New View on Pathogenesis of Cardiovascular Calcification

Elena Aikawa MD, PhD
Director, Heart Valve Translational Research Program

Cardiovascular Medicine
Brigham and Women's Hospital
Harvard Medical School

AP VALVE 2016
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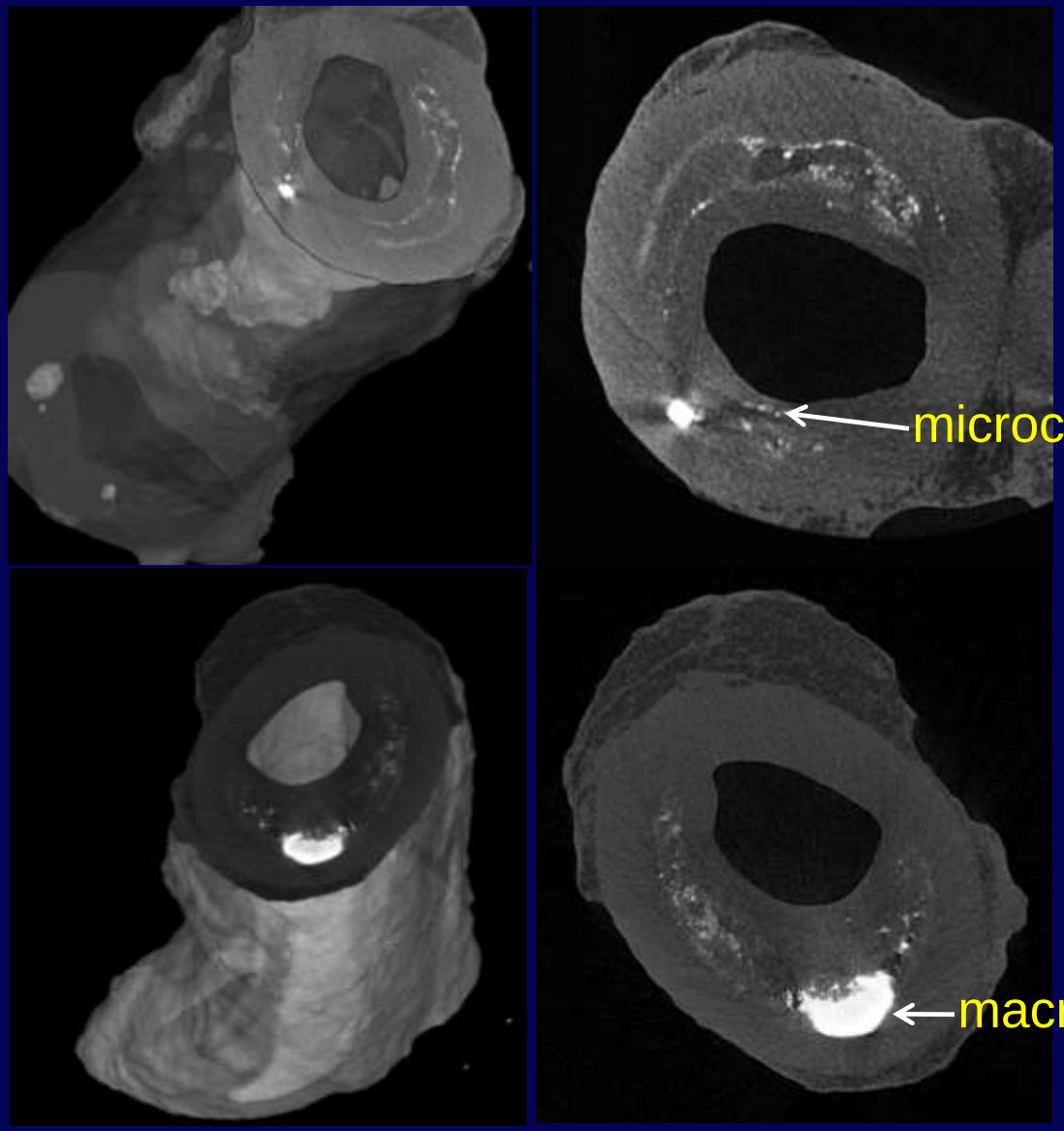
BRIGHAM AND WOMEN'S HOSPITAL

Harvard Medical School

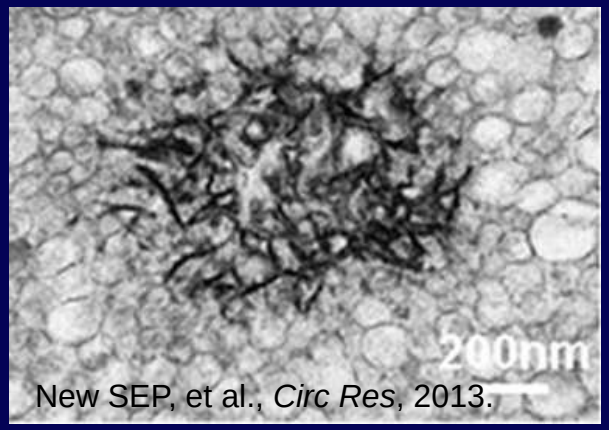
Macrocalcification and microcalcification in human plaque detected by micro-CT



Collaboration with Dr. Sheldon Weinbaum

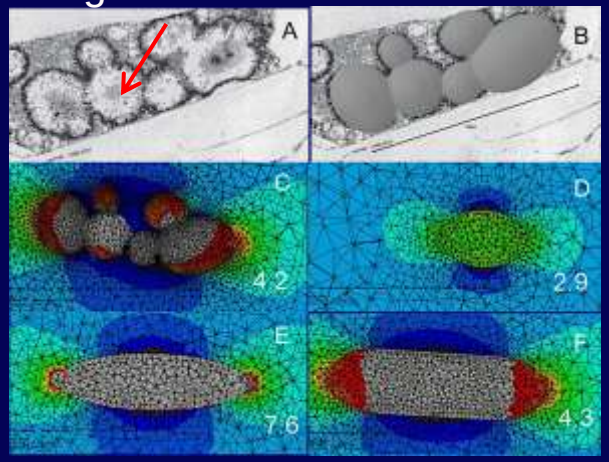
Microcalcifications contribute to plaque instability

Exstracellular vesicles generate microclacifications

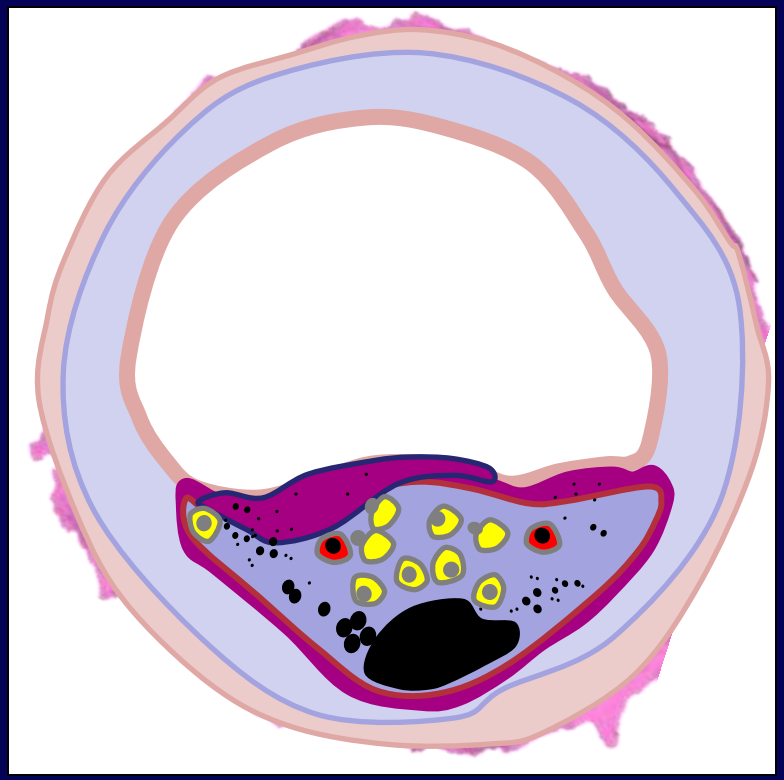


New SEP, et al., *Circ Res*, 2013.

Microclacifications generate high stress concentrations



EM-based FEA by Natalia Maldonado, *PNAS* 2013

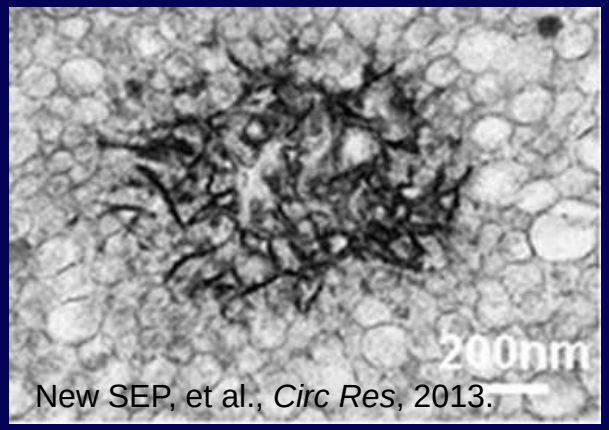


Courtesy of Dr. Sasha Singh

We currently do not understand the mechanism underlying microcalcification formation!

Microcalcifications contribute to plaque instability

Matrix vesicles
generate microclacifications



New SEP, et al., *Circ Res*, 2013.

Microclacifications generate
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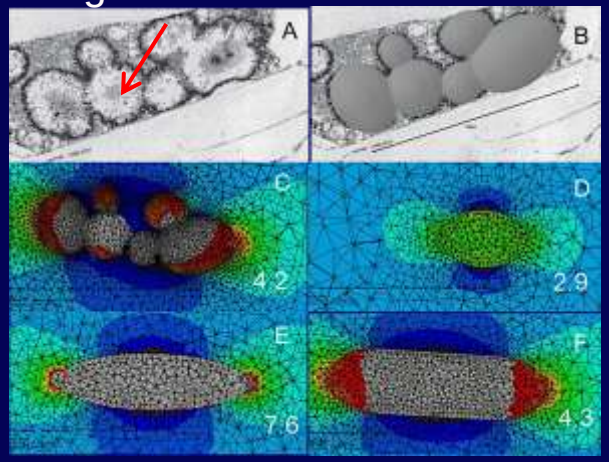

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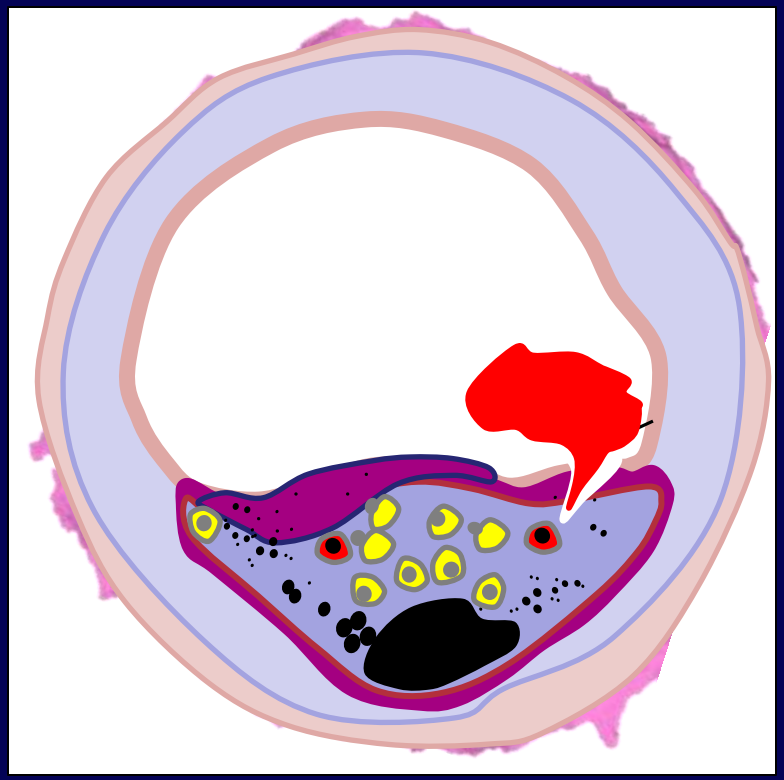


Courtesy of Dr. Sasha Singh

We currently do not understand the mechanism underlying microcalcification formation!

