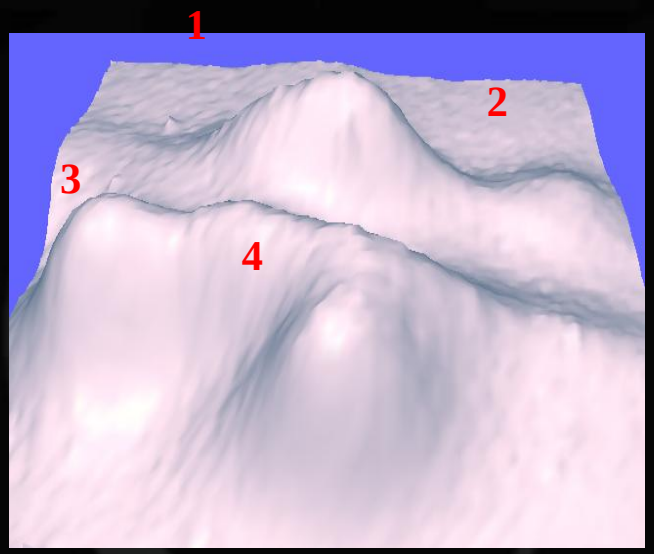
TOP 16 PROTEINS: IPA-GRAPH-CONNECTIVITY

Symbol	Protein name
YWHAE	14-3-3 protein epsilon
EEF1A1	Elongation factor 1-alpha 1
ACTB	Actin, cytoplasmic 1
TP53	Cellular tumor antigen p53
GRB2	Growth factor receptor-bound protein 2
HDAC1	Histone deacetylase 1
MTA1	Metastasis-associated protein MTA1
ACTA1	Actin, alpha skeletal muscle
HSPD1	60 kDa heat shock protein, mitochondrial
CAV1	Caveolin-1
ANXA2	Annexin A2
APP	Amyloid beta A4 protein
AR	Androgen receptor
CDKN2A	Cyclin-dependent kinase inhibitor 2A, isoforms 1/2/3
SHC1	SHC-transforming protein 1
EIF2AK2	Interferon-induced, double-stranded RNA-activated protein kinase

