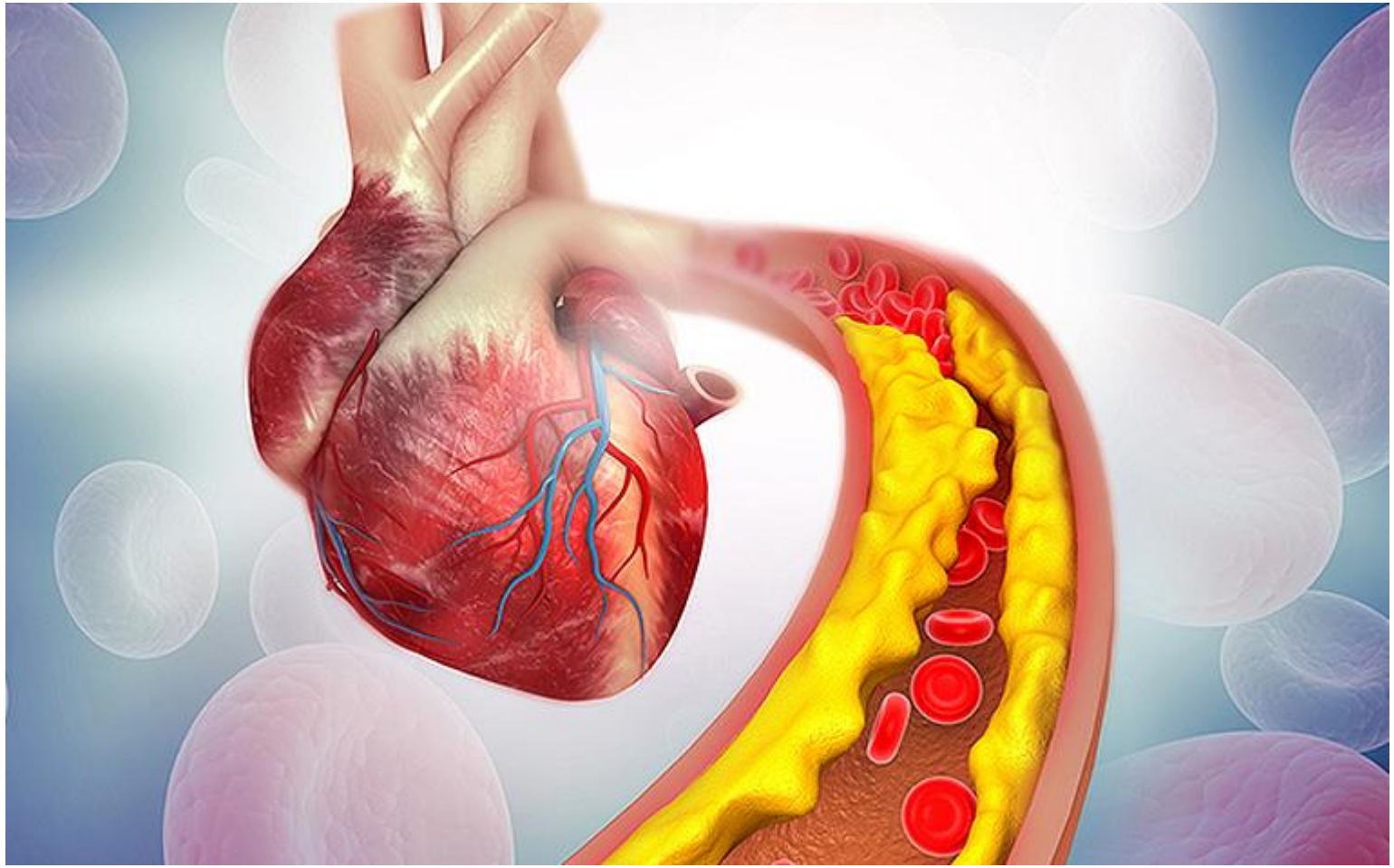
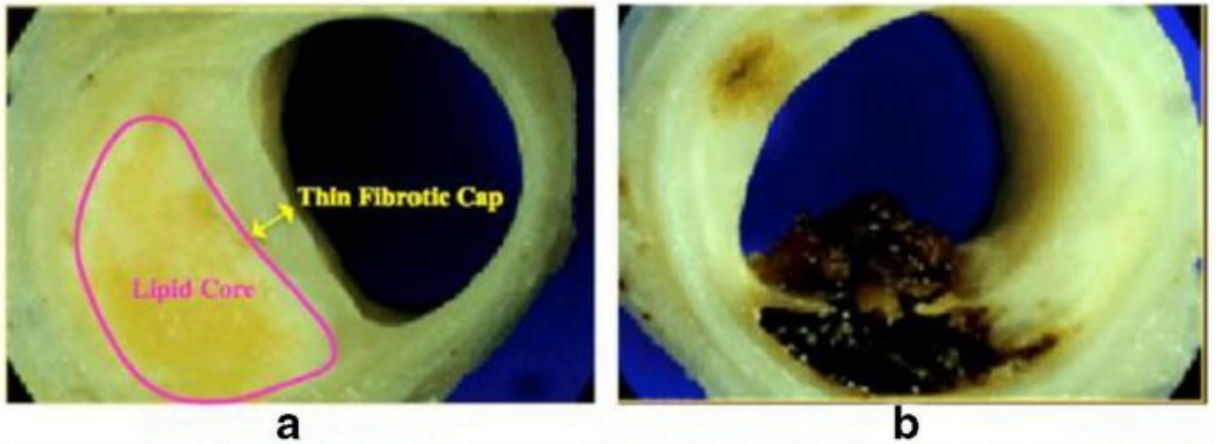
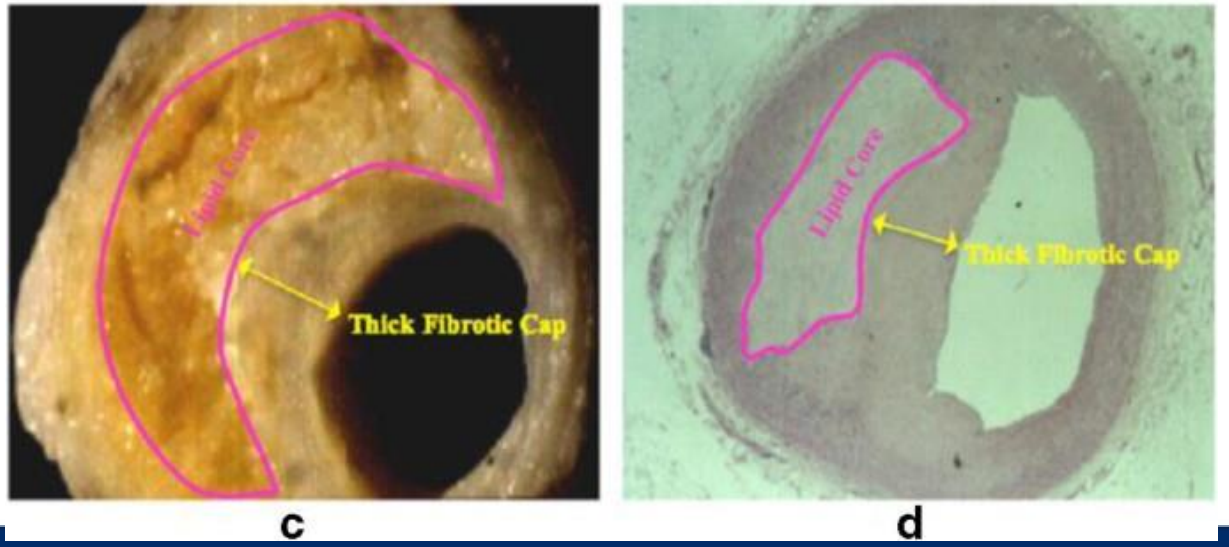
Mouse Models: Atherosclerosis



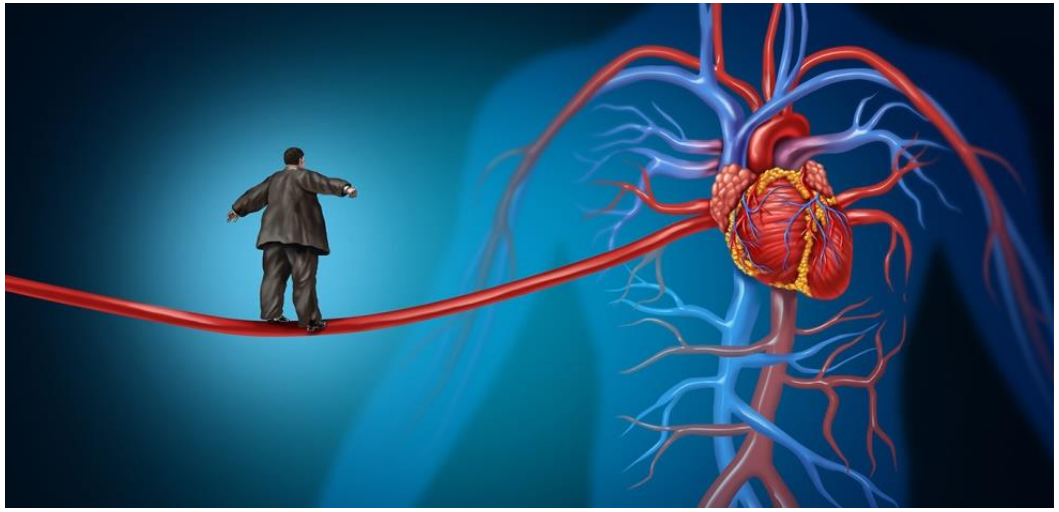
Hans Bluyssen, 14.03.2023

What is Atherosclerosis?





What is Atherosclerosis?



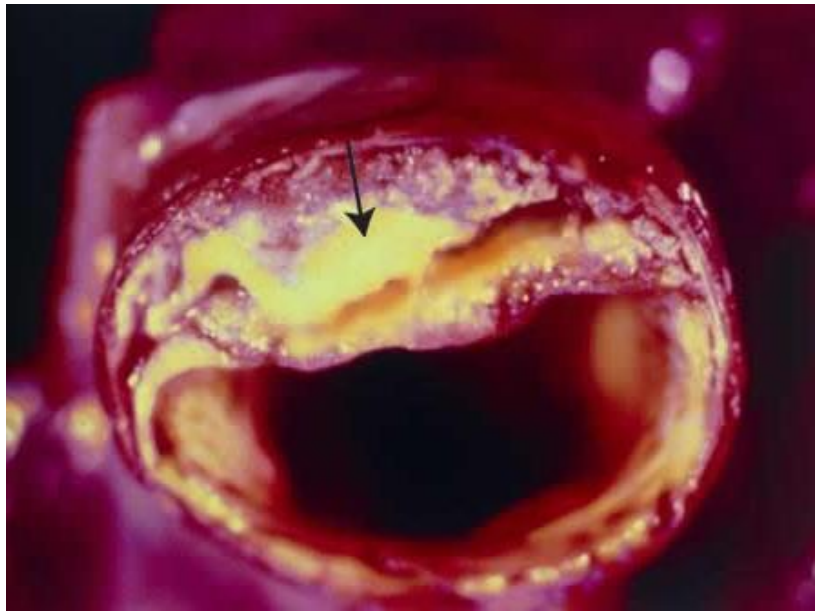
Atherosclerosis generally starts when a person is young and worsens with age.[2] Almost all people are affected to some degree by the age of 65.[6] It is the number one cause of death and disability in the developed world.[10]
Though it was first described in 1575,[11] there is evidence that the condition occurred in people more than 5,000 years ago.[11]

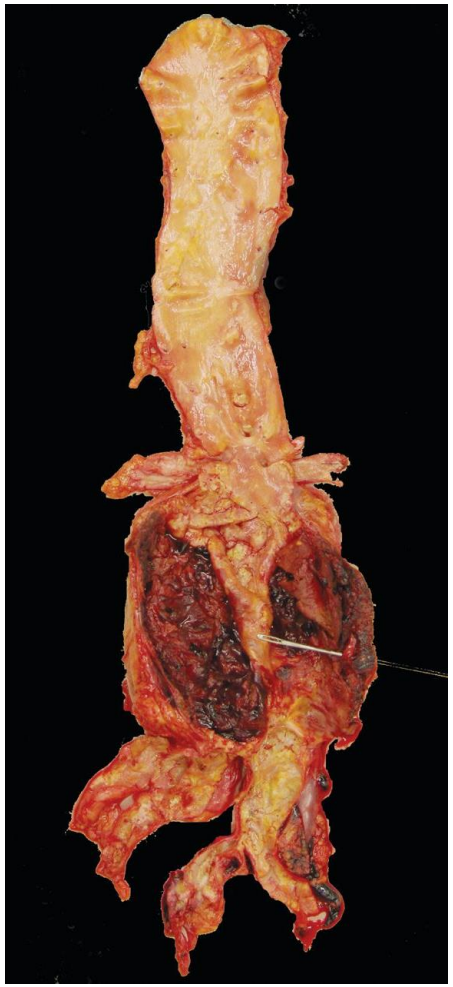
ATHEROSCLEROSIS
Atherosclerosis means thickening and hardening of medium sized vessel due to involvement of intima.