Calcification Program

Sophie E.P. New, PhD

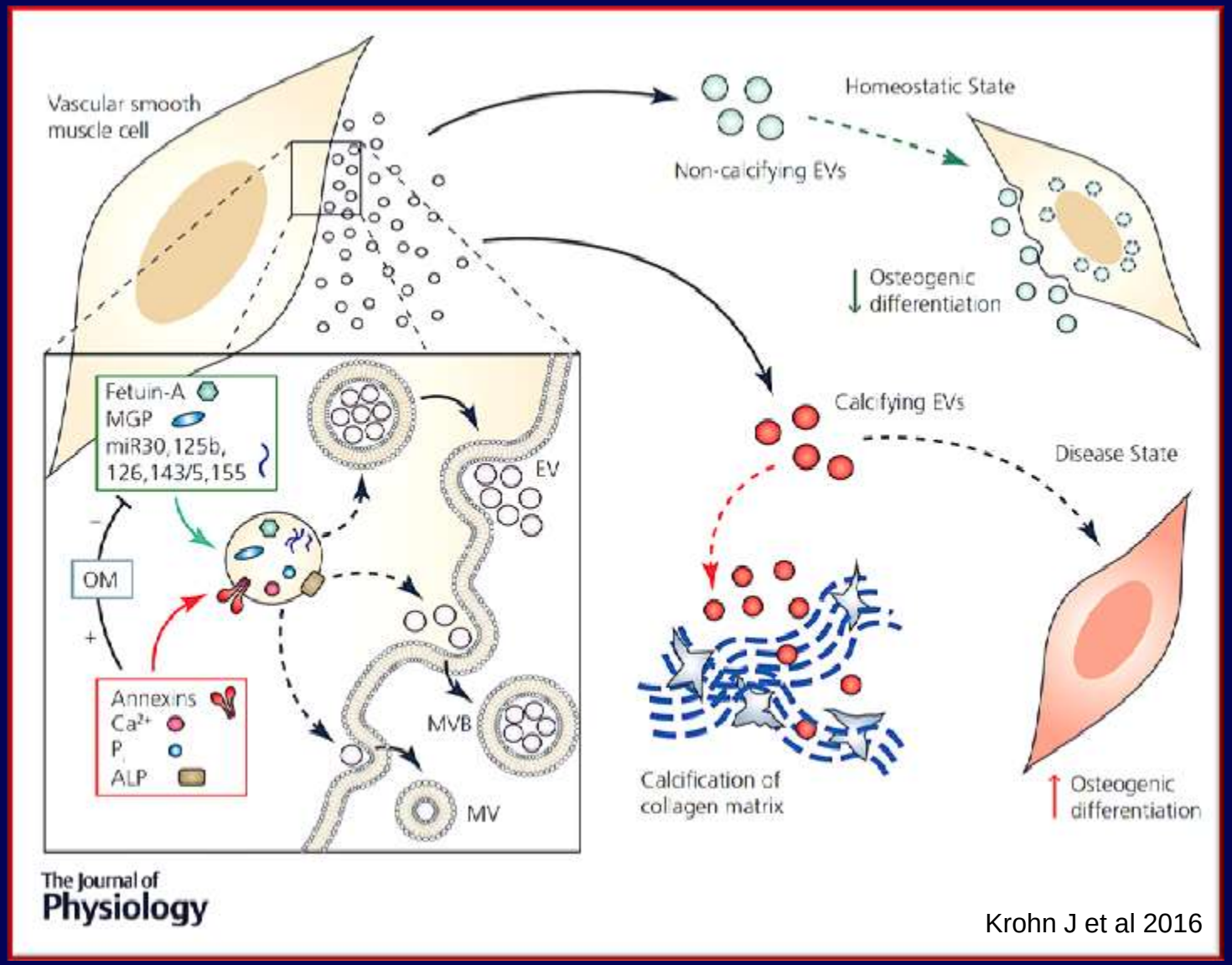
Joshua Hutcheson, PhD

Claudia Goettsch, PhD

Payal Vyas, PhD

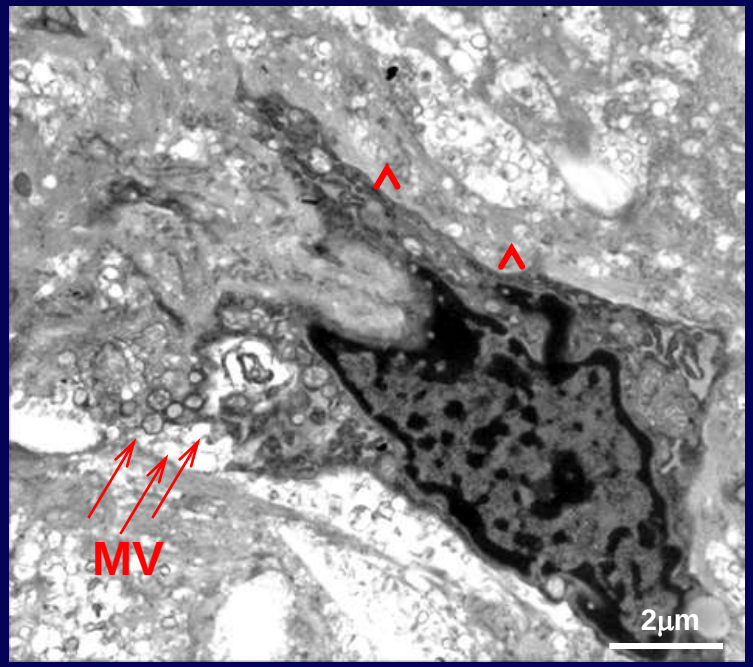
The role of extracellular vesicles (exosomes) in the formation of microcalcifications

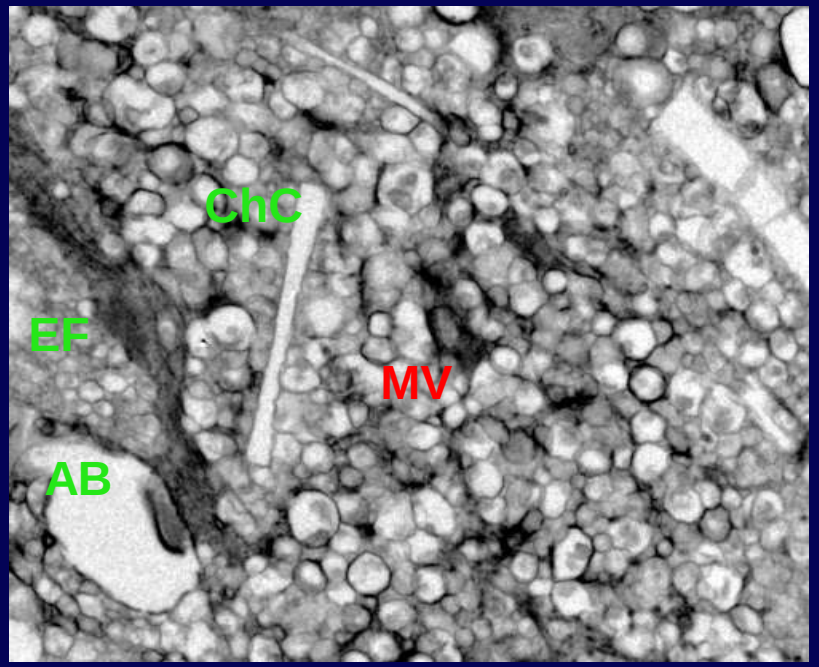
Extracellular vesicles contribute to physiology and pathology



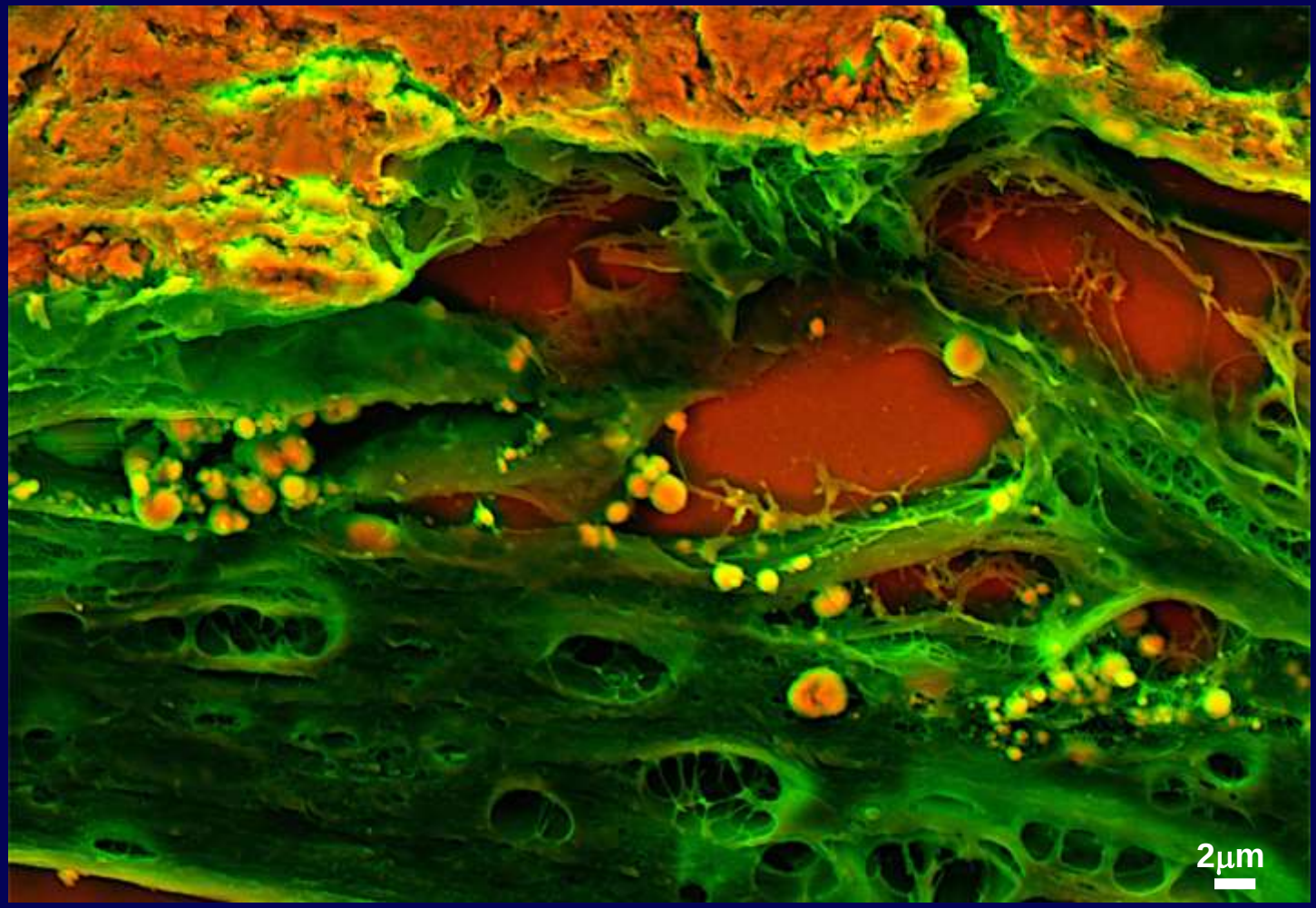
Krohn J et al 2016

Extracellular vesicles observed in calcifying atherosclerotic plaques





Calcifying arteries show macro- and microcalcification detected by density-dependent scanning electron microscopy