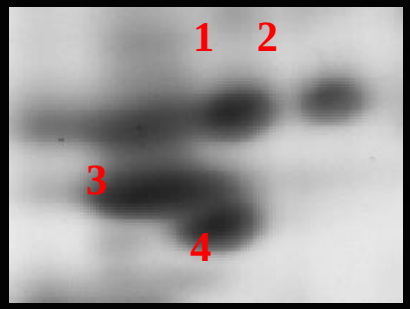
14-3-3 Proteins

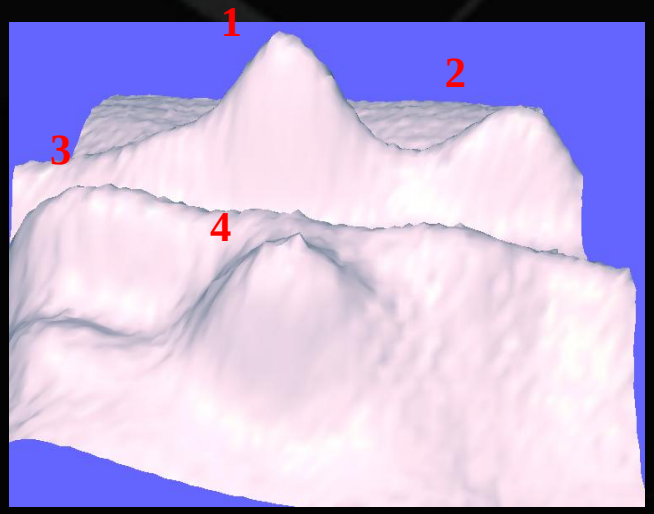
CTRL





+ LDL 180 mg/dl





Mean Values in 2D

		Ratio +LDL/ctrl
1	gamma	2,94
2	epsilon	4,30
3	tsheta	0,83
4	teta/ delta	0,93

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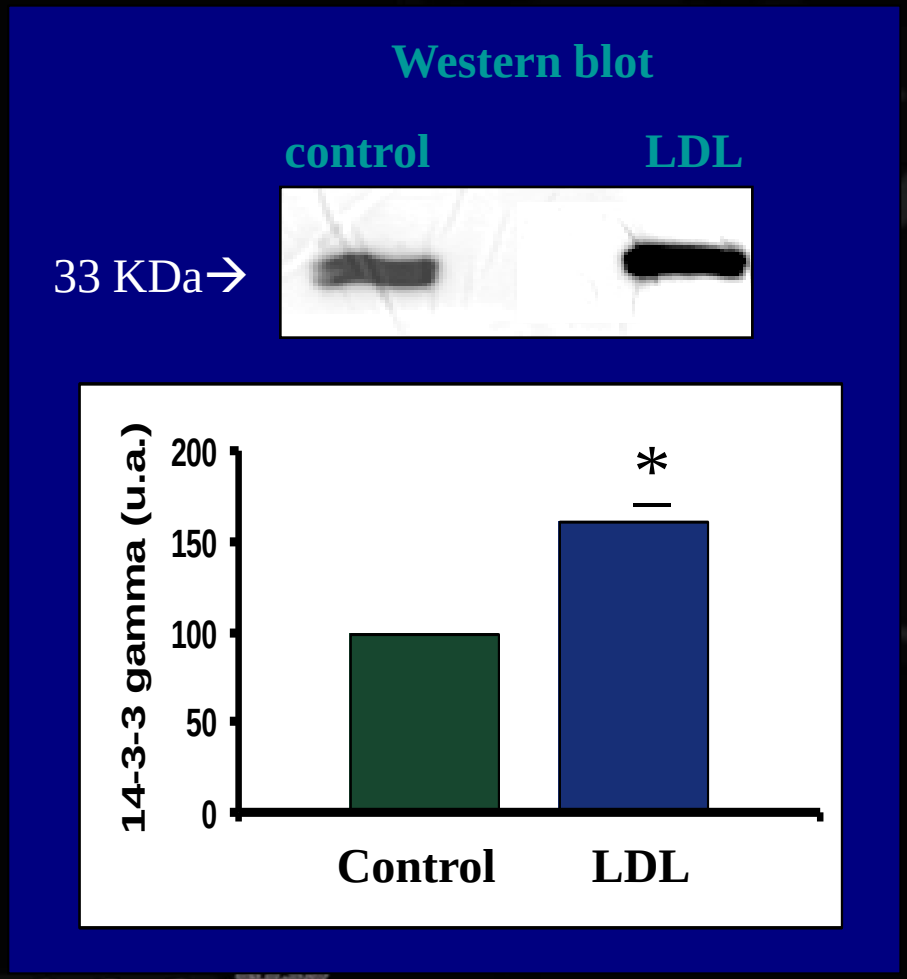
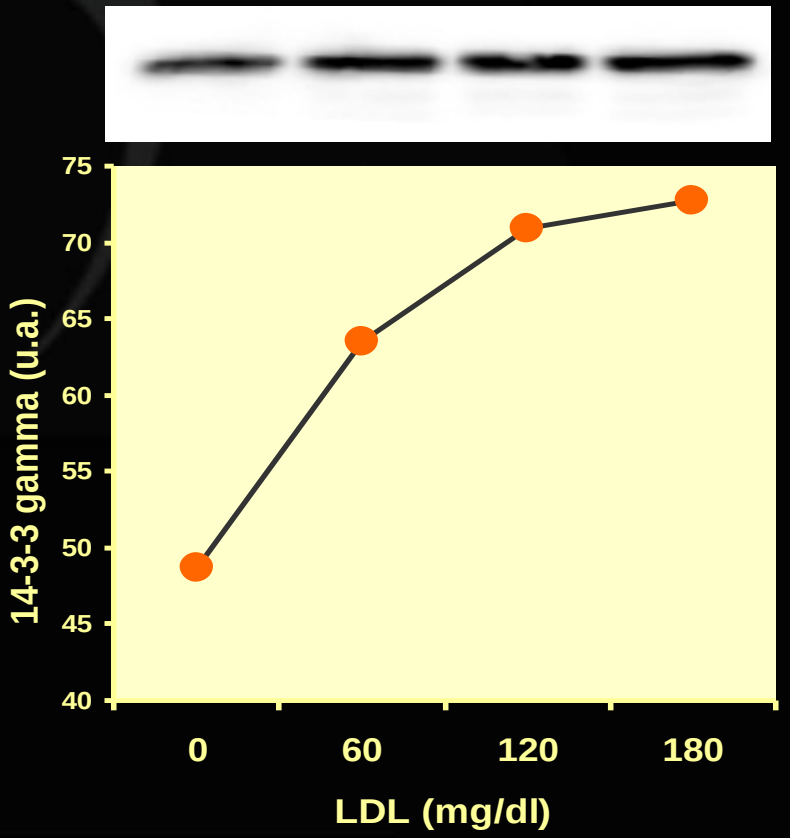
14-3-3γ Proteins



Dose-response effects of LDL on 14-3-3 gamma levels



LDL: 180 mg/dl

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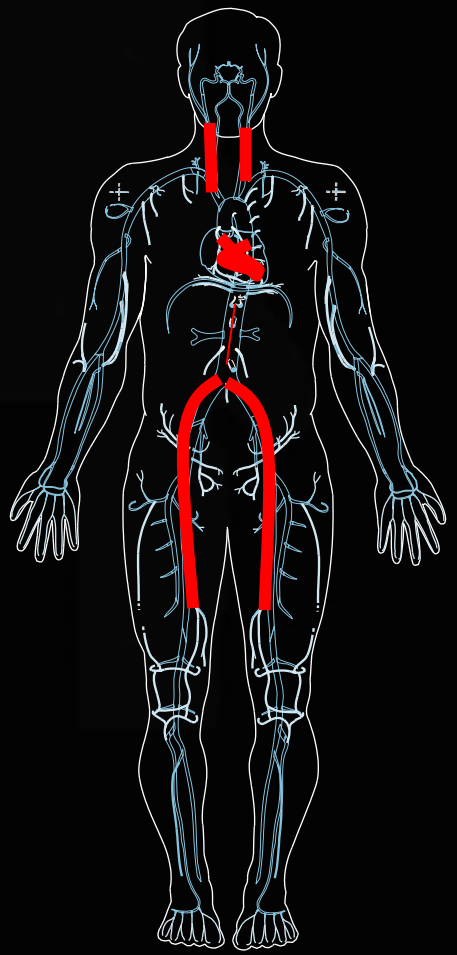
ATHEROSCLEROSIS

- VESSEL REMODELING
- NEOVESSEL FORMATION
- INFLAMMATION
- REGULATORY EFFECTS
  ENDOTHELIAL CELLS



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