Atherosclerosis term is derived from Greek word “Athero” means “gruel or porridge” and “sclerosis” means “hardening”.

Incidence – high in developed countries and low in Africa, Asia, Central and south America
Sites – large and medium sized arteries are involved. Most commonly involved are aorta, coronary arteries, carotid artery and iliac arteries

What is Atherosclerosis?

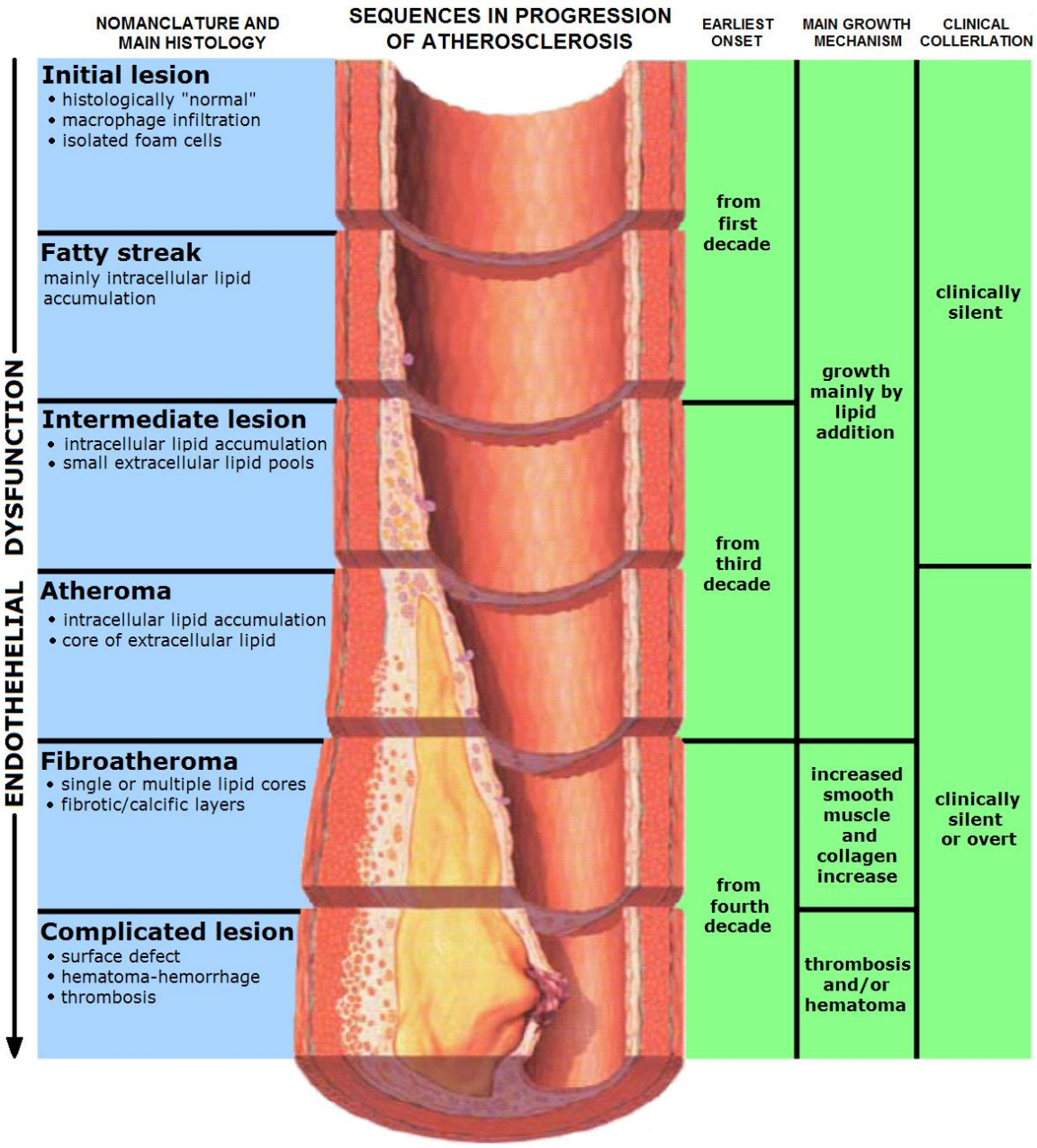


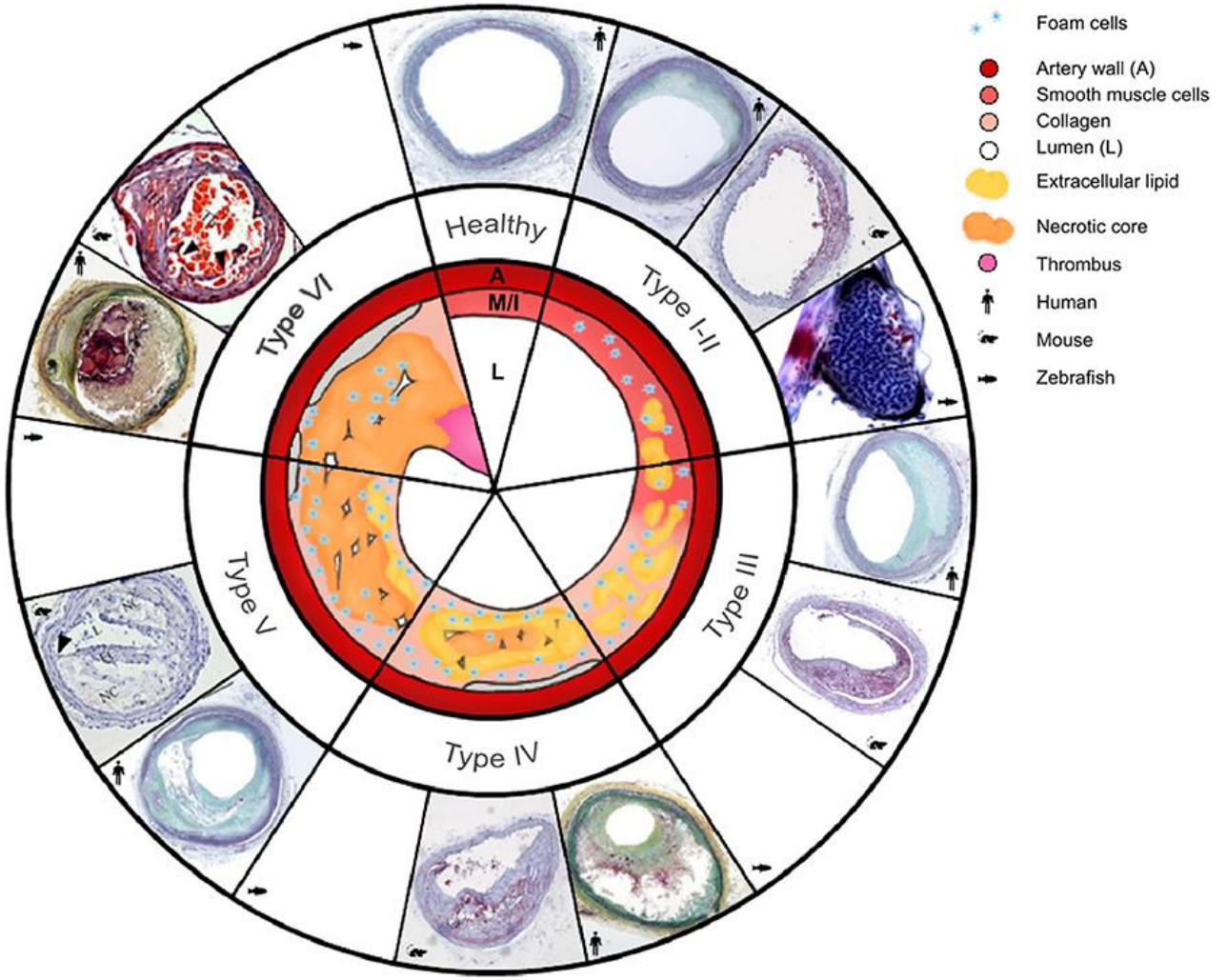


The wall of the artery develops abnormalities, called lesions.
These lesions may lead to narrowing due to the buildup of atheromatous plaque.

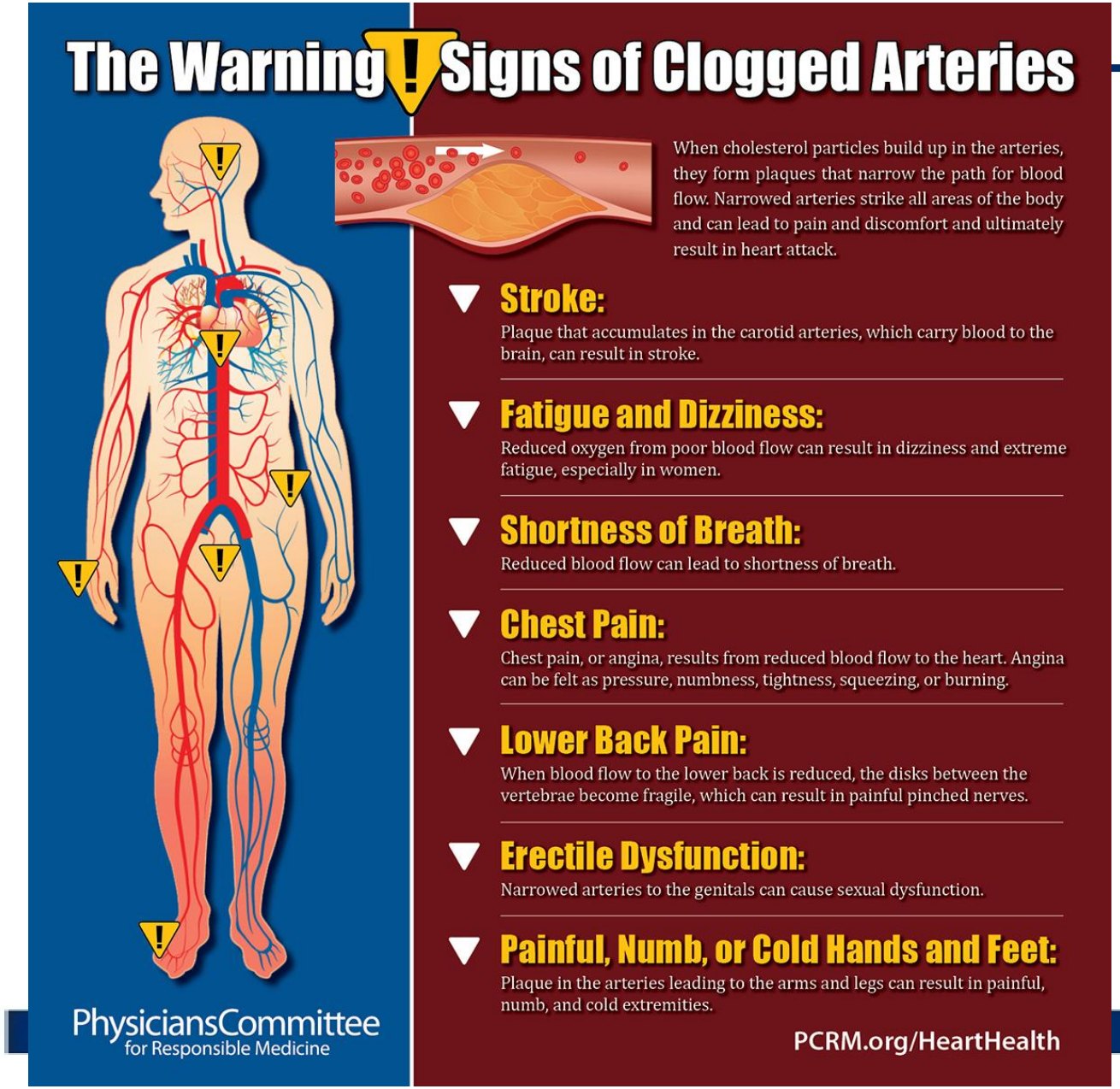
Plaque is made up of fat, cholesterol, calcium, and other substances found in the blood.[7]
The narrowing of arteries limits the flow of oxygen-rich blood to parts of the body.