Krohn J et al, *ATVB* 2016

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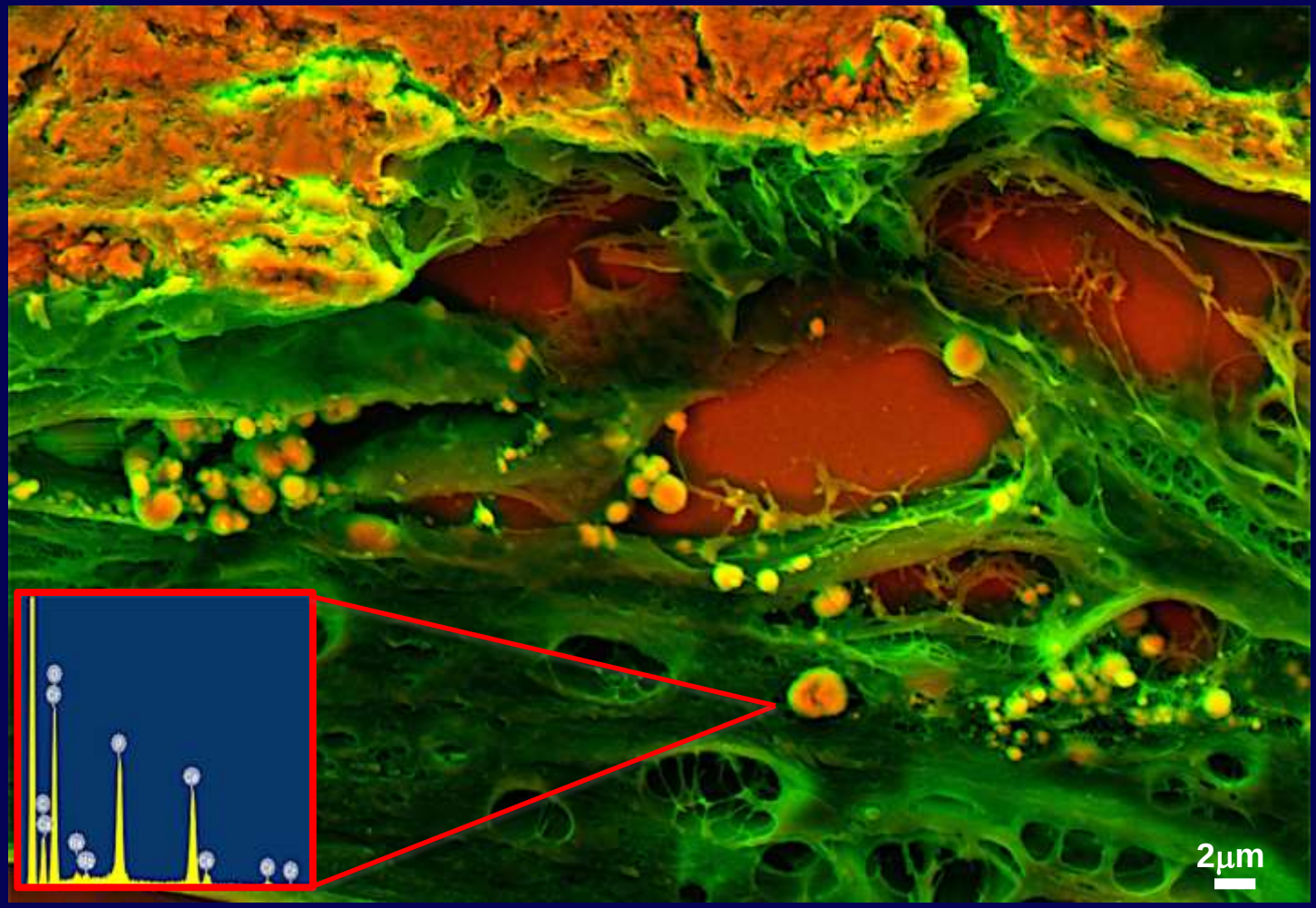
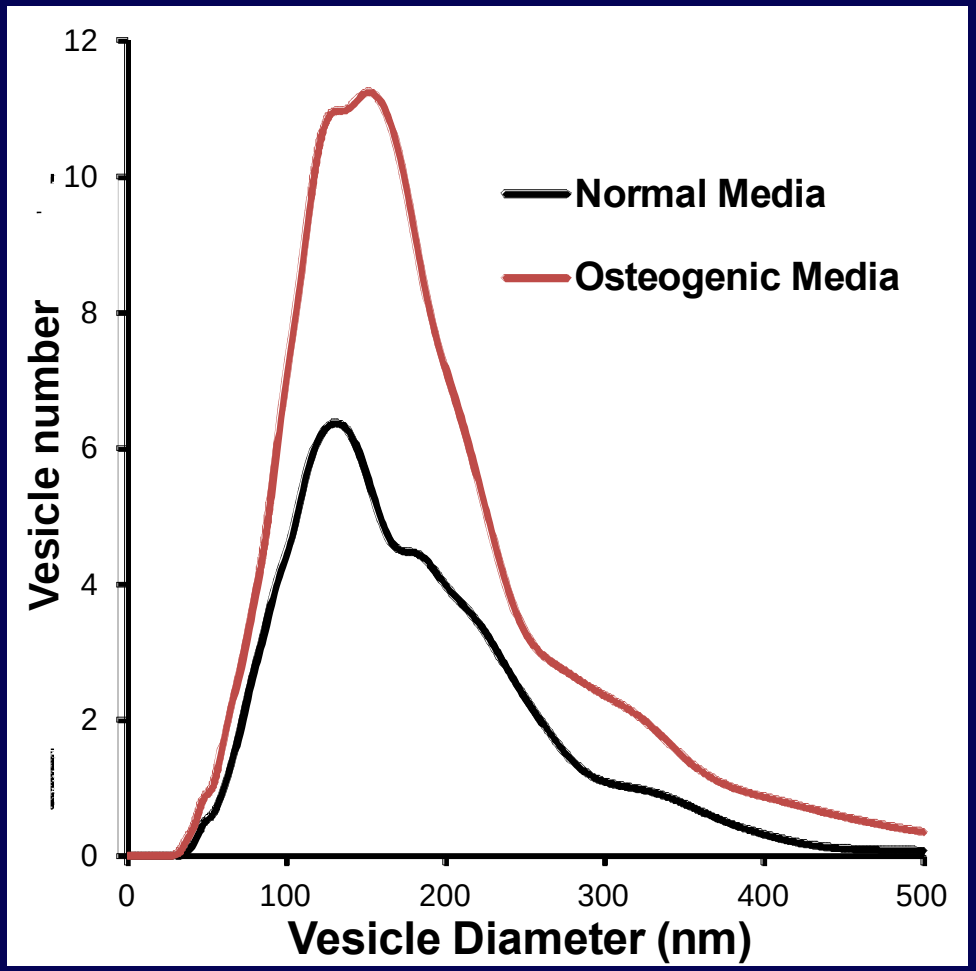
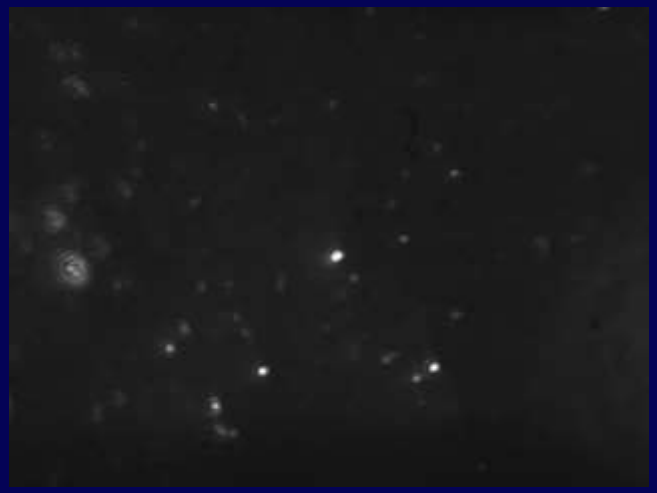


Krohn J et al, *ATVB* 2016

NanoSight nanoparticle tracking analysis reveals size and numbers of extracellular vesicles