Stages of atherosclerosis



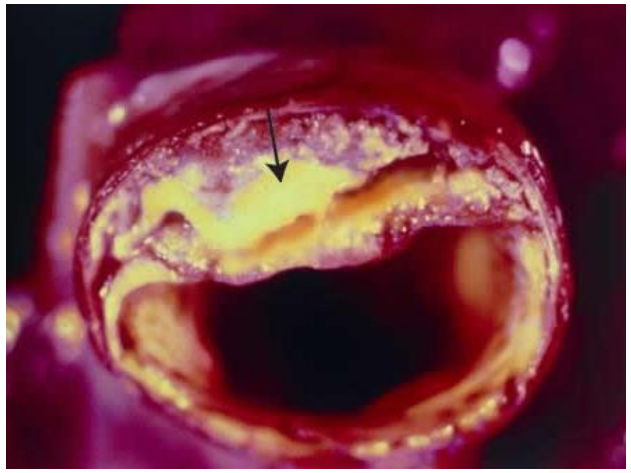


Symptoms of atherosclerosis



Initially, there are generally no symptoms.[1] When severe, it can result in coronary artery disease, stroke, peripheral artery disease, or kidney problems, depending on which arteries are affected.[1] Symptoms, if they occur, generally do not begin until middle age.[3]

Atherosclerosis



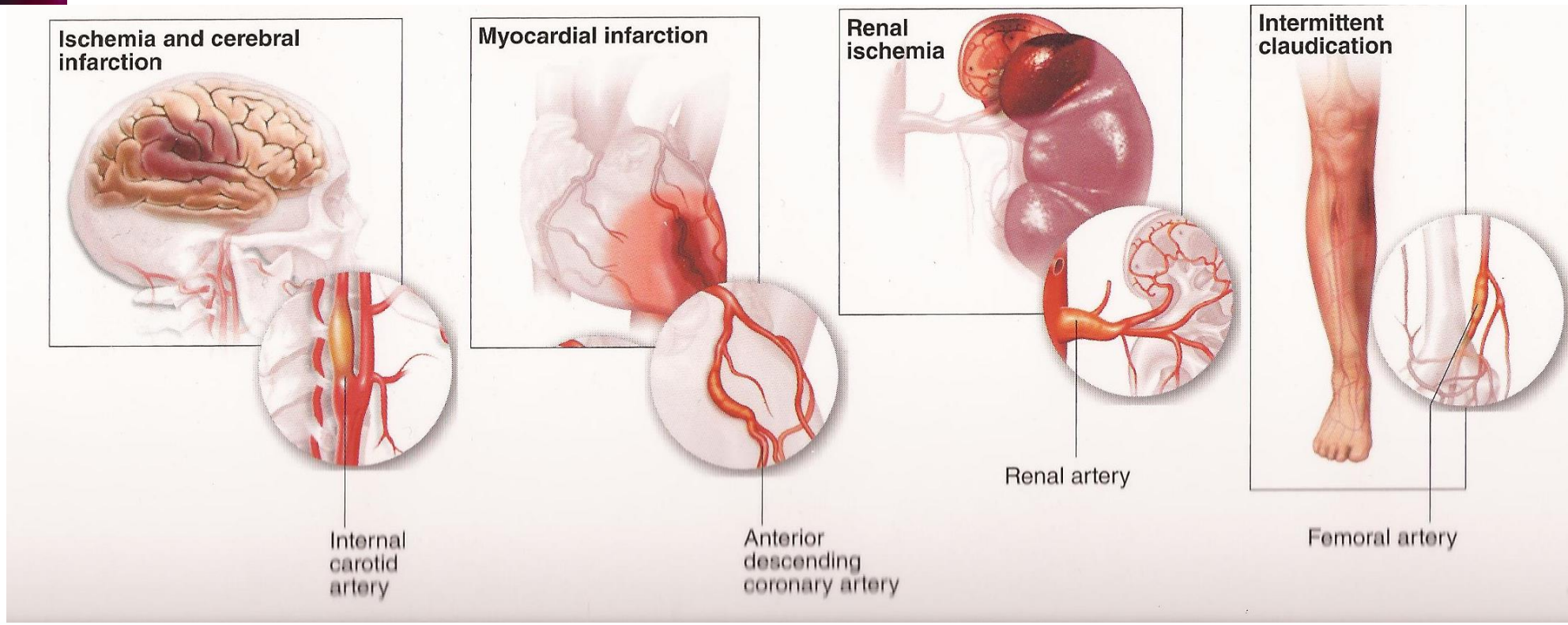
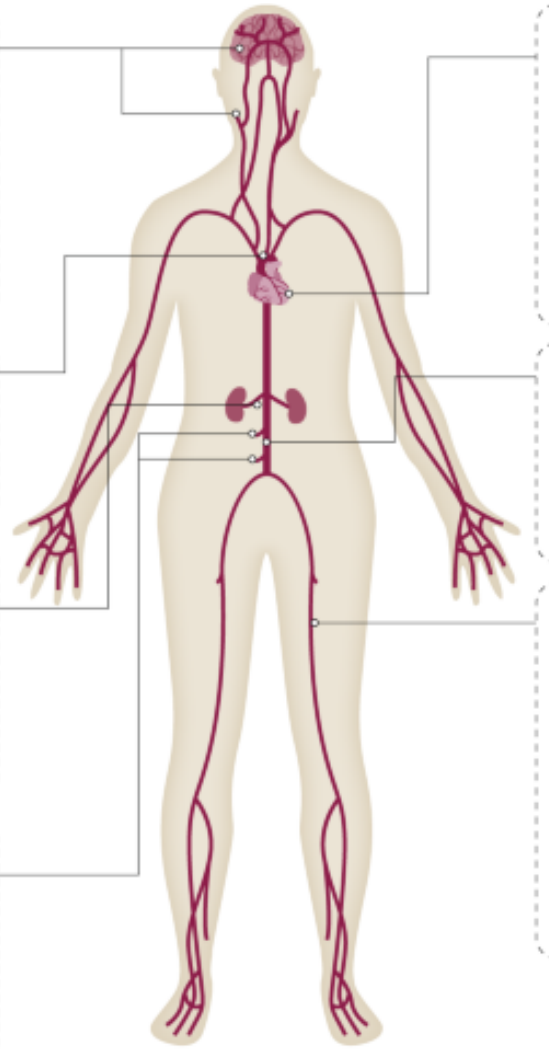


Fig. Atherosclerosis complications. Dr Philip Barlow Mills FCP (SA).

Atherosclerosis



Carotid arteries and cerebral arteries

- Stroke
- Transient ischaemic attack (TIA)
- Recurrent TIAs
- Vascular dementia

Thoracic aorta

- Aortic rupture
- Aortic dissection

Renal arteries

- Renal artery occlusion (rare)
- Worsening renal function
- Renovascular hypertension

Superior and inferior mesenteric arteries

- Acute mesenteric ischaemia
- Chronic mesenteric ischaemia
- Abdominal angina

Coronary arteries

- Acute coronary syndromes (unstable angina and myocardial infarction)
- Stable angina
- Silent ischaemia

Abdominal aorta

- Aortic occlusion (rare)
- Aortic aneurysm

Peripheral arteries (aortoiliac, common or superficial femoral arteries; popliteal, tibial or peroneal arteries)

- Acute peripheral arterial occlusion
- Chronic limb ischaemia
- Intermittent claudication

Acute presentation
Chronic presentation

Atherosclerosis: Diagnosis

Tests and diagnosis

Doctors may find signs of narrowed, enlarged or hardened arteries during a physical exam. These include:

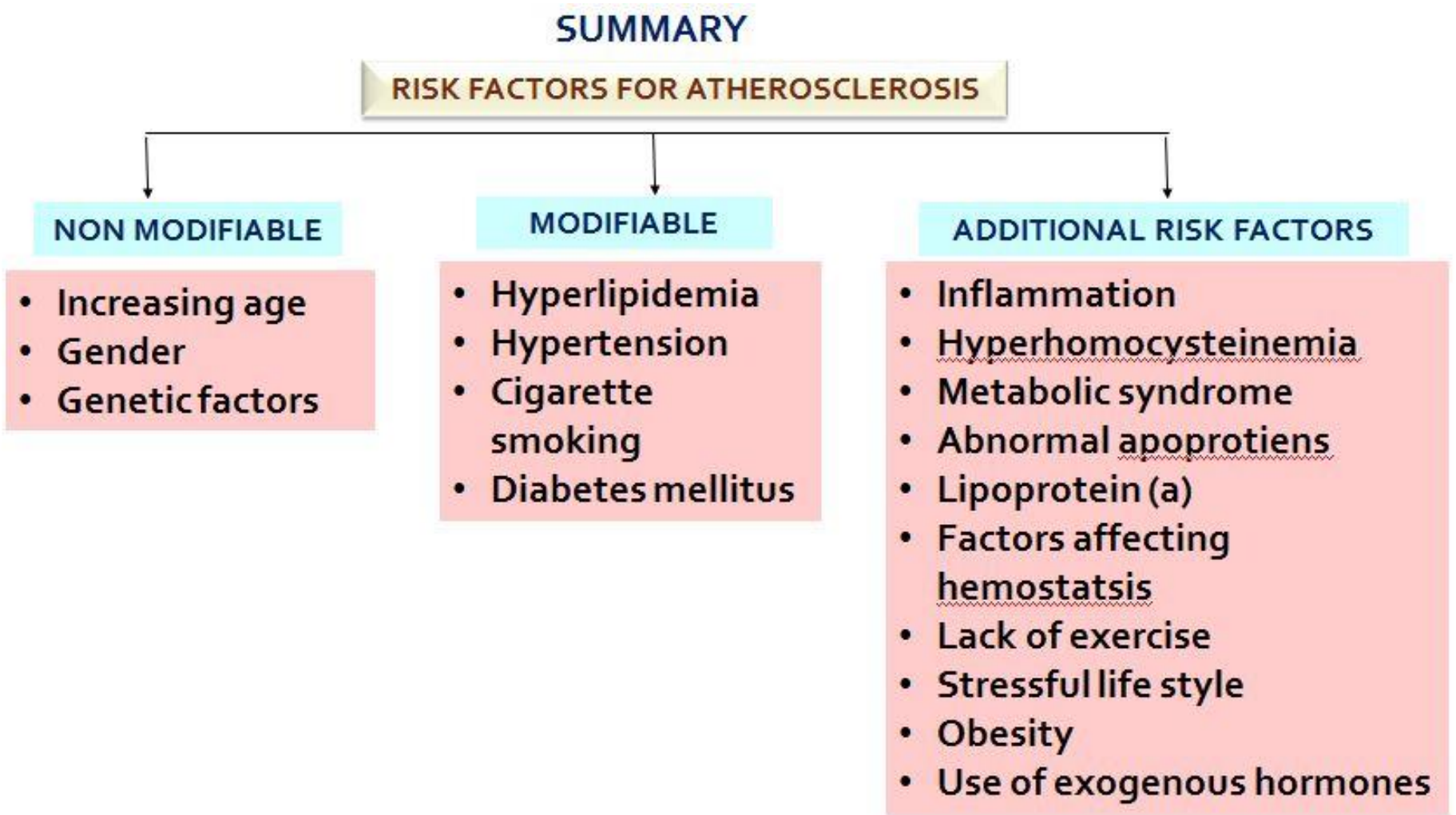
- A weak or absent pulse below the narrowed area of the artery
- Decreased blood pressure in an affected limb
- Whooshing sounds (bruits) over the arteries, heard with a stethoscope
- Signs of a pulsating bulge (aneurysm) in the abdomen or behind knee
- Evidence of poor wound healing in the area where blood flow is restricted

Tests and diagnosis

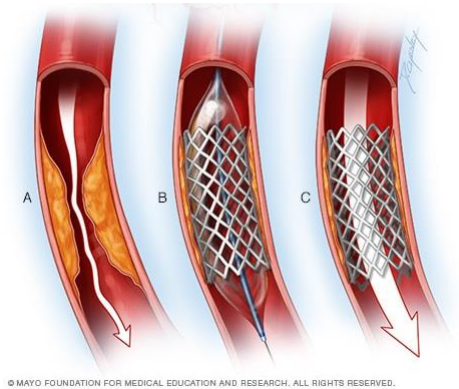
Depending on the results of the physical exam, doctors may suggest one or more diagnostic tests, including:

- **Blood tests.**
- **Doppler ultrasound**
- **Ankle-brachial index.**
- **Other imaging tests.**
- **Angiogram.**
- **Electrocardiogram (ECG).**

Risk factors of atherosclerosis

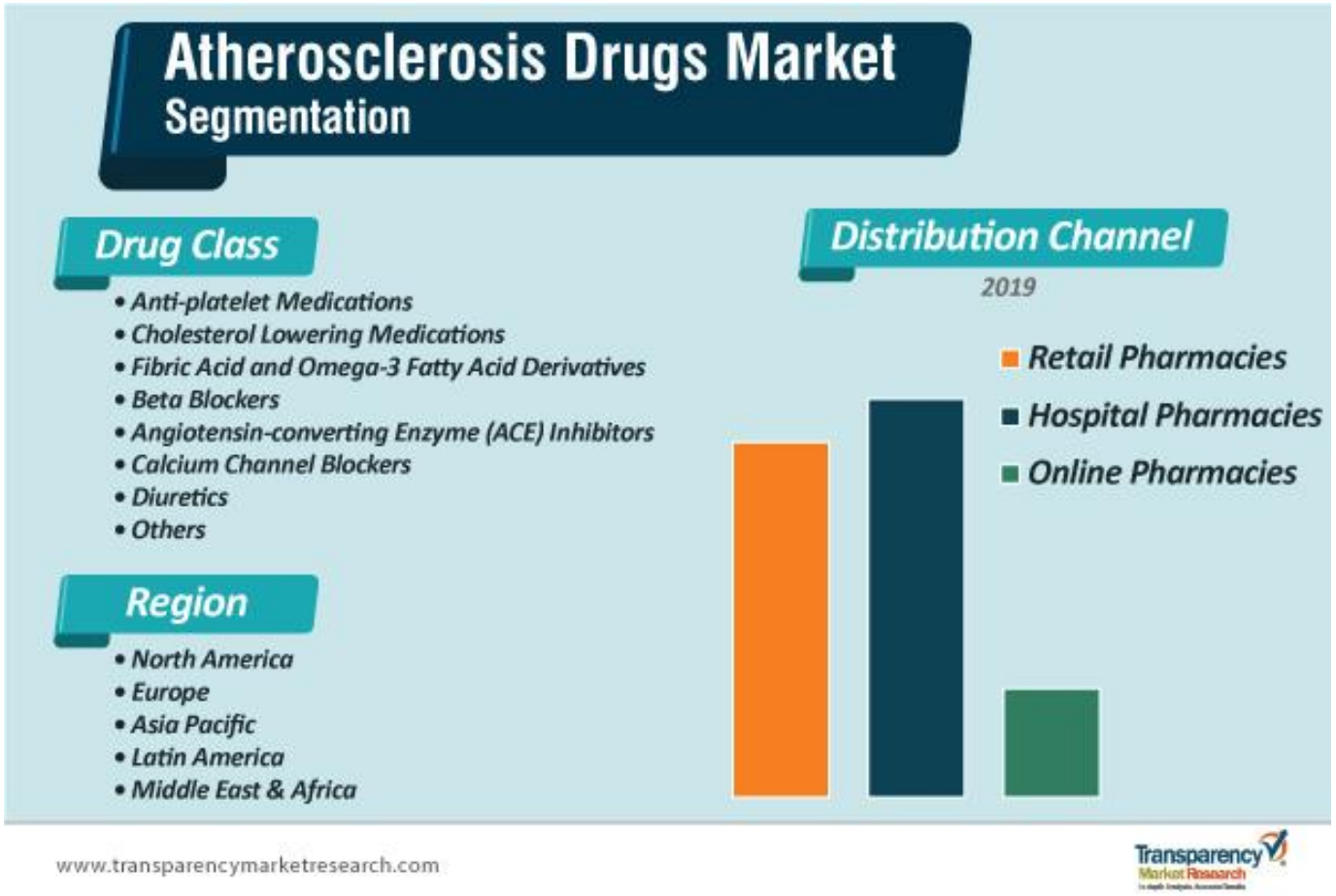


Treatment of atherosclerosis



Treatments and drugs

- **Thrombolytic therapy.** If you have an artery that's blocked by a blood clot, your doctor may insert a clot-dissolving drug into your artery at the point of the clot to break it up.
- **Bypass surgery.** Your doctor may create a graft bypass using a vessel from another part of your body or a tube made of synthetic fabric. This allows blood to flow around the blocked or narrowed artery.

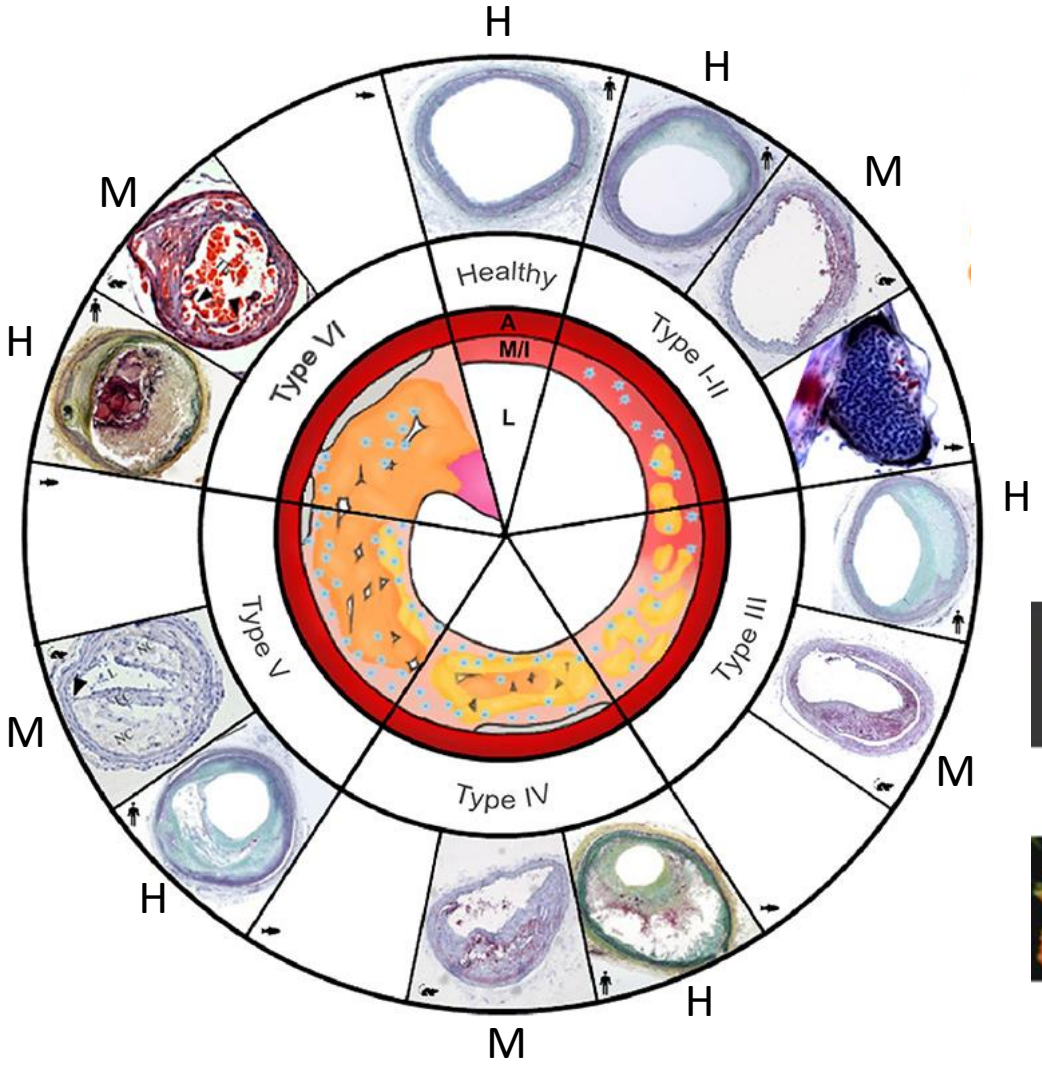


Atherosclerosis

Clinical Trials (2012)

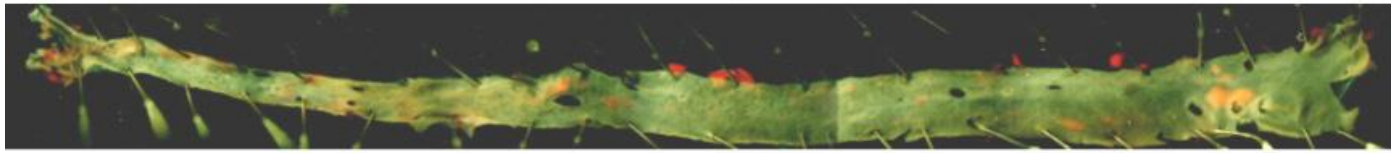
Clinical trial acronym	Clinical trial name	Drugs tested	Refs
ACCORD	Action to control cardiovascular risk in diabetes	Multiple diabetic agents	17
AFCAPS/TexCAPS	Air Force/Texas coronary atherosclerosis prevention study	Statins	31
ARISE	Aggressive reduction of inflammation stops events	Succinobucol (AGI-1067)	40
CARE	Cholesterol and recurrent events	Pravastatin (Pravachol; Bristol-Myers Squibb)	30
CIRT	Cardiovascular inflammation reduction trial	Methotrexate	81
ENHANCE	Simvastatin with or without ezetimibe in familial Hypercholesterolaemia	Simvastatin, ezetimibe (Ezetrol; Merck)	16
IBIS-2	Integrated biomarker imaging study 2	Darapladib	46
ILLUMINATE	Investigation of lipid level management to understand its impact in atherosclerotic events	Torcetrapib	15
JUPITER	Justification for the use of statin in prevention: an intervention trial evaluating rosuvastatin	Rosuvastatin (Crestor; AstraZeneca)	22
MRC-ILA-HEART	Medical research council interleukin-1 receptor antagonist — HEART study	Interleukin-receptor 1 antagonist	85
PROVE IT-TIMI 22	The pravastatin or atorvastatin evaluation and infection therapy thrombolysis in myocardial infarction 22 trial	Pravastatin, atorvastatin (Lipitor; Pfizer)	19
SOLID-TIMI 52	The stabilization of plaques using darapladib — thrombolysis in myocardial infarction 52 trial	Darapladib ClinicalTrials.gov identifier: NCT01000727	
STABILITY	The stabilization of atherosclerotic plaque by initiation of darapladib therapy trial	Darapladib	86
VISTA-16	Vascular inflammation suppression to treat acute coronary syndrome for 16 weeks	Varespladib ClinicalTrials.gov identifier: NCT01130246	

Stages of atherosclerosis: Human = Mouse

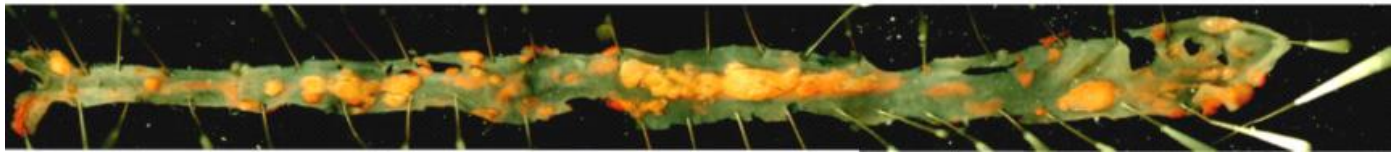


APOE and LDLR are important for the clearance of cholesterol and triglyceride-rich lipoprotein particles from the blood.