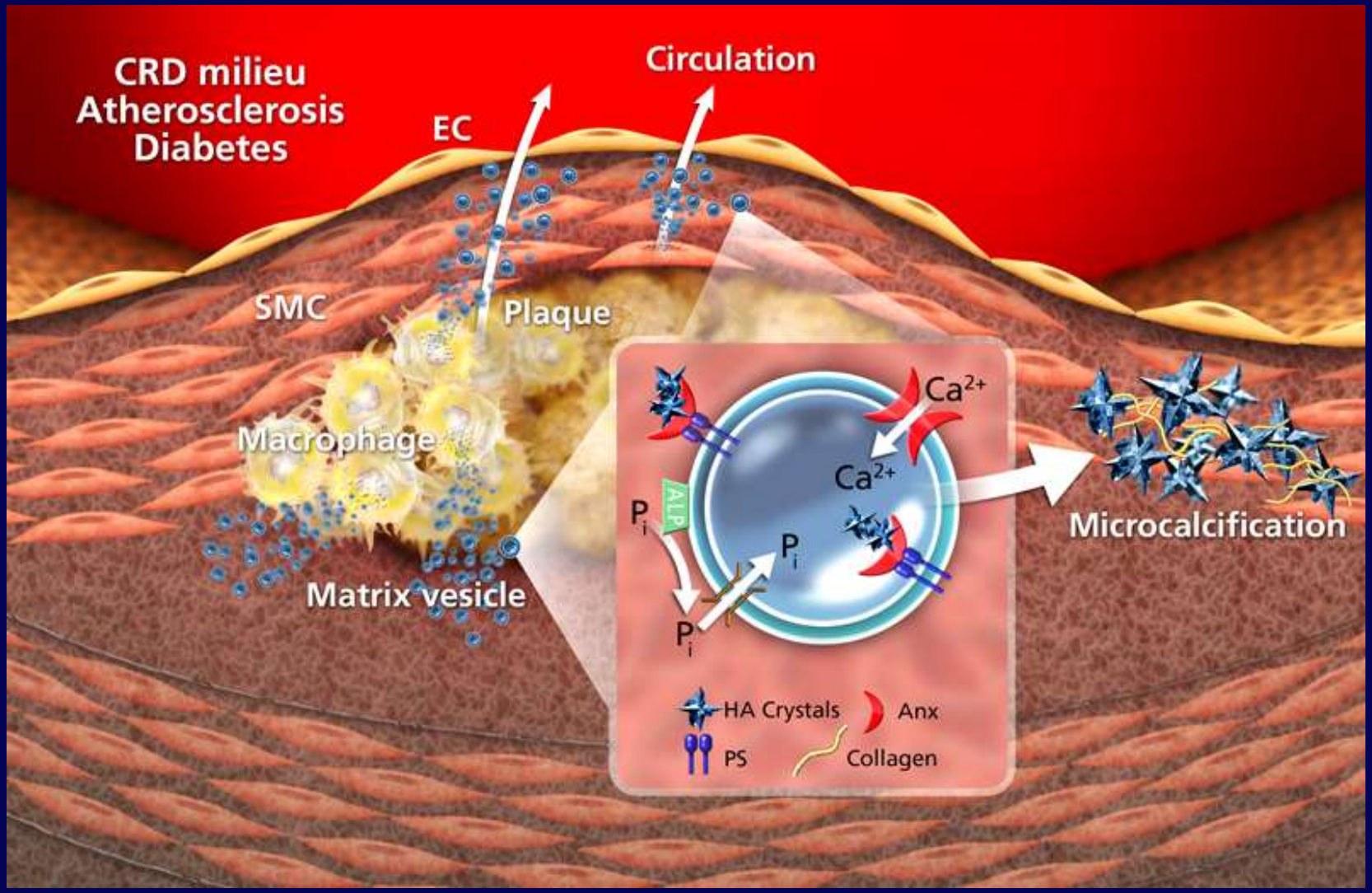


Normal Media



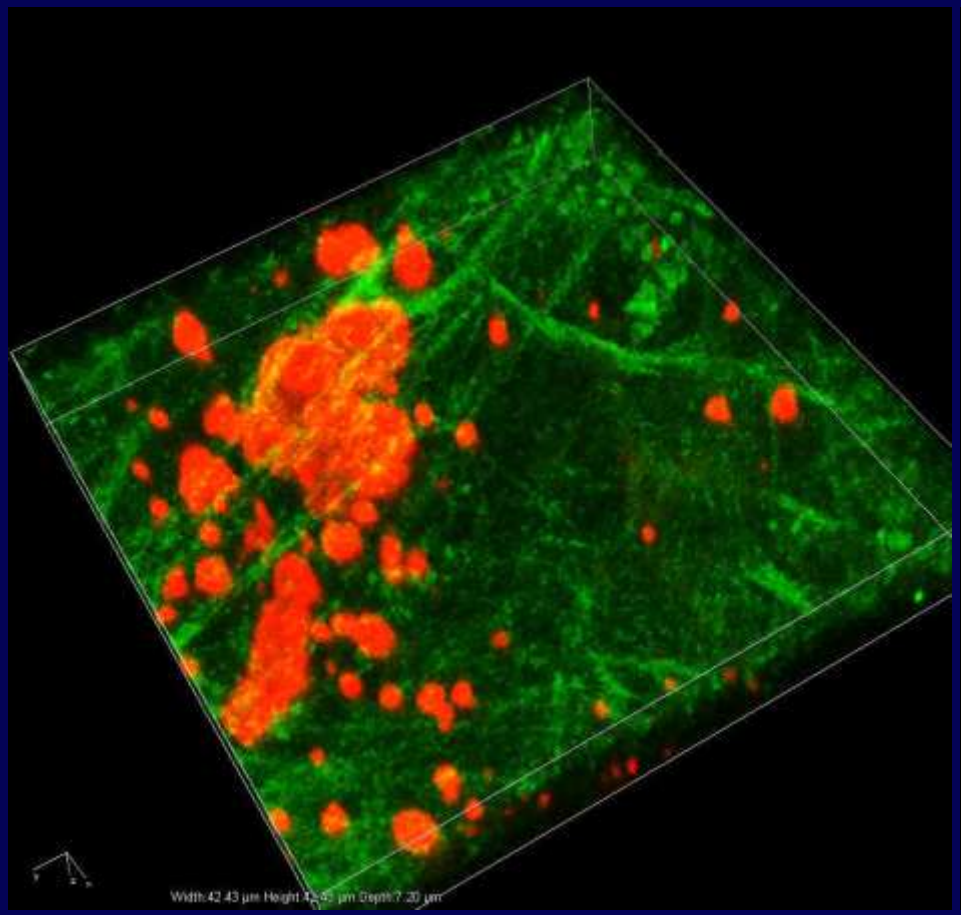
Osteogenic Media

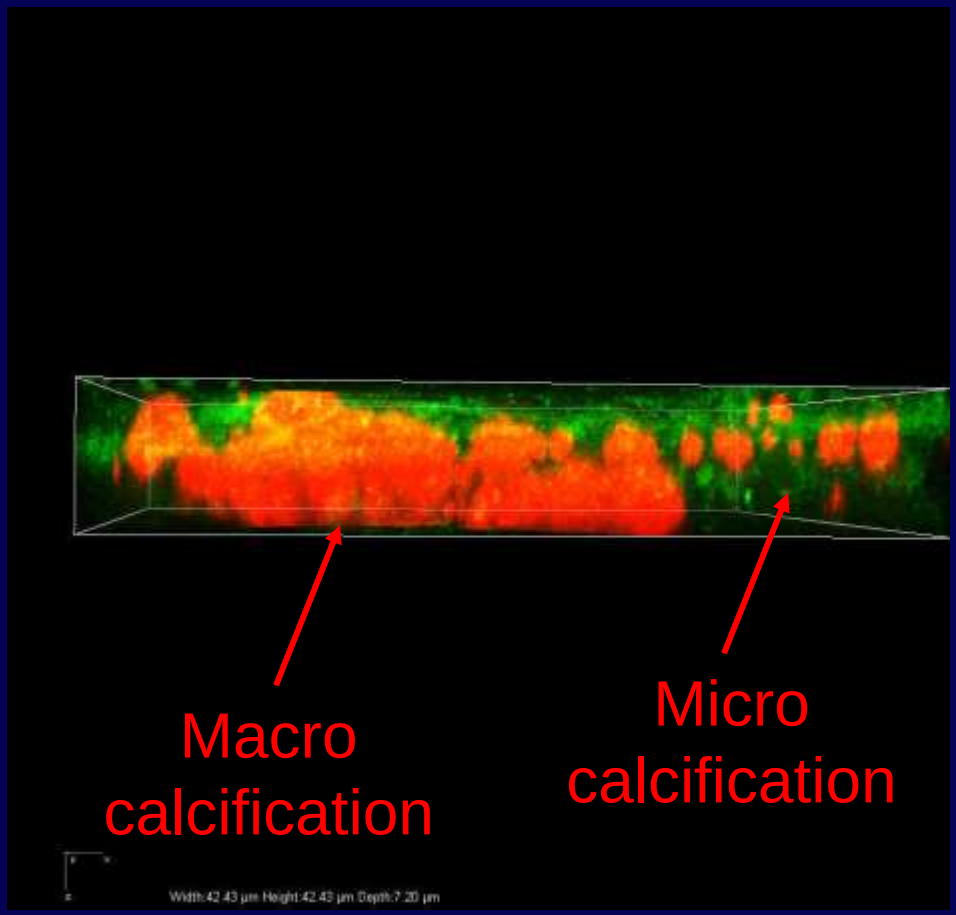
Mechanism of extracellular vesicle calcification



New, SEP & Aikawa E, *ATVB* 2013

Smooth muscle cells in osteogenic conditions recapitulate fibrotic and calcific responses





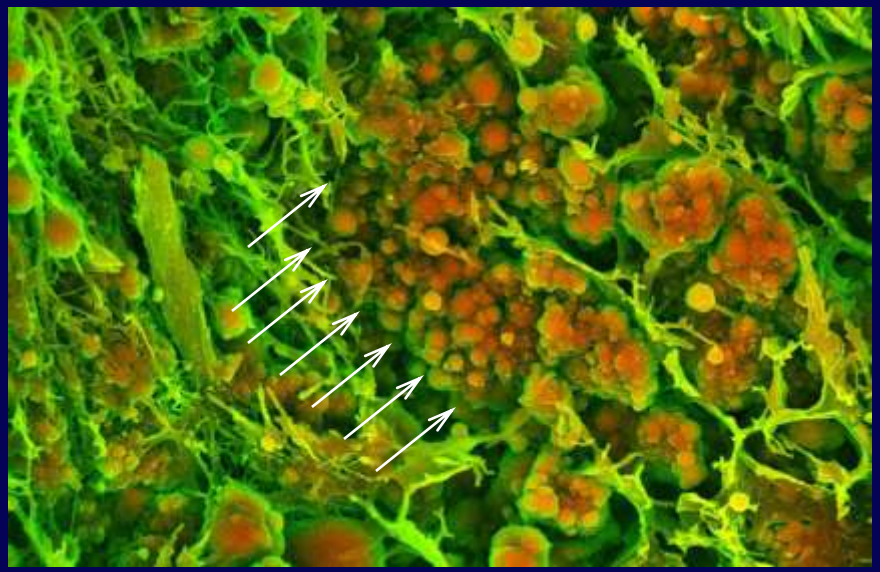
Collagen - Collagen Probe

Calcification - Near Infrared Calcium Tracer

Hutcheson et al, *Nature Materials* 2016

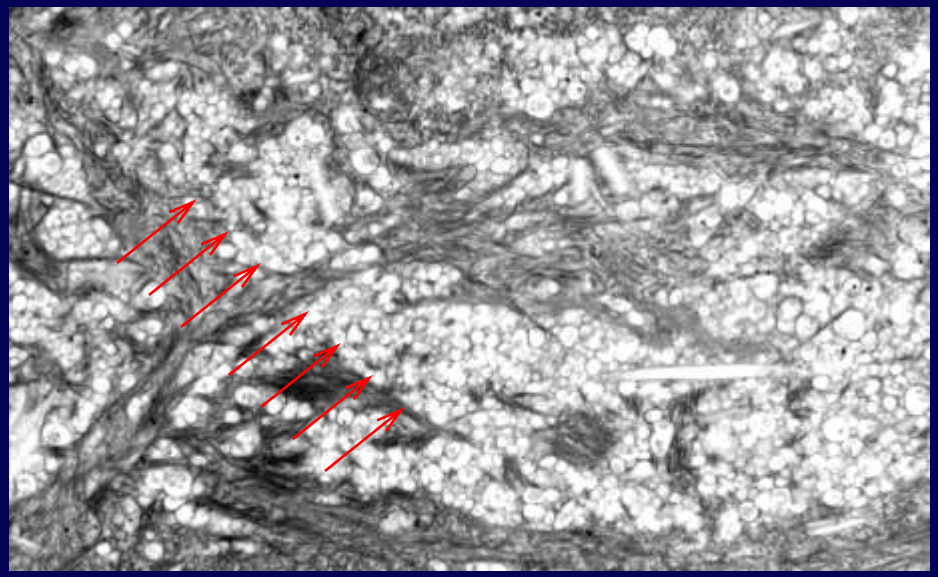
Extracellular vesicles accumulate between collagen fibers