C57BL6/J mouse aorta – 26 weeks of age



ApoE KO mouse aorta – 26 weeks of age

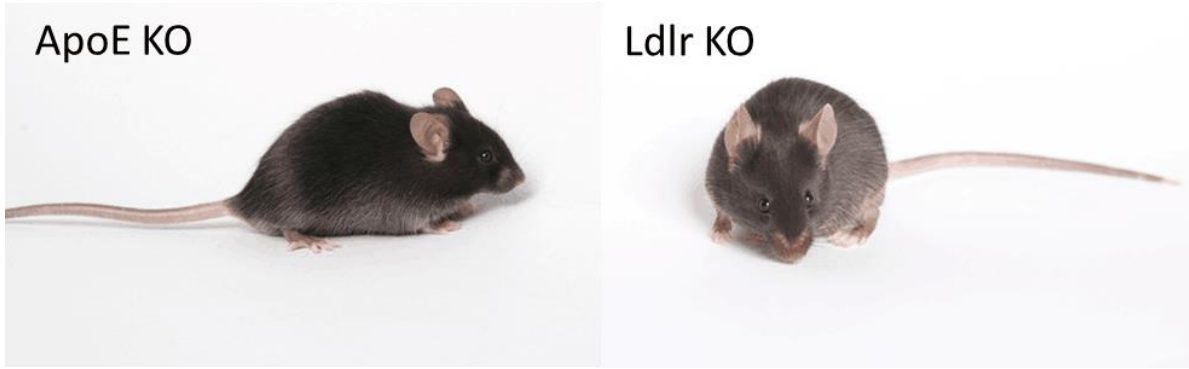


+ HFD

Mouse models of Atherosclerosis

Aortic arch

Model	Lipid profile	Plaque distribution and characteristics (20 weeks WD)	Advantages & limitations
ApoE$^{-/-}$ Disruption of the ApoE gene	**Plasma cholesterol:** 400-600 mg/dl on ND >1000 mg/dl on WD **Lipoproteins:** ↑↑ VLDL ↑ LDL ↓ HDL	**Fibrous plaques:** Smooth muscle cells Extracellular matrix Inflammatory cells Necrotic core	⊕ Develops atherosclerosis on ND ⊖ No human-like lipid profile ⊖ ApoE plays a role in inflammation → influence plaque development ⊖ No spontaneous plaque rupture, thrombosis and complications
LDLr$^{-/-}$ Disruption of the LDL receptor gene	**Plasma cholesterol:** 200-300 mg/dl on ND >1000 mg/dl on WD **Lipoproteins:** ↑ VLDL ↑↑ LDL = HDL	**Fibrous plaques:** Smooth muscle cells Extracellular matrix Inflammatory cells Necrotic core	⊕ Human-like lipid profile (LDL) ⊕ Functional ApoE → no impact on inflammation ⊖ Complex lesion development requires a WD ⊖ No spontaneous plaque rupture, thrombosis and complications
ApoE$^{-/-}$ LDLr$^{-/-}$ Disruption of the ApoE and the LDL receptor gene	**Plasma cholesterol:** 400-600 mg/dl on ND >1000 mg/dl on WD **Lipoproteins:** ↑↑ VLDL ↑↑ LDL ↓ HDL	**Fibrous plaques:** Smooth muscle cells Extracellular matrix Inflammatory cells Necrotic core	⊕ Develops atherosclerosis on ND ⊖ No spontaneous plaque rupture, thrombosis and complications
ApoE3-Leiden ApoE3-Leiden mutation via DNA construct (ApoE, ApoC1) from the ApoE3-Leiden proband	**Plasma cholesterol:** 100-200 mg/dl on ND >1000 mg/dl on WD **Lipoproteins:** ↑↑ VLDL ↑ LDL ↑ HDL (only on WD)	**Fibrous plaques:** Smooth muscle cells Extracellular matrix Inflammatory cells Necrotic core	⊕ Functional ApoE → no impact on inflammation ⊖ Complex lesion development requires a WD ⊖ No spontaneous plaque rupture, thrombosis and complications



	Treatment	Lipid profile	plauqe characteristics
Apoe-/- mice [27]	HFHC diet (21% fat, 0.15% cholesterol) for 20 weeks	TC:>1000mg/dL on HFHC diet 300-500mg/dl on chow diet Lipoprotein: VLDL↑ CM↑ HDL↓	 Large lesions Fibroatheroma lesions: necrotic core; fibrous cap; foam cell; smooth muscle cell extracellular matrix
Ldlr-/- mice [25]	Atherogenic diet (7.5% fat, 1.25% cholesterol,7.5% casein,0.5% cholic acid) for 24 weeks	TC:>1000mg/dL on atherogeic diet 400-600mg/dL on chow diet Lipoprotein: LDL↑↑ VLDL↑ HDL	 Large lesions Fibroatheroma lesions: necrotic core; fibrous cap; foam cell; smooth muscle cell extracellular matrix

Risk factors of atherosclerosis

CAD: Coronary Artery Disease

Table 2. Consistency of Human CAD Risk Factors in Atherosclerosis Mouse Models

Concordant Risk Factors	Effect on Atherosclerosis	Reference
Hypercholesterolemia	↑	Plump et al., 1992; van Ree et al., 1994
Elevated lipoprotein levels: LDL	↑	Huszar et al., 2000; Powell-Braxton et al., 1998
Elevated lipoprotein levels: VLDL	↑	Knouff et al., 2004; VanderLaan et al., 2009
Elevated lipoprotein levels: HDL	↓	Bérard et al., 1997; Feig et al., 2014;
Elevated lipoprotein levels: LPA	↑	Callow et al., 1995; Schneider et al., 2005; Pedersen et al., 2010
Hypertriglyceridemia	↑	Voyiaziakis et al., 1998
Hypertension	↑	Leong et al., 2015; Weiss et al., 2001; Wiesel et al., 1997
Inflammatory diseases: arthritis	↑	Rose et al., 2013
Inflammatory diseases: lupus	↑	Ma et al., 2008
Inflammatory diseases: psoriasis	↑	Karbach et al., 2014
Smoking	↑	Boué et al., 2012; Gairola et al., 2001; Lietz et al., 2013
Air pollution	↑	Araujo, 2010; Soares et al., 2009; Sun et al., 2005
T1D	↑	In't Veld, 2014; Kunjathoor et al., 1996; Shen and Bornfeldt, 2007
T2D	↑	Jun et al., 2011; King, 2012; Renard et al., 2004; Schreyer et al., 1998
Aging	↑	Merat et al., 2000; Rosenfeld et al., 2000
Distress	↑	Kumari et al., 2003; Najafi et al., 2013; Roth et al., 2015
TMAO	↑	Gregory et al., 2015; Hartiala et al., 2014; Wang et al., 2011
Thrombosis	↑	Schafer et al., 2003
Lack of physical activity	↑	Meissner et al., 2011; Pellegrin et al., 2009
Bacterial presence	↑	Gibson et al., 2004; Lalla et al., 2003
Renal failure	↑	Bro et al., 2003; Hewitson et al., 2015; Neven and D'Haese, 2011
Metabolic syndrome	↑	Kennedy et al., 2010

Human

=

Mouse

Genetics of atherosclerosis: Human vs Mouse

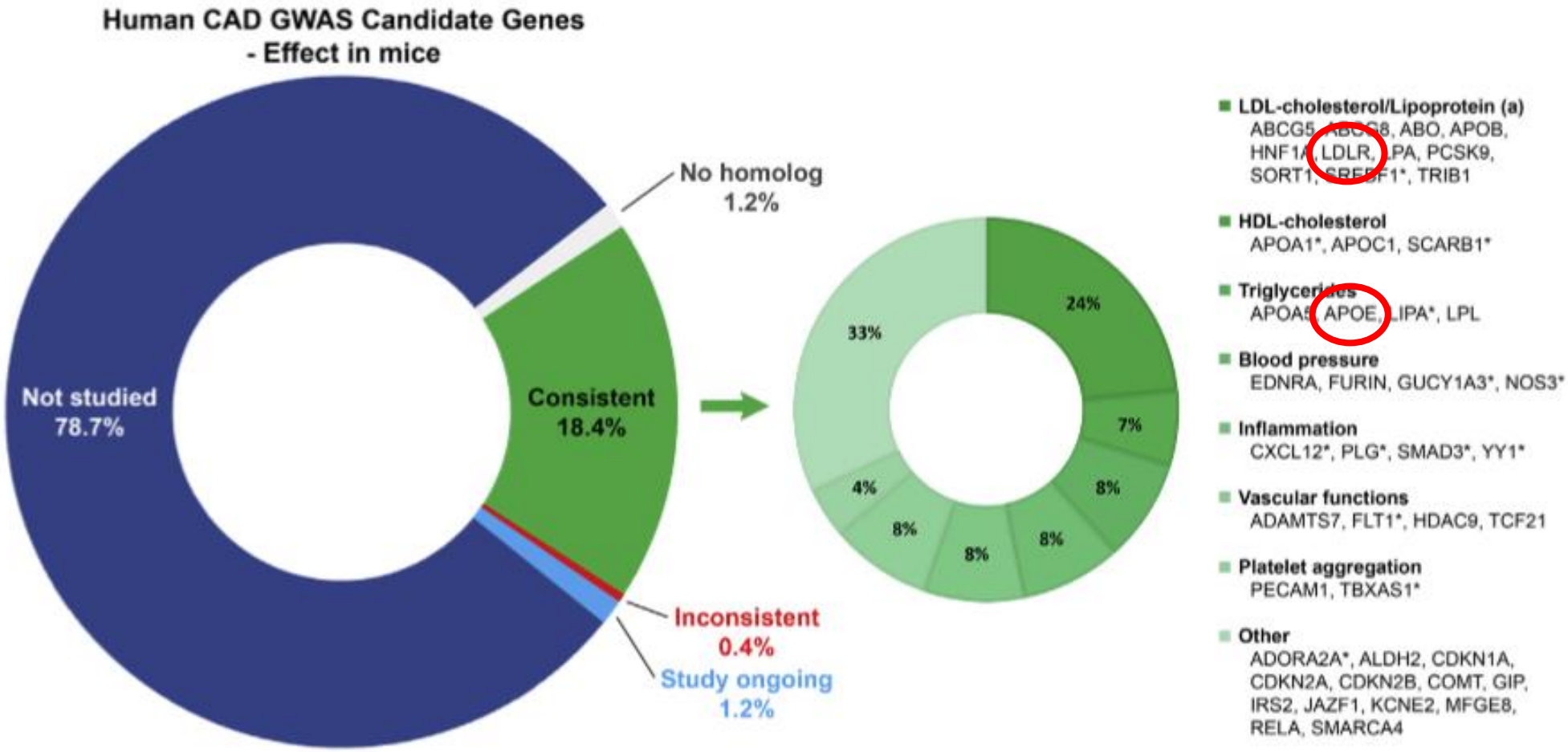


Figure 1. Human CAD GWAS Candidate Genes: Focus on Genes Already Validated in Mice

GWAS: Genomw Wide Association Study

Genetics of atherosclerosis: Human vs Mouse

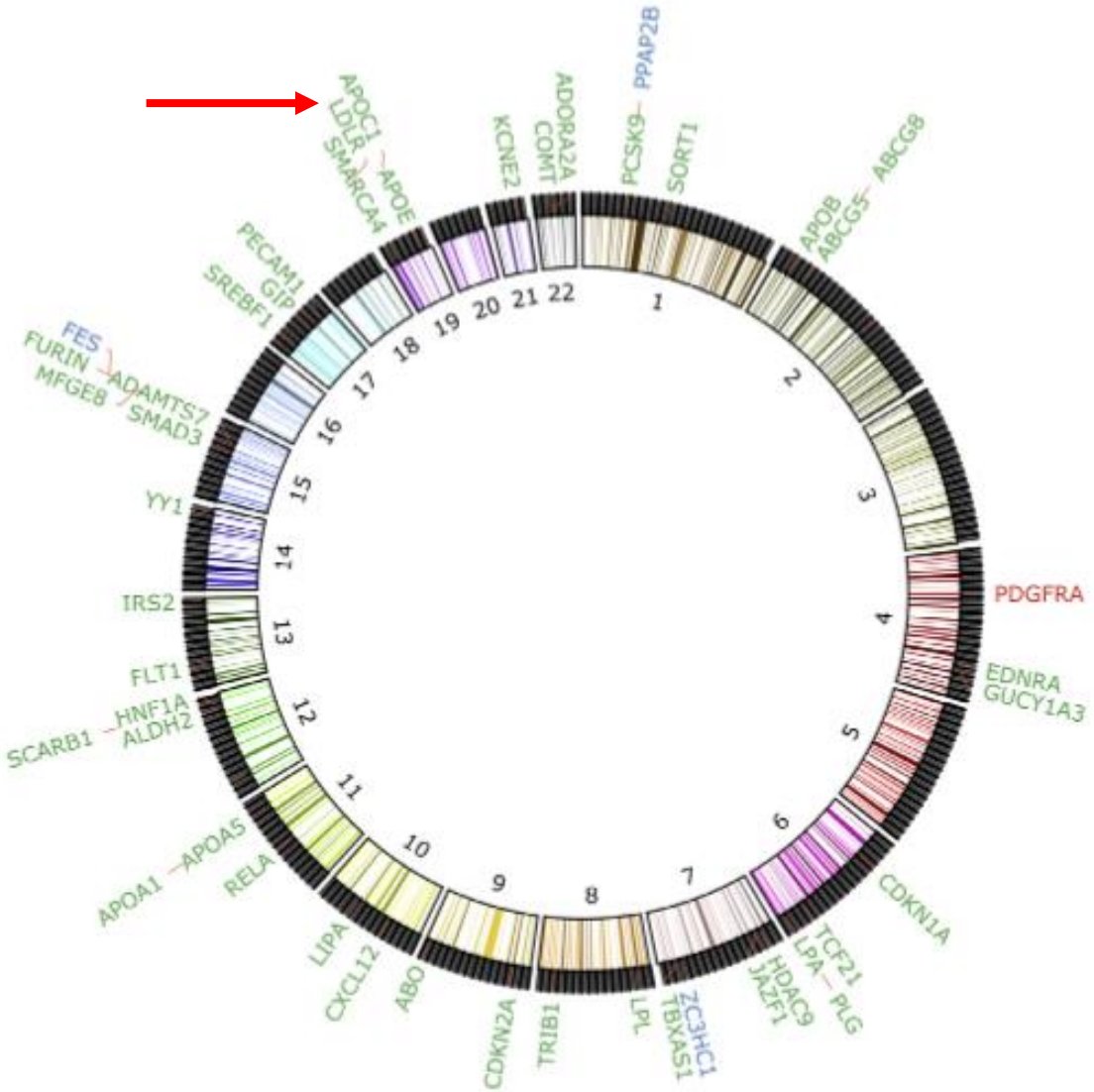
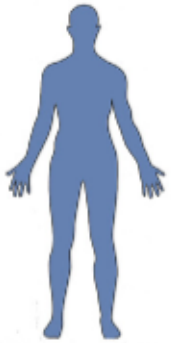


Figure 2. Human CAD GWAS Candidate Genes that Have Been Tested in Mouse Animal Models.
This circular plot shows a fraction of 244 human CAD GWAS candidate genes that have been tested in animal models. The numbers within the circle represent the 22 human autosome pairs. Candidate genes are arranged according to GWAS peak SNPS. Genes labeled green have already been studied in mouse models and show significant effects on atherosclerosis.

Pathways of atherosclerosis: Human vs Mouse

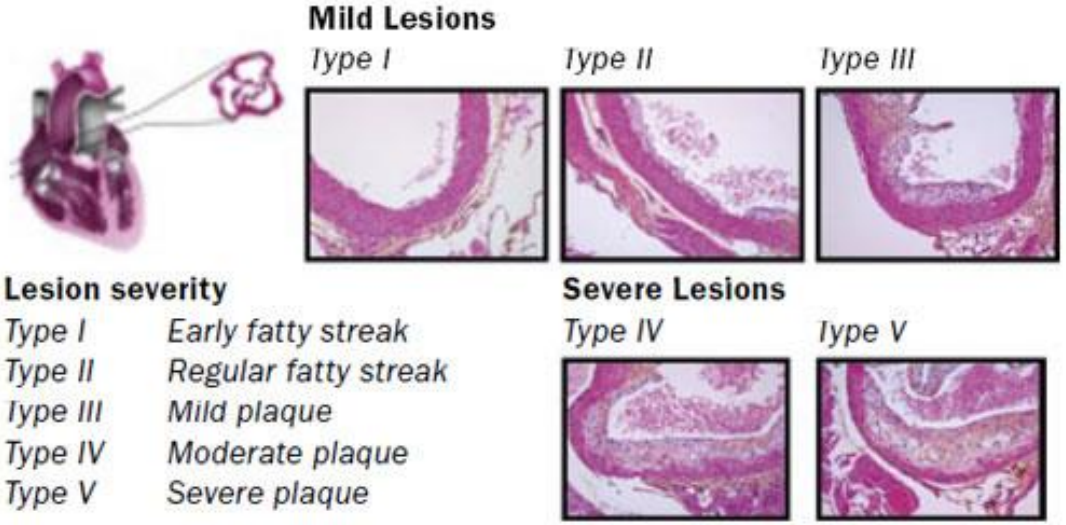


Top ranked mouse Atherosclerosis Pathways		Overlapping human CAD Pathways
Immune system	———	Immune system
Chemokine signaling pathway	———	Chemokine signaling pathway
Class A1 rhodopsin like receptors	———	Class A1 rhodopsin like receptors
Pathways in cancer	———	Pathways in cancer
GPCR ligand binding	———	Class A1 rhodopsin like receptors
Cytokine cytokine receptor interaction	- - - -	Chemokine receptors bind chemokines
Hemostasis	———	Hemostasis
GPCR downstream signaling	- - - -	G beta g. signaling through PI3K gamma
Metabolism of lipids and lipoproteins	———	Metabolism of lipids and lipoproteins
Peptide ligand binding receptors	- - - -	Chemokine receptors bind chemokines
Platelet activation signaling and aggregation	———	Platelet activation signaling and aggregation
Focal adhesion	———	Focal adhesion
TOLL receptor cascades	———	TOLL receptor cascades
G alpha i signaling events	- - - -	Adenylate cyclase inhibitory pathway
Inflammation pathway	———	Cytokine pathway
TGF beta signaling pathway	———	TGF beta signaling pathway
JAK STAT signaling pathway	———	JAK STAT signaling pathway
Lipid digestion mobilization and transport	———	Lipid digestion mobilization and transport
Cytokine signaling in immune system	———	Cytokine signaling in immune system
Signaling by NGF	- - - -	PI3K AKT activation

Fewest overlap to human pathways (%)		Fewest overlap to mouse pathways (%)	
Calcium signaling pathway	(27.5%)	Sulfur amino acid metabolism	(0%)
Amyotrophic lateral sclerosis ALS	(22.6%)	Organic cation anion zwitterion transp.	(0%)
Regulation of gene expr. in B. cells	(20.0%)	Metabolism of polyamines	(0%)
Developmental biology	(18.2%)	Phenylalanine metabolism	(0%)

Figure 3. Top-Ranked Mouse Atherosclerosis Pathways: Overlap with Human CAD pathways

Treatment of atherosclerosis



Lesion severity

Type I	*Early fatty streak*
Type II	*Regular fatty streak*
Type III	*Mild plaque*
Type IV	*Moderate plaque*
Type V	*Severe plaque*

*Atherosclerosis development in APOE*3-Leiden.hu CETP transgenic mice. Classification of lesion phenotype according to AHA*

	ApoE$^{-/-}$		LDLr$^{-/-}$		E3L	
Pharmaceutical Modifiers	chol	athero	chol	athero	chol	athero
Statins	↔	va	va	va	↓	↓
ACE inhibitors	↔	↓	↔	↔	nd	nd
AT-$_1$R antagonists	↔	↓	nd	nd	↓	↓
Statins + hypotensives	va	↓	nd	nd	↓	↓
PPAR agonists						
PPARα	↑	↔	va	↓	↓	↓
PPARγ	↔	↓	va	↓	nd	nd
PPARδ	nd	nd	↔	va	↓	↓
PPARα/γ	va	↓	↔	↓	↓	↓
LXR agonists						
LXRα,β	↓	↓	↓	↓	↑	↓
Miscellaneous						
Ezetimibe	↓	↓	↓	↓	↓	↓
ACAT-inhibitors	↓	↓	nd	nd	↓	↓

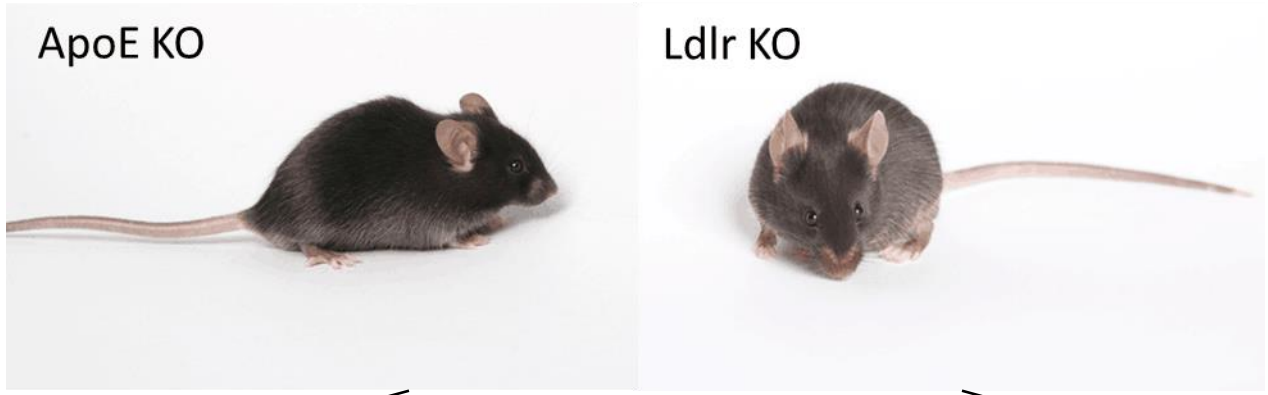
va indicates variable; nd, not determined.