Density Dependent Color
Scanning Electron Microscopy



Human

Electron Microscopy



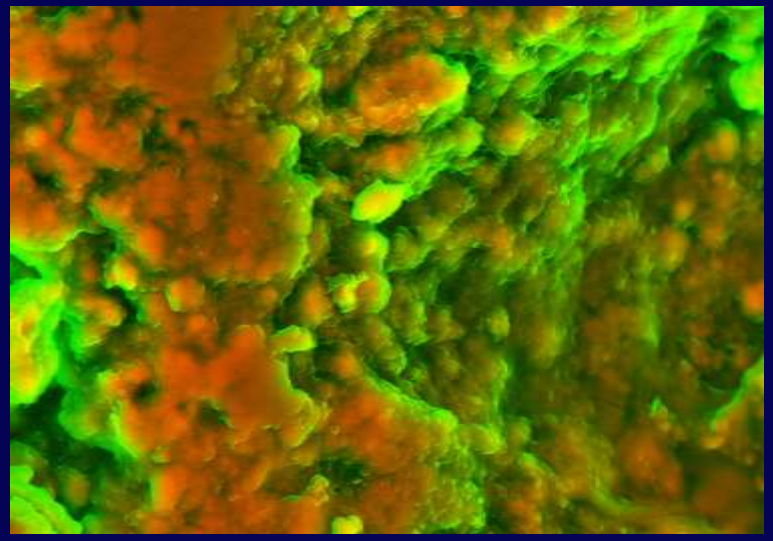
Mouse

Scanning electron microscopy combined with energy-dispersive X-ray spectroscopy

Human tissue

Early

Advanced



Collagen

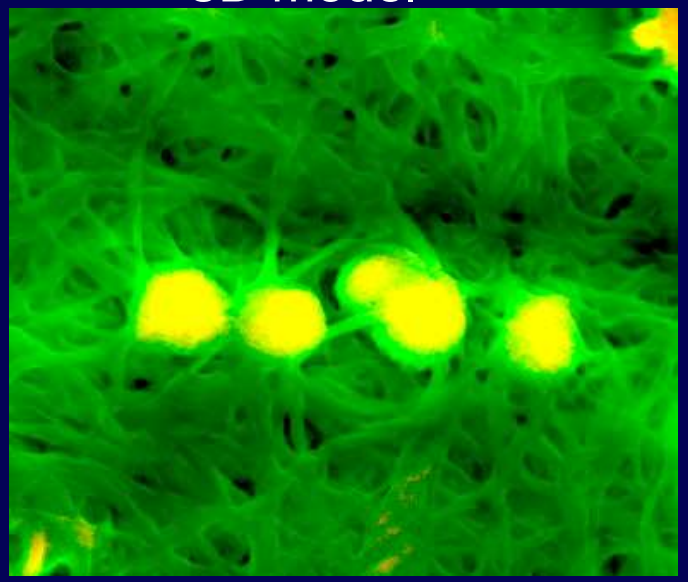
Calcification

Scanning electron microscopy combined with energy-dispersive X-ray spectroscopy

Human tissue

3D model

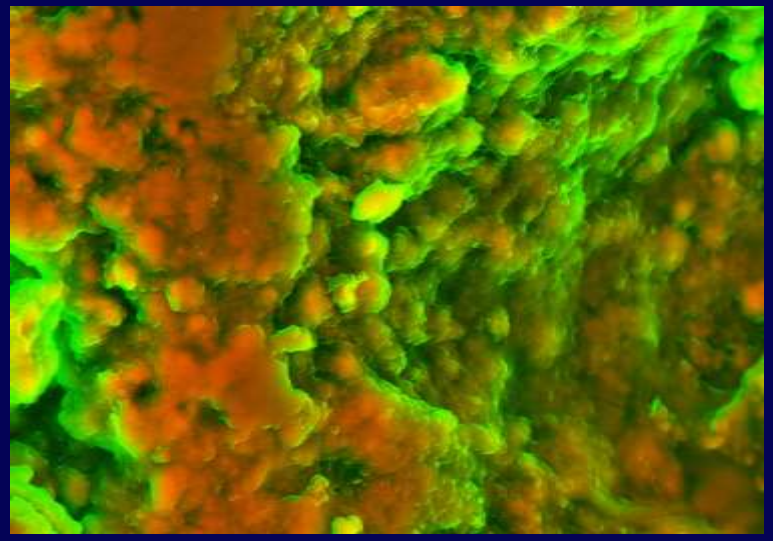
Early

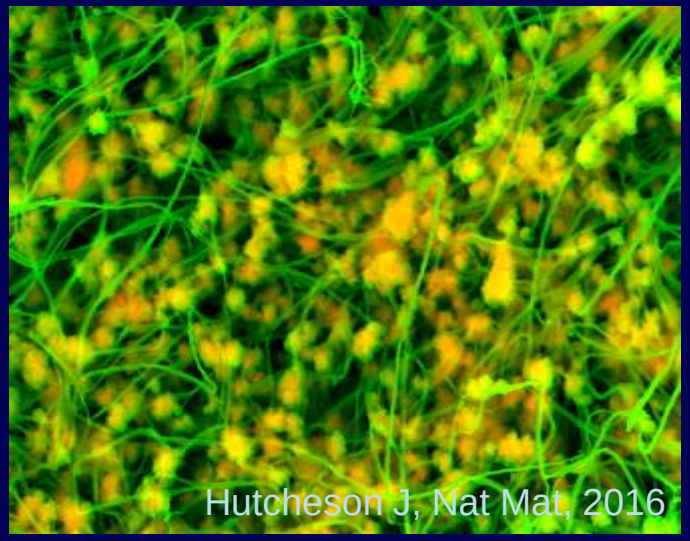


Collagen

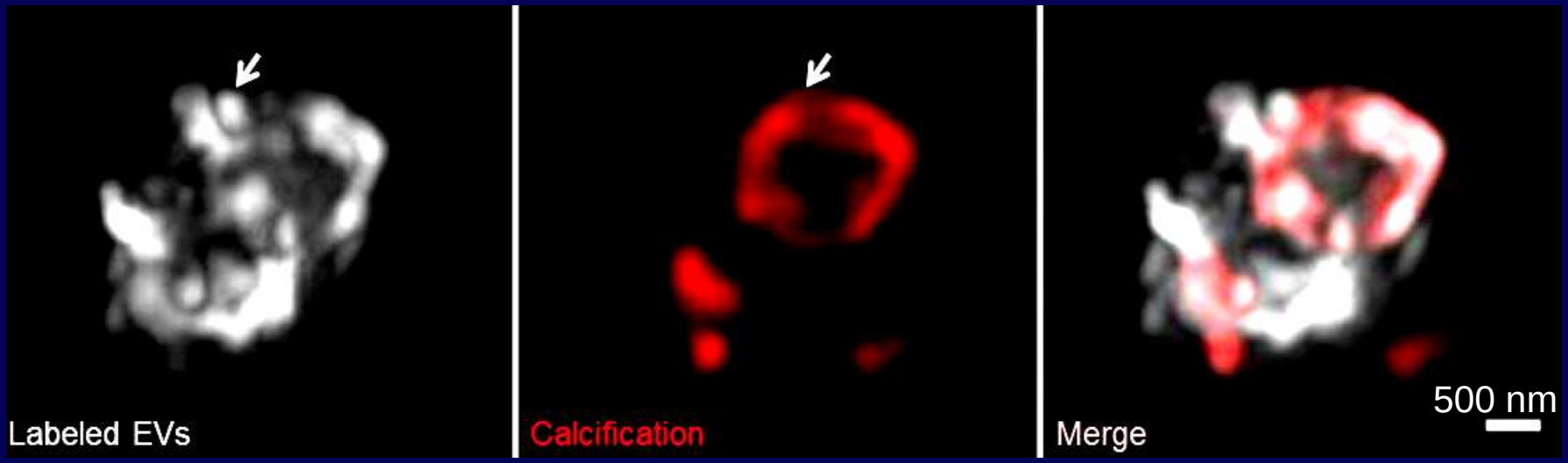
Calcification

Advanced





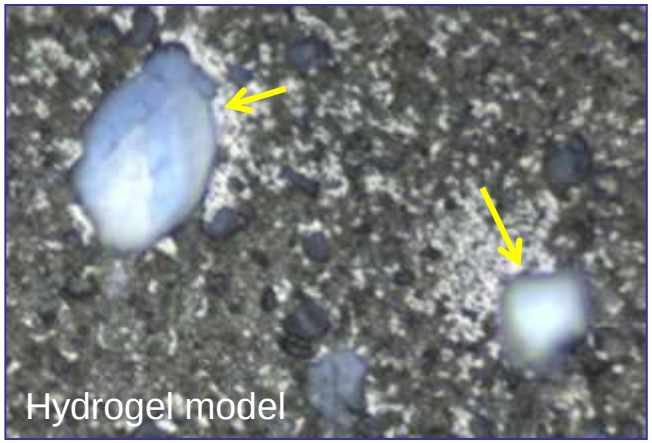
NIRF signal indicates calcification in EV aggregates after 5 days in collagen hydrogels

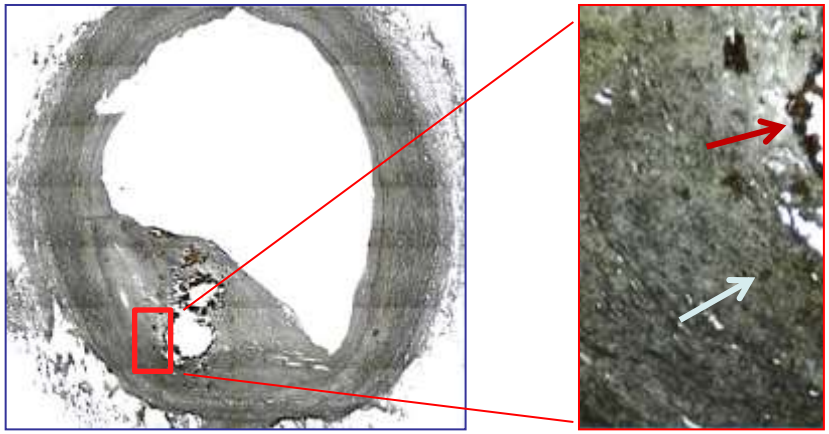


Previous knowledge was limited to TEM in calcified tissues! We have provided time course and 3-D observations using structure illumination microscopy.

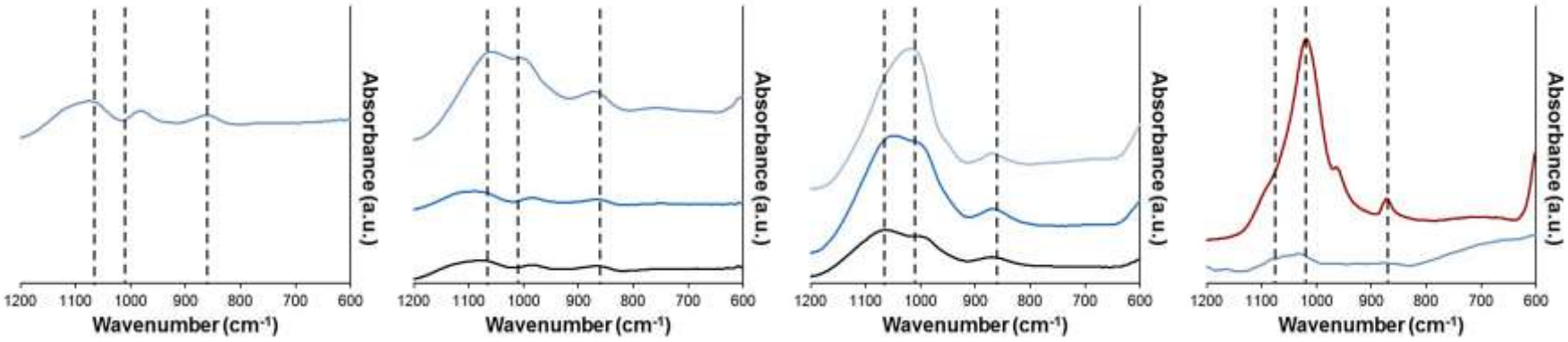
Hutcheson et al, *Nature Materials* 2016

FTIR spectra indicates microcalcification maturation after 5 days in collagen hydrogels





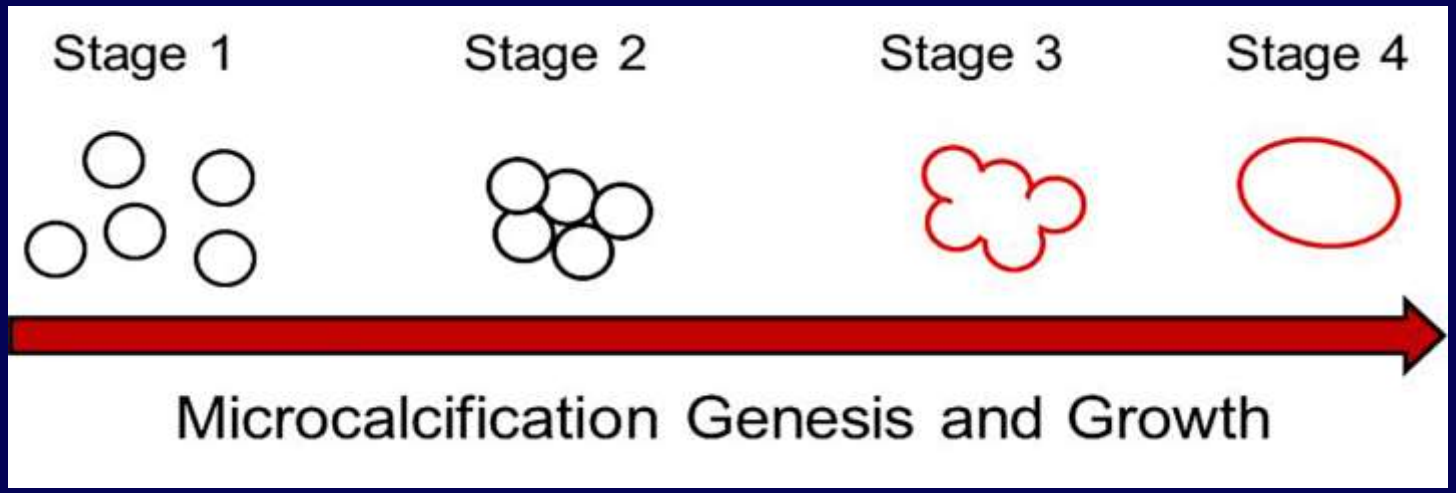
Day 0 Day 1 Day 5 Calcified Tissue



Hutcheson et al, *Nature Materials* 2016

A model of microcalcification genesis and growth

Ruiz, Hutcheson, Aikawa E. (unpublished)



NanoSight
Zeta Potential

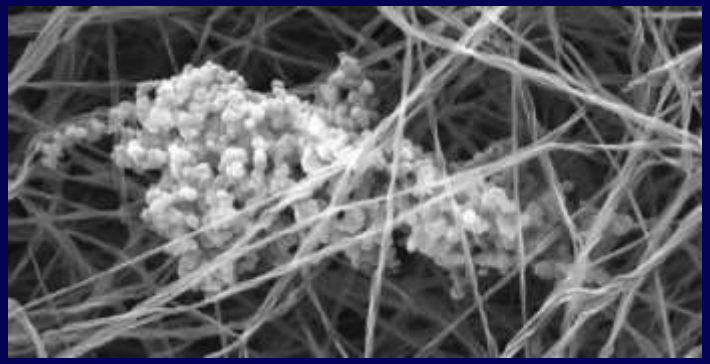
Structure Illumination
Confocal
Zeta Potential