Mouse responds to
similar drugs as humans

Mouse models of Atherosclerosis: Applications

Patho-mechanism

Diagnostics



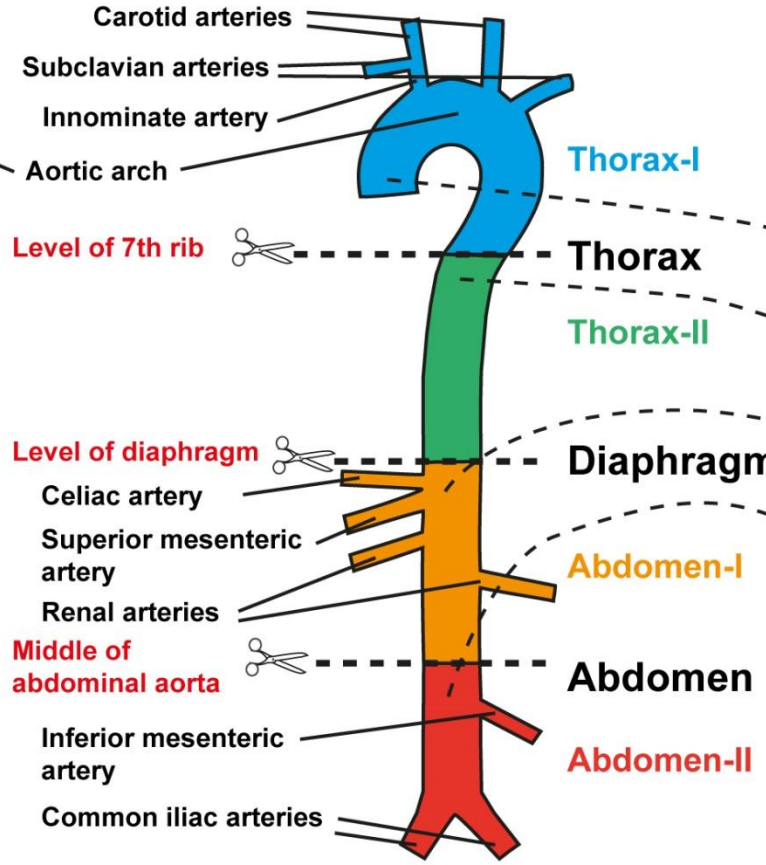
Therapeutics

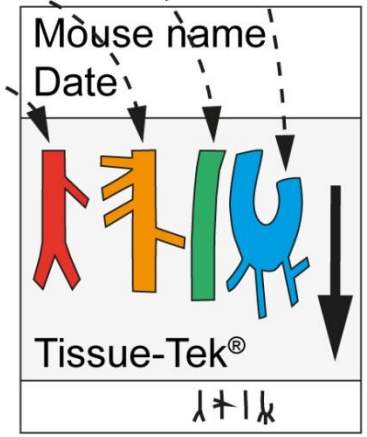
Bio Imaging

Mouse models of Atherosclerosis: Applications

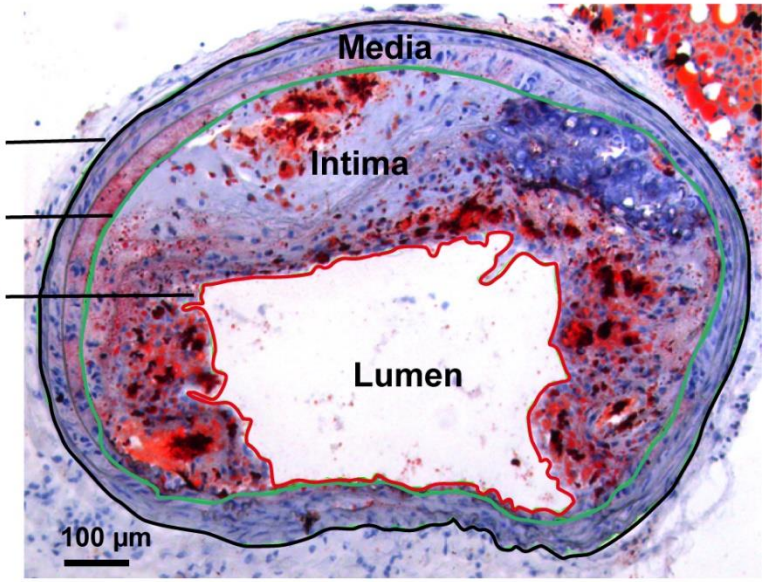
Aorta with adipose tissue

Scheme of aorta parts



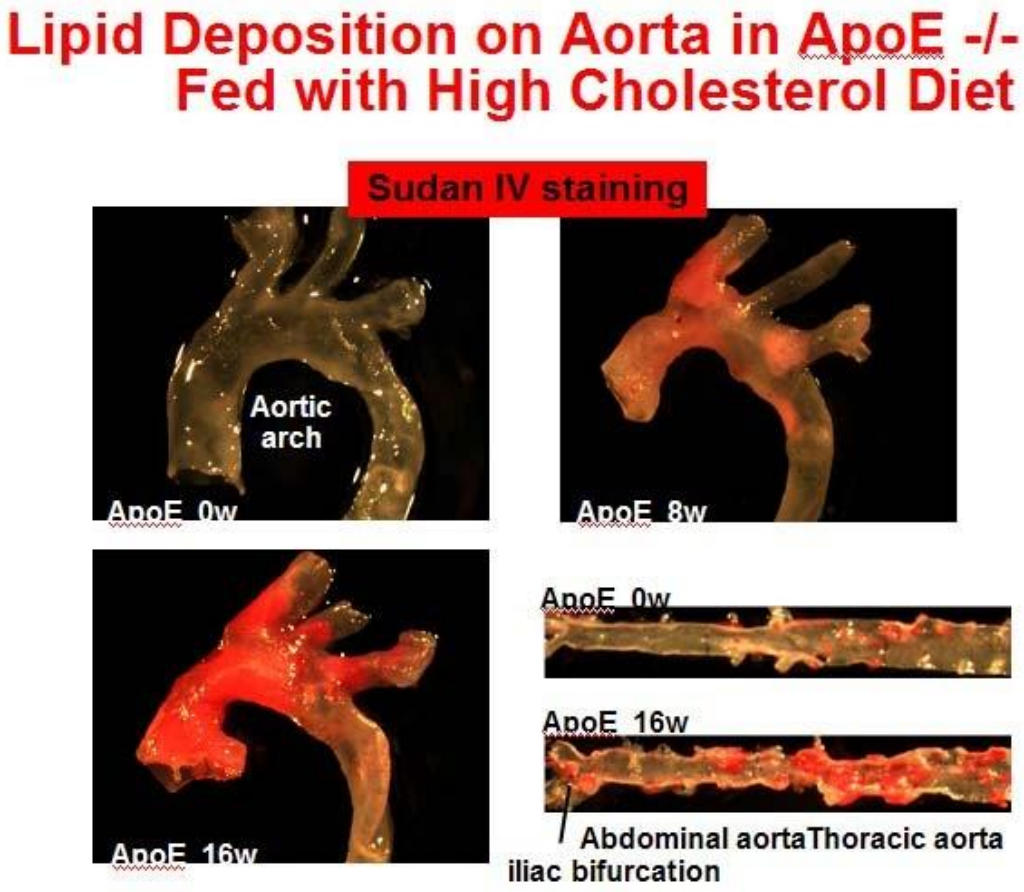


Innominate artery cross section

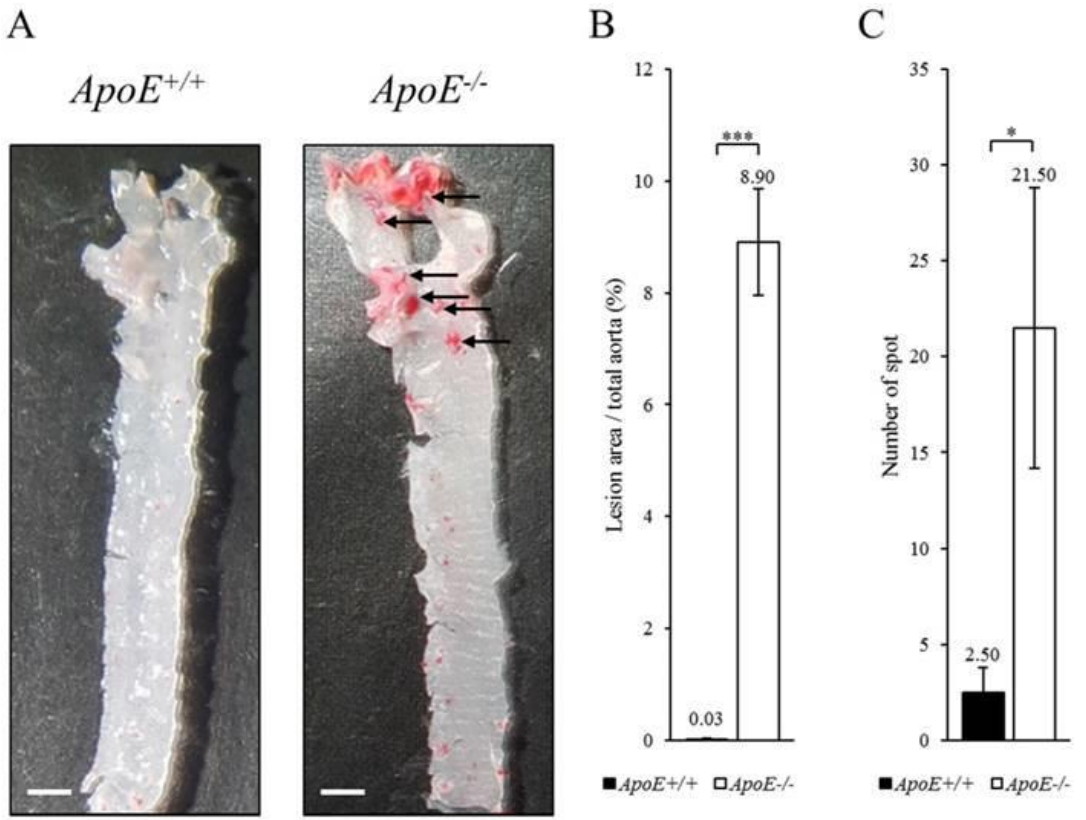