Structure Illumination
FTIR
Confocal
X-ray Spectroscopy
SEM
TEM
Electron Diffraction
FE-SEM

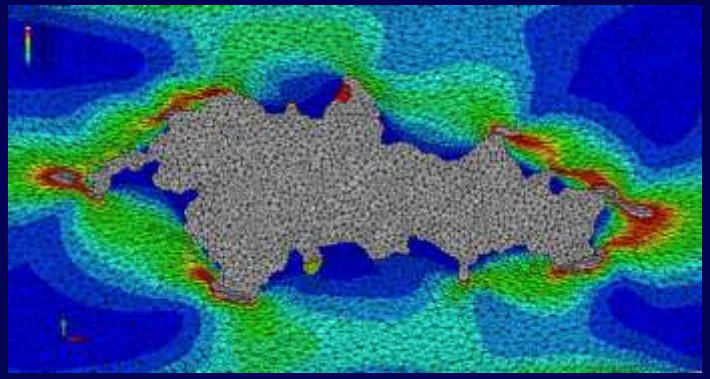
FTIR
Confocal
Two-photon
FE-SEM

Hutcheson et al, *Nature Materials* 2016

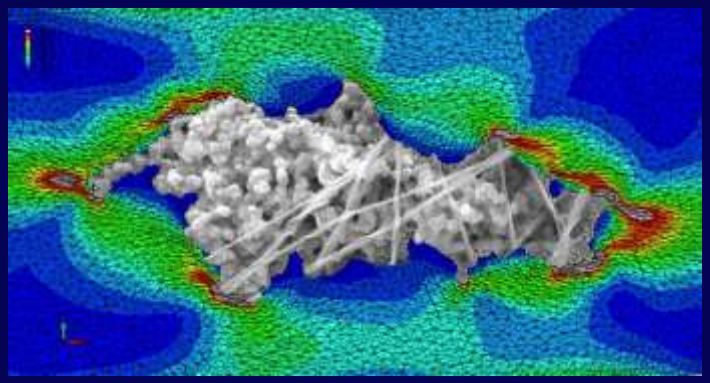
“Harmful” microcalcifications were detected in a 3D hydrogel model by FEA



SMC-derived microcalcification formed in a 3D hydrogel model



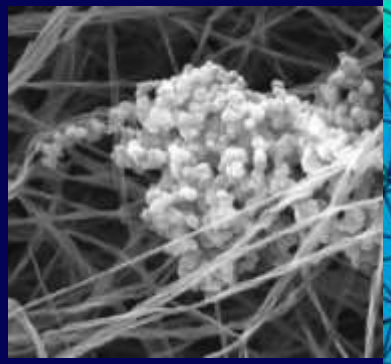
FEA: Stress concentration (red color) around microcalcification. Stress concentration factor = 6 (600% increase in stresses)

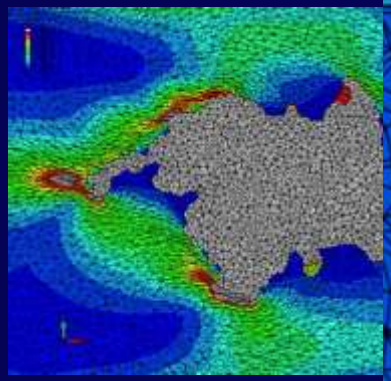


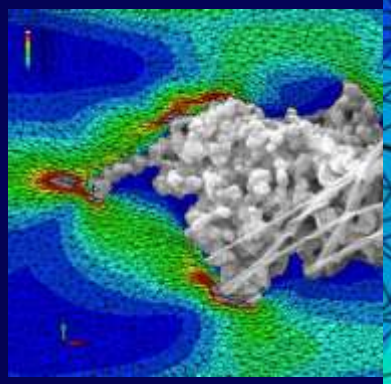
Same as above, but with the image of the microcalcification superposed on the stress concentration image

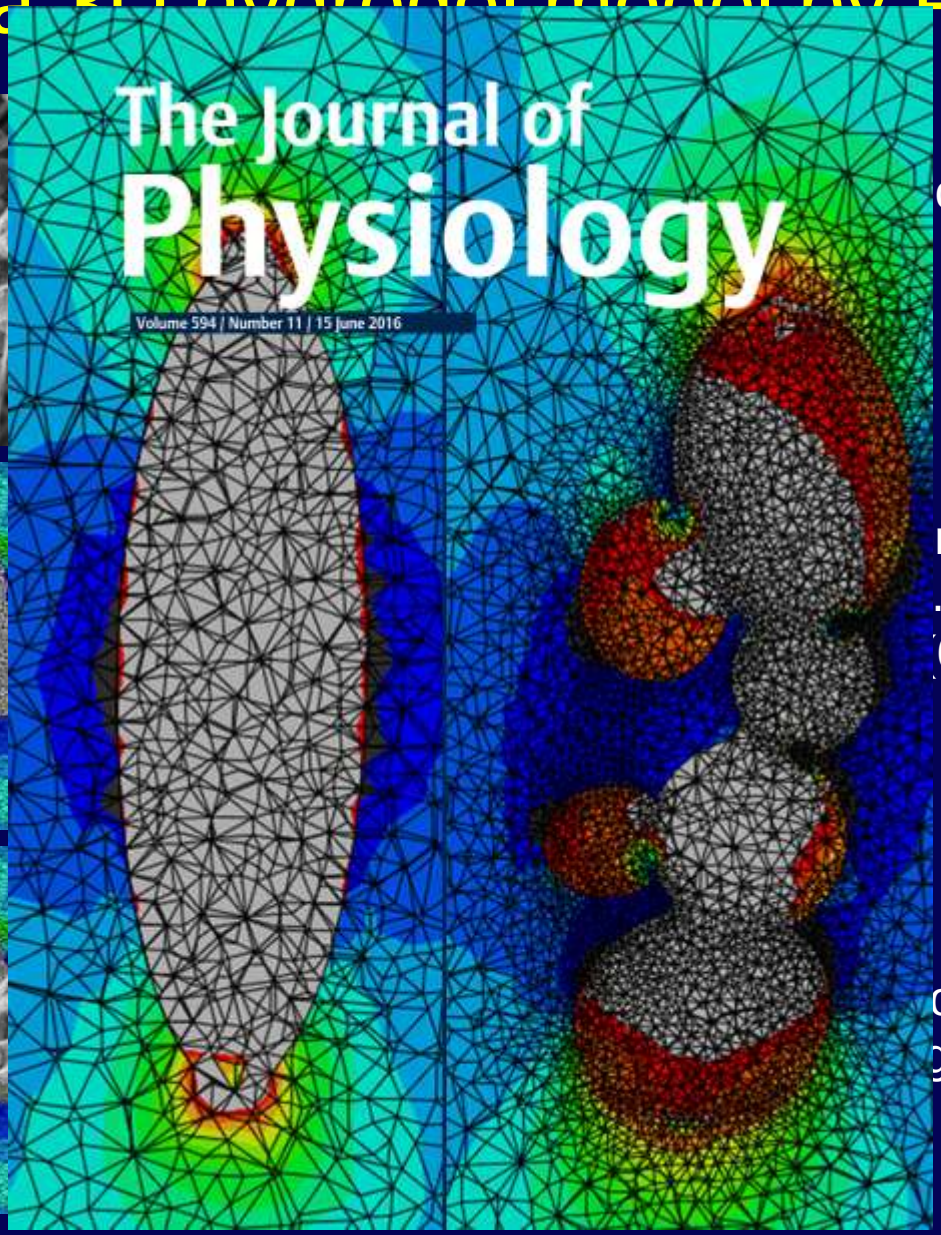
Collaboration with Dr. Sheldon Weinbaum

“Harmful” microcalcifications were detected in a 3D hydrogel model by FEA









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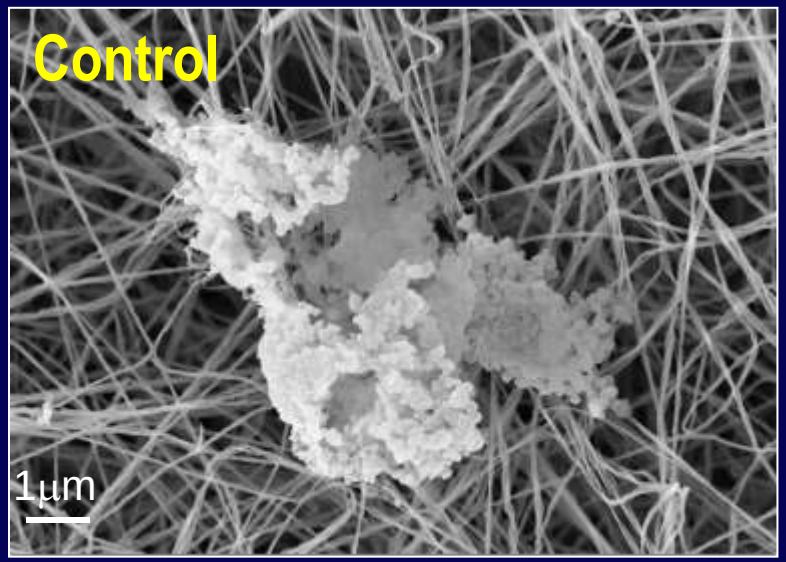
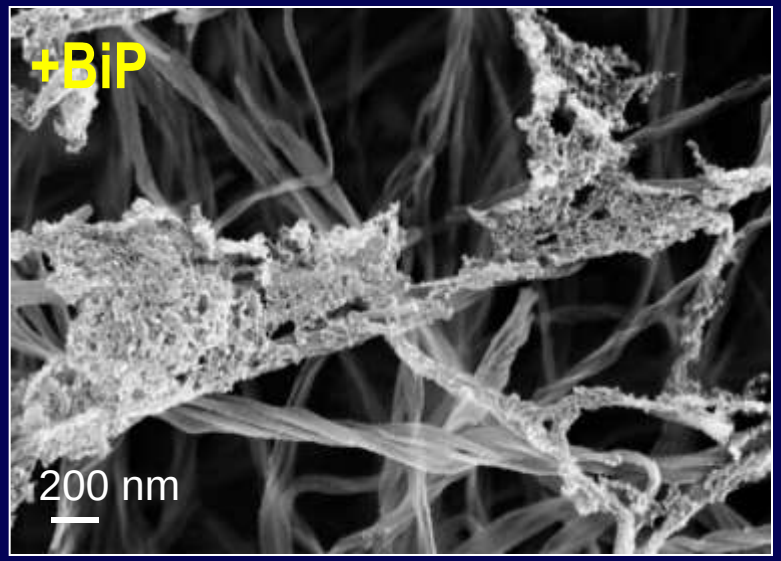
Altered morphology and size of microcalcifications growing in a 3D hydrogels after BiP treatment



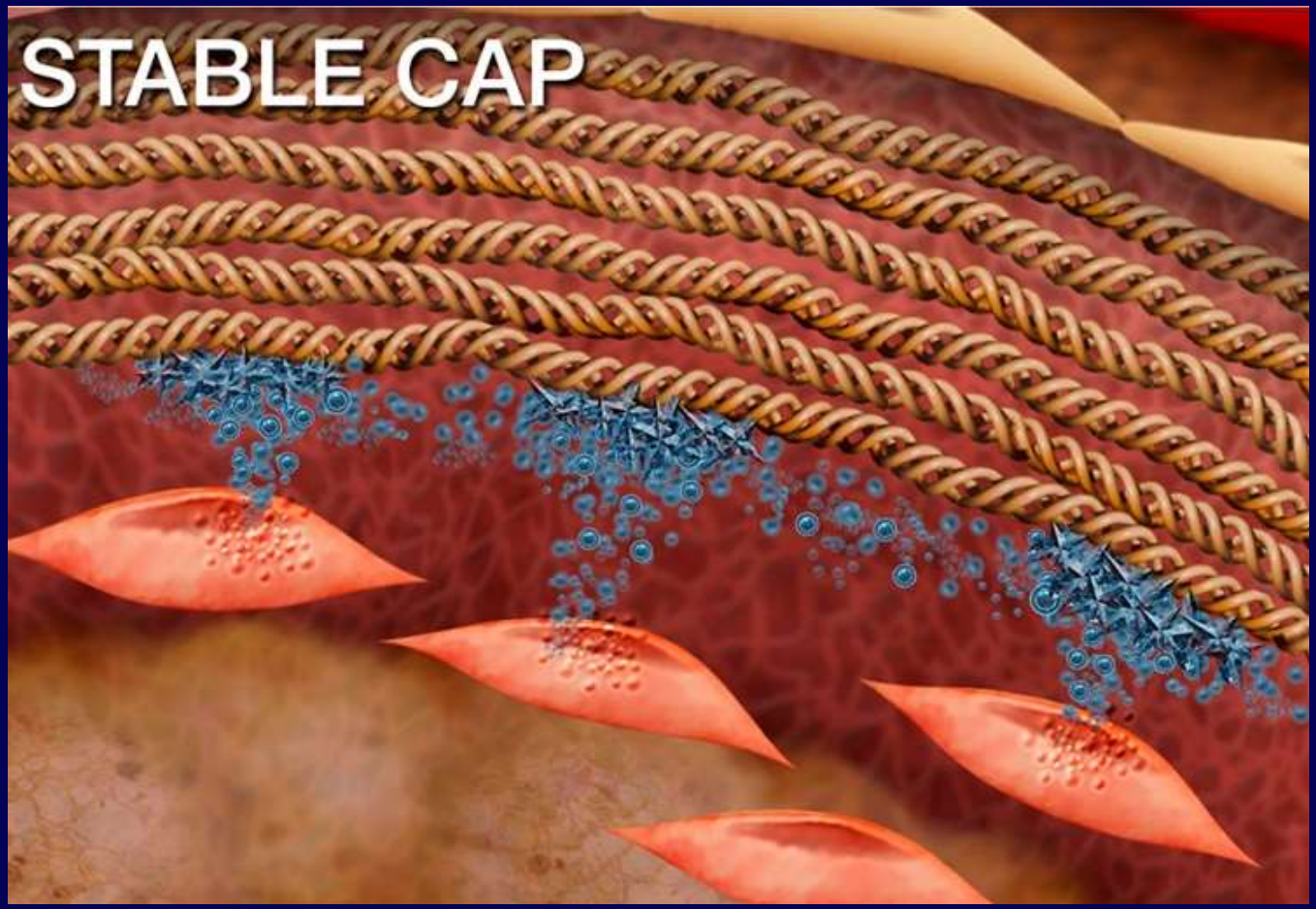


J. Ruiz, J. Hutcheson, E. Aikawa, unpublished data

Collagen degradation within the fibrous cap leads to microcalcification formation

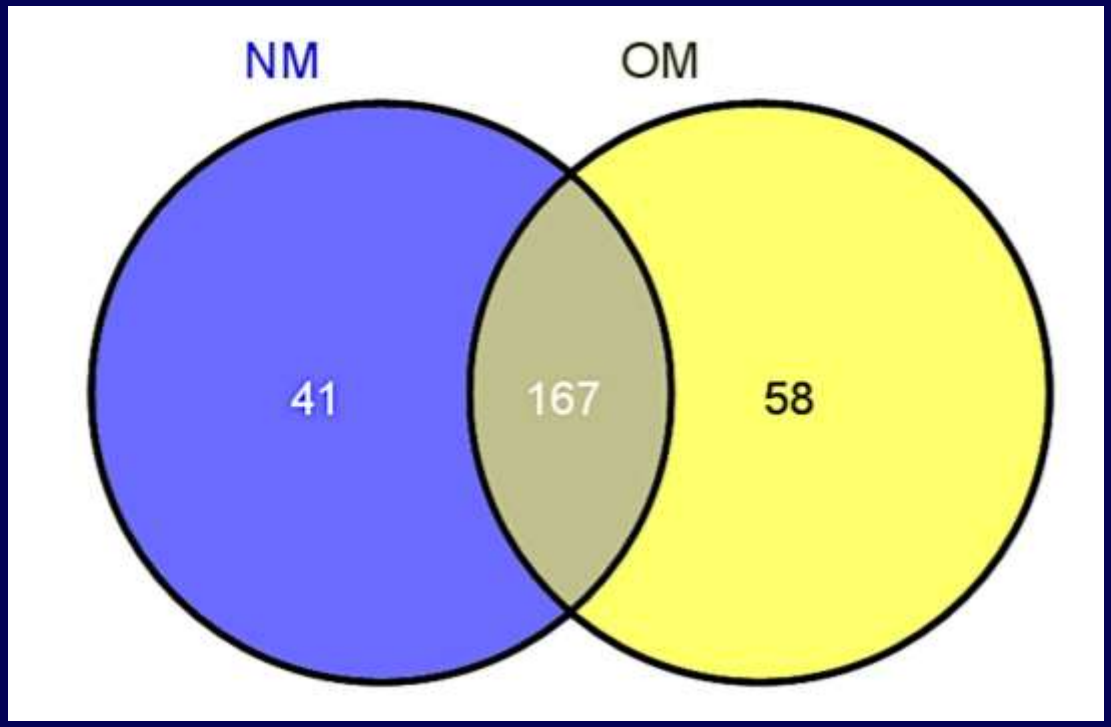
Hutcheson et al, *Nature Materials* 2016

Collagen degradation within the fibrous cap leads to microcalcification formation

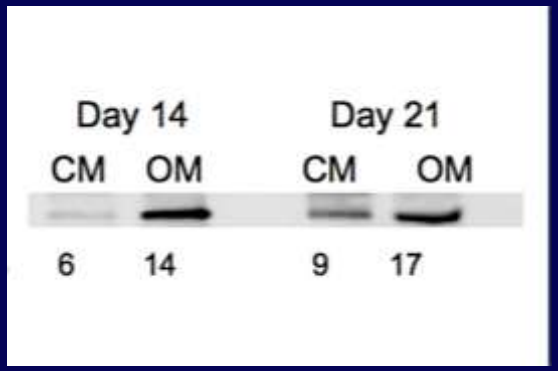


Hutcheson et al, *Nature Materials* 2016

Number of protein groups identified in extracellular vesicles



Sortilin mass spectrometry



Exclusive	Total	Label
58	225	Osteogenic Media (OM)
41	208	Normal Media (NM)
167	167	OM/Control

Human Genome Wide Association Study (GWAS) showed association of Sortilin with cardiovascular calcification

Sort1 gene encoding sortilin protein is associated with the risk of cardiovascular diseases.

Nat Genet. 2008;40

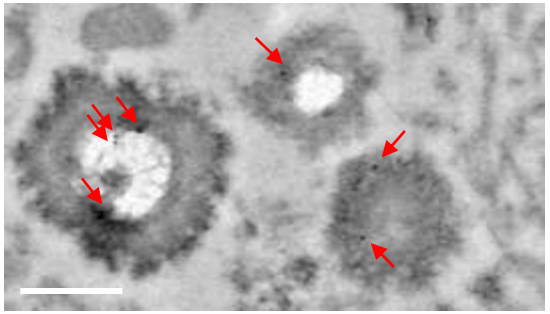
Nature. 2010;466

Genome-wide association study for coronary artery calcification with follow-up in myocardial infarction.

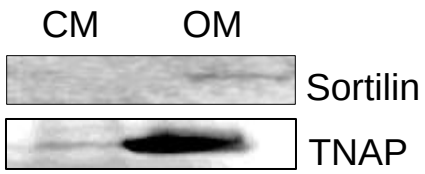
Circulation. 2011;124

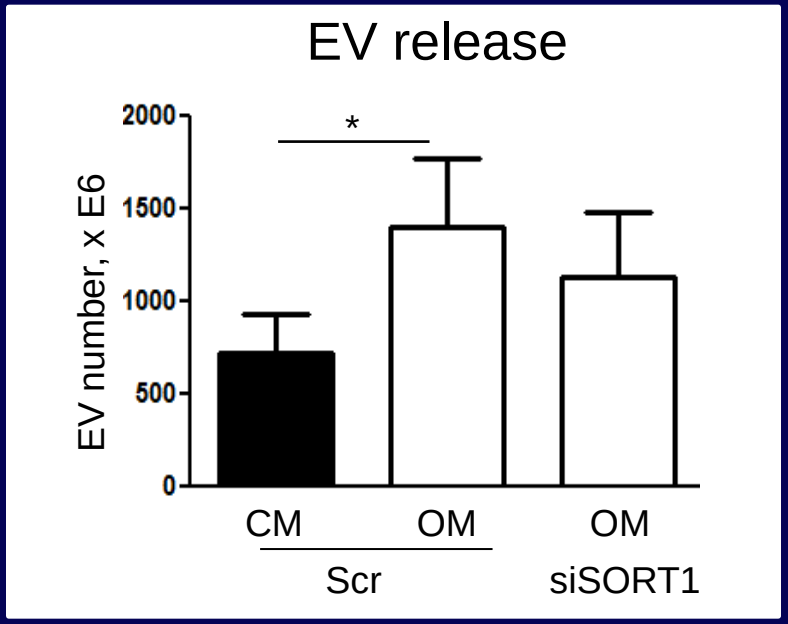
Osteogenic environment promotes loading of sortilin into extracellular vesicles (EV)

Sortilin immunogold



Sortilin western blot



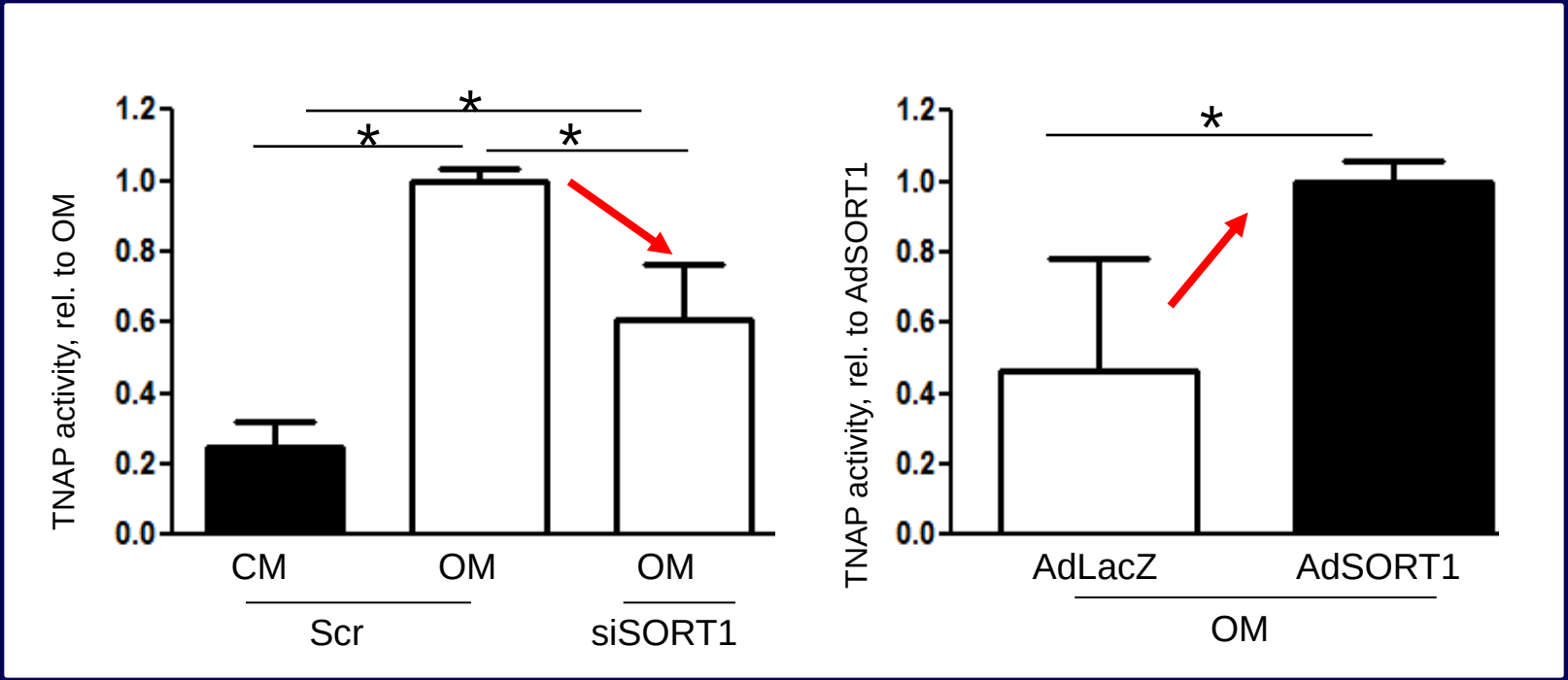


Mean ± SD
n≥3, *P<0.05

Goettsch et al, *JCI* 2016

Sortilin promotes calcification potential of EV

TNAP activity

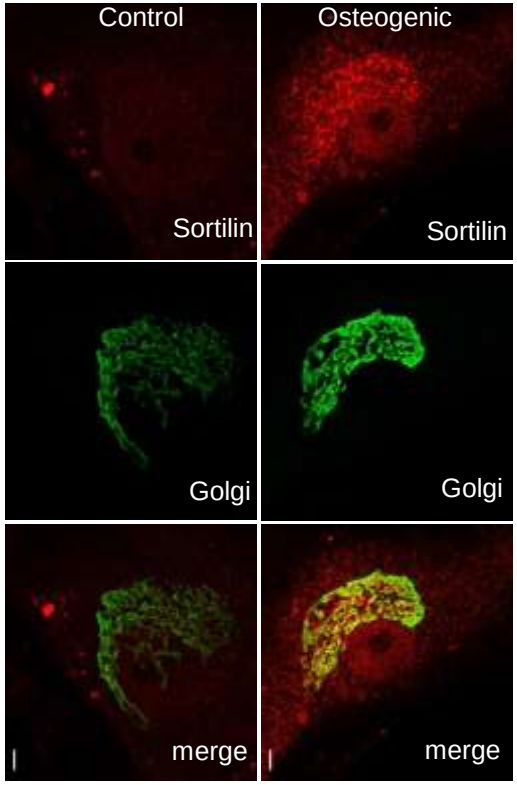


Mean ± SD
n≥3, *P<0.05

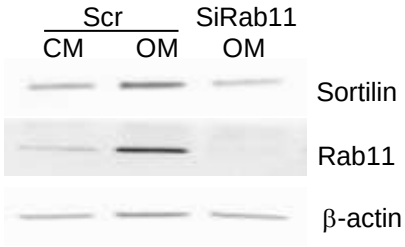
Goettsch et al, *JCI* 2016

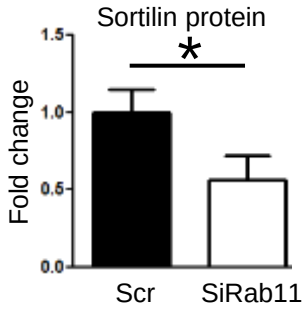
Rab11 regulates Golgi-to-plasma membrane transport

Calcifying SMC

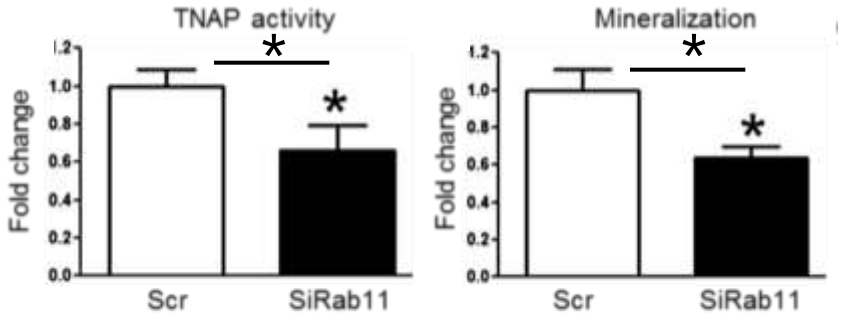


Silencing of Rab 11 reduces Sortilin protein



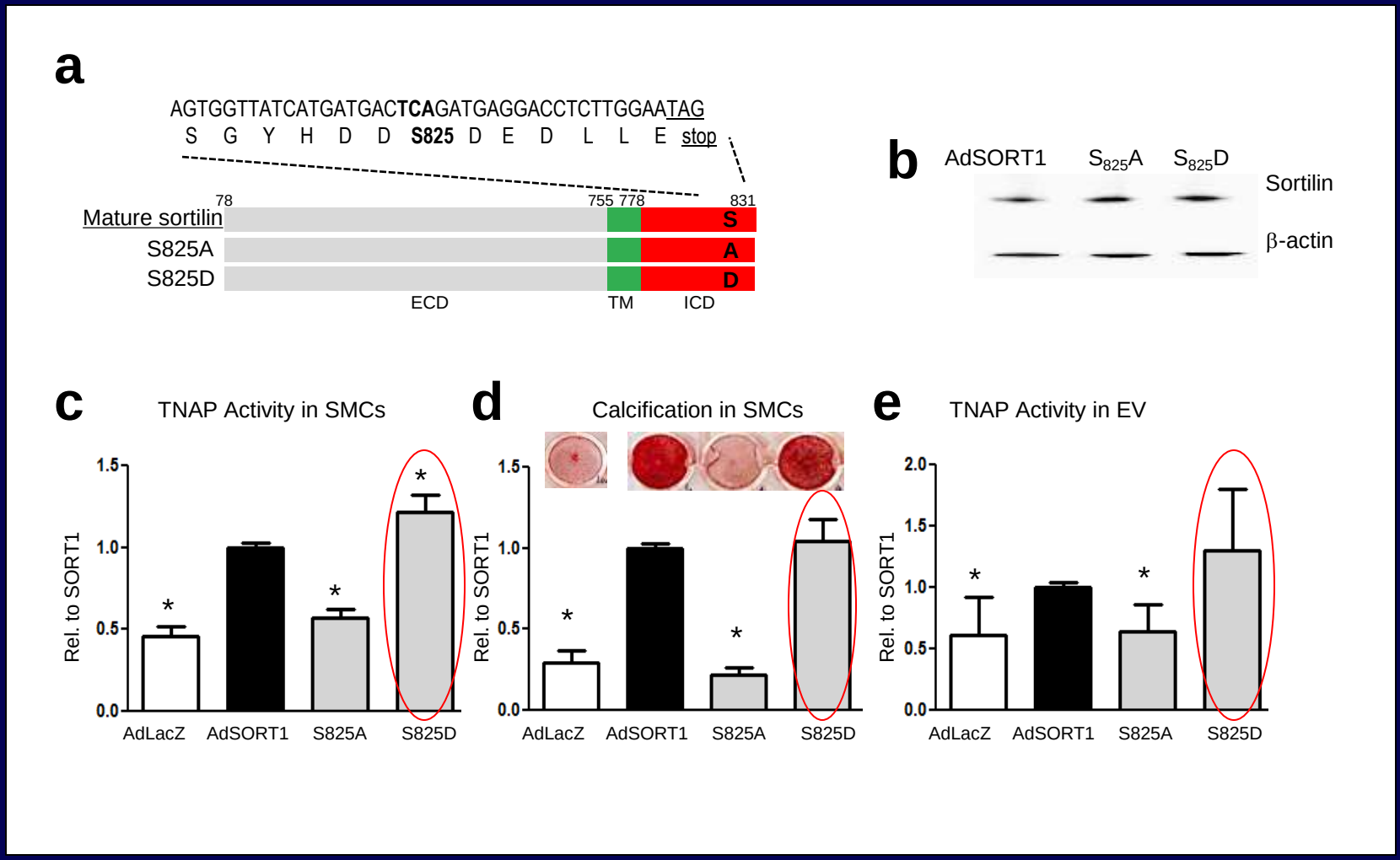


Silencing of Rab 11 reduces SMC calcification



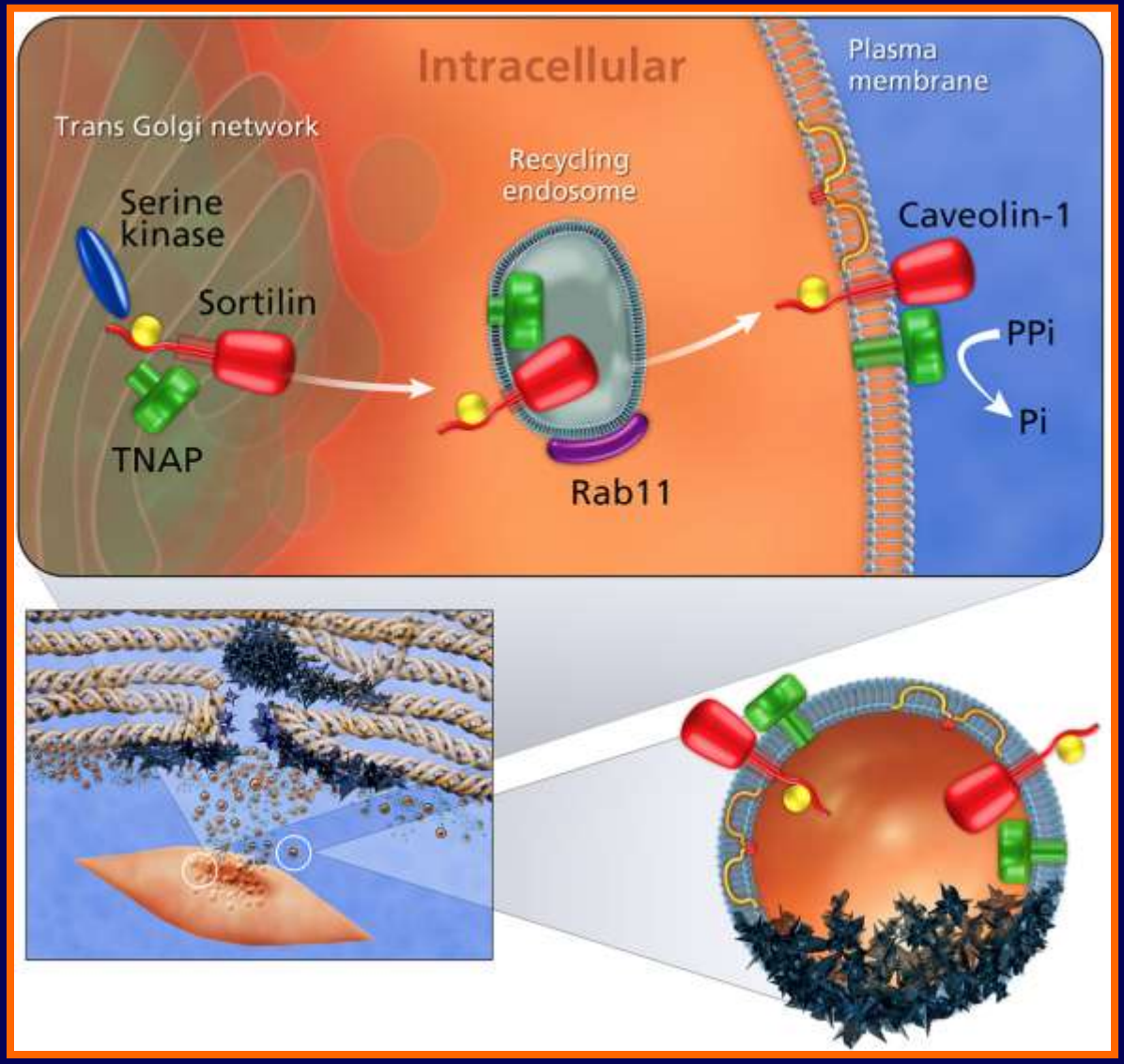
Goettsch et al, *JCI* 2016

Phosphorylation of sortilin C-terminus accelerates SMC calcification and EV calcification potential



Goettsch et al, *JCI* 2016

Novel biology of Sortilin



Goettsch et al, *JCI* 2016

Novel biology of Sortilin

Goettsch et al, *JCI* 2016

Acknowledgments

Brigham and Women's Hospital

Claudia Goettsch
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Matthias Nahrendorf
Ralph Weissleder

Imperial College London

Sergio Bertazo

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