Embedding in Cryomold → Oil Red O / Hematoxylin Staining and Morphometry

Mouse models of Atherosclerosis: Applications

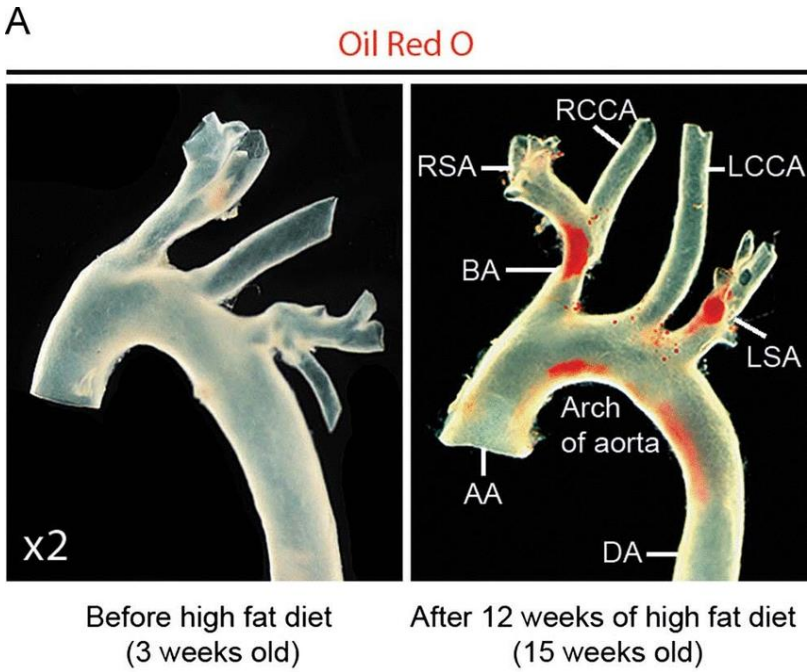


Staining

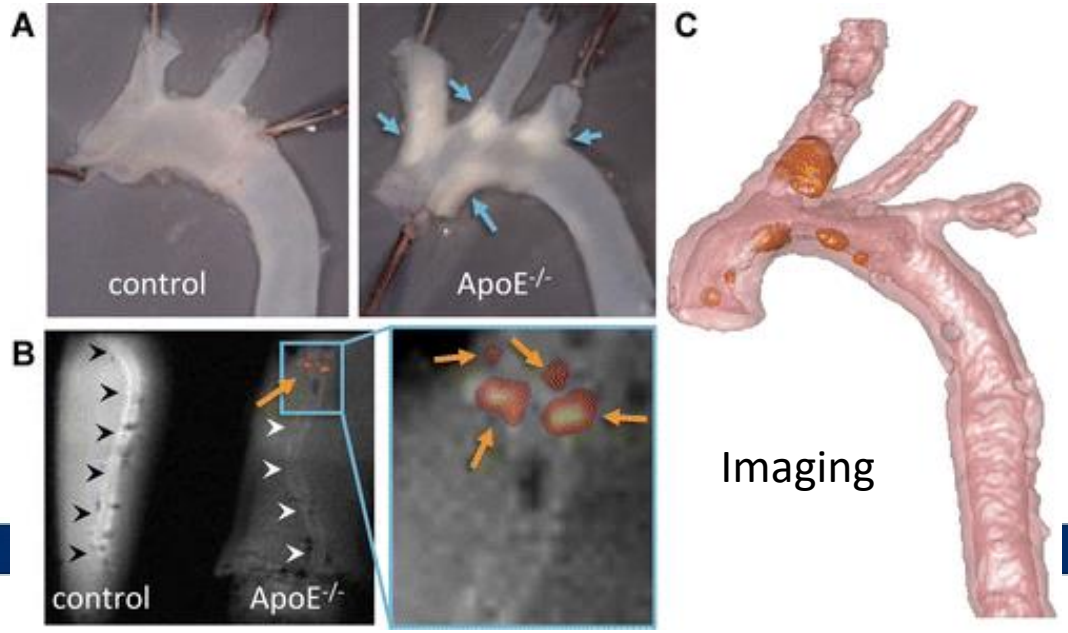


Staining Quantification

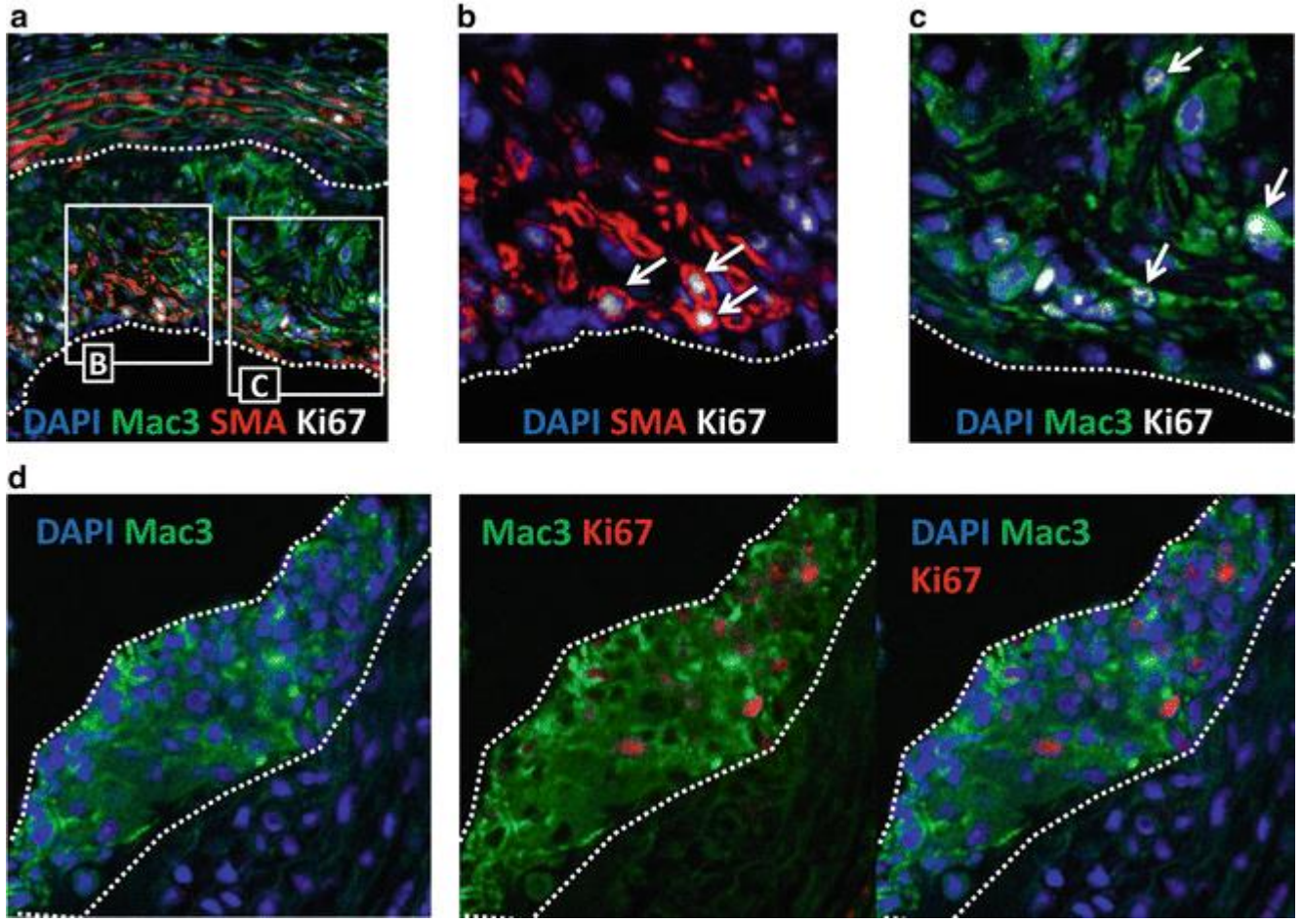
Visualization



Chemical Staining

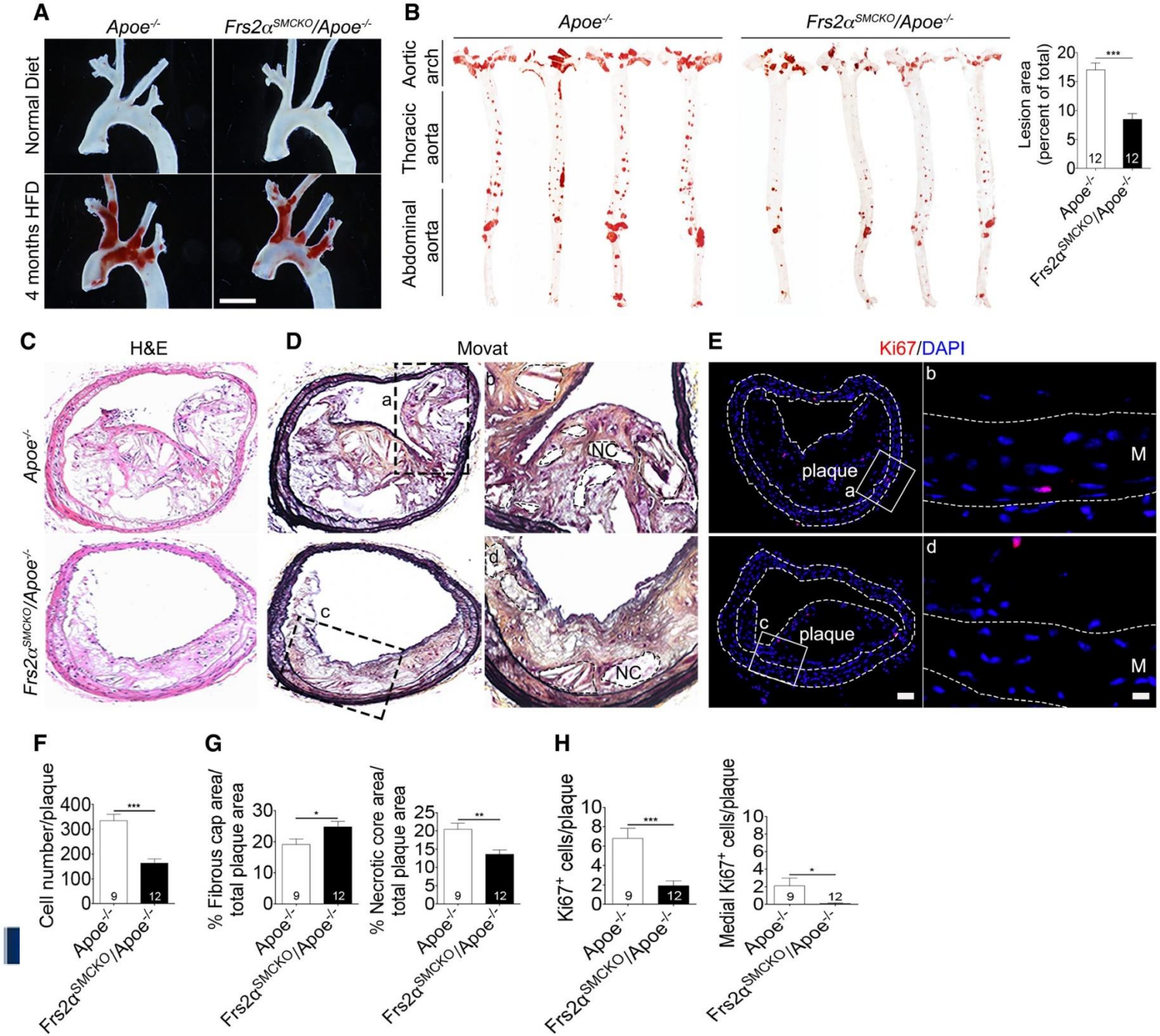


Imaging



Fluorescence

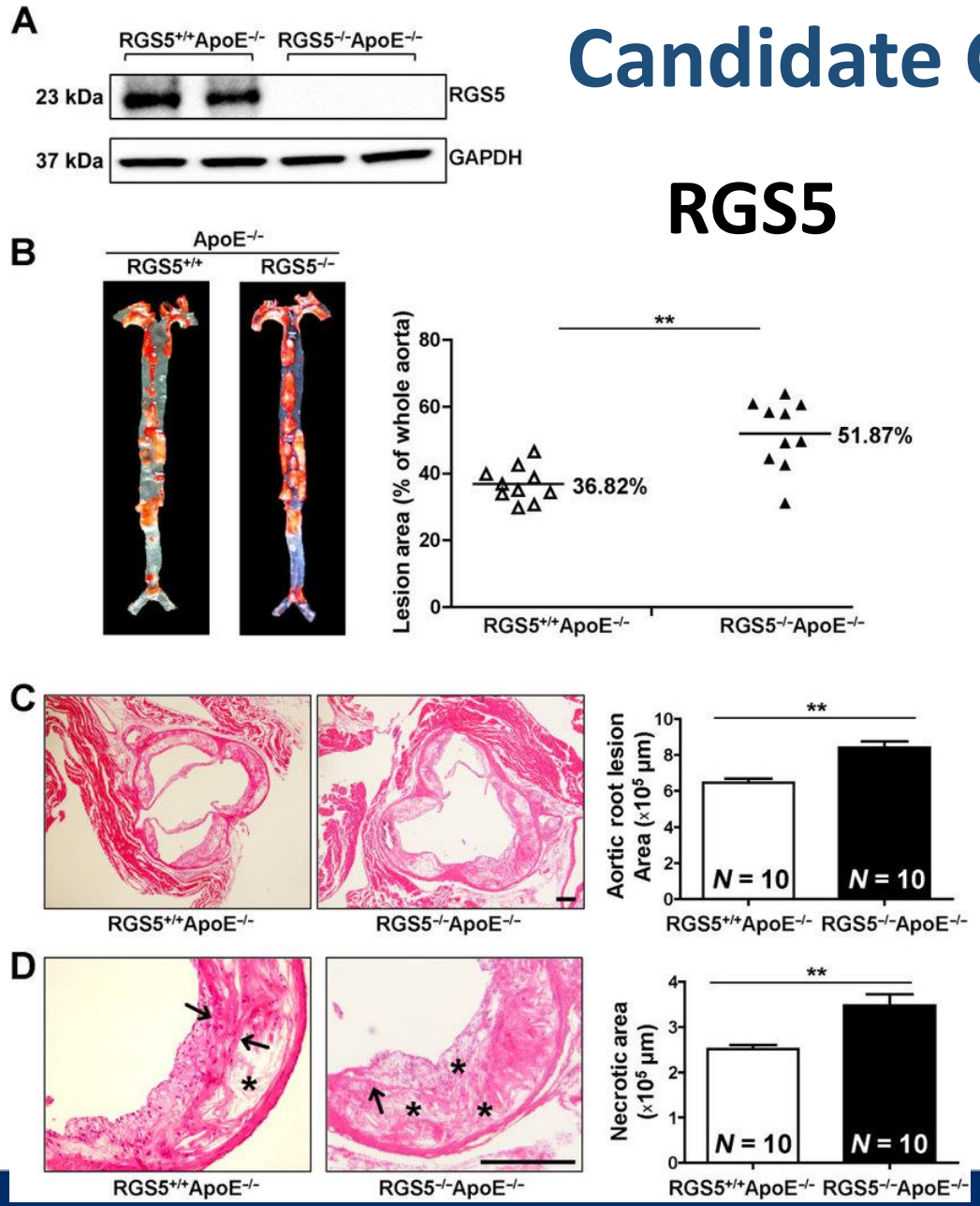
Quantification



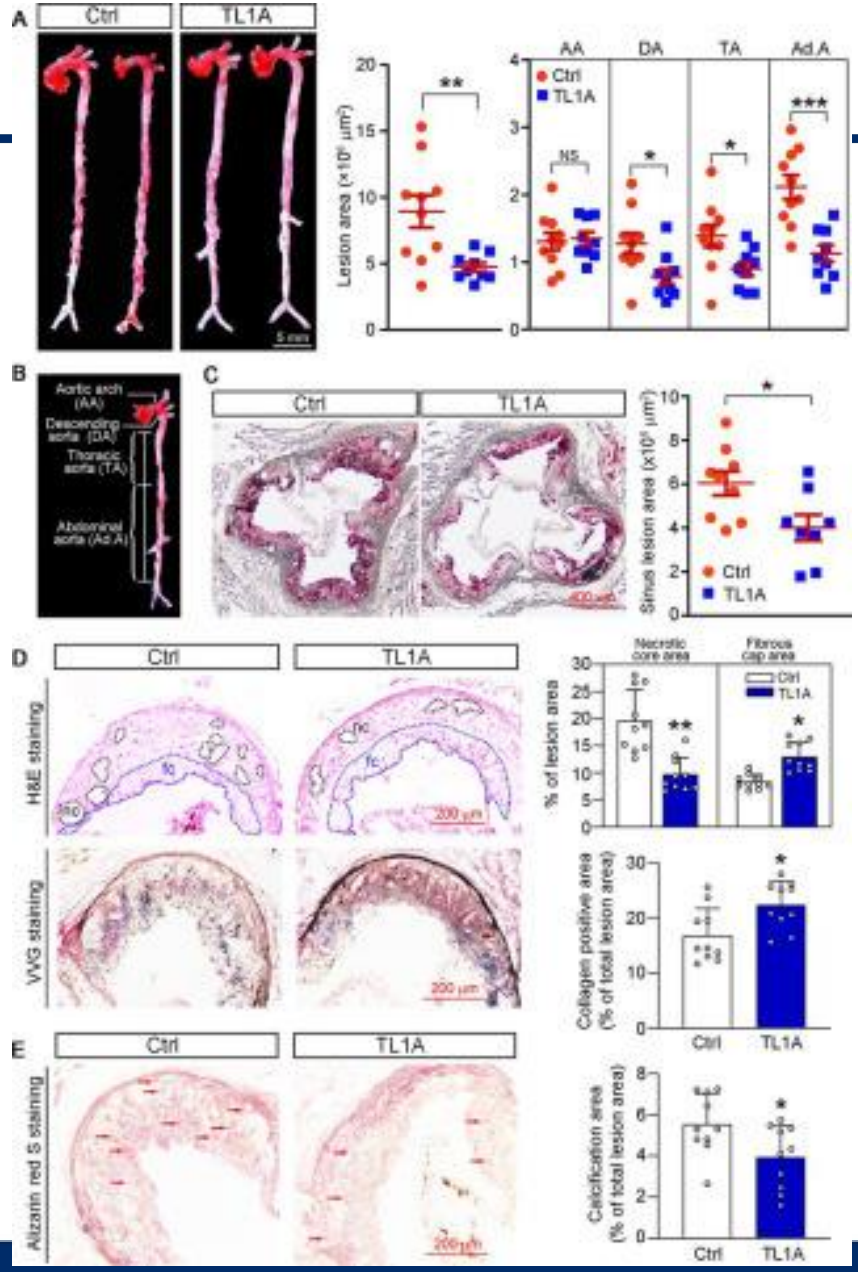
Visualization
vs
Biology
vs
Pathology

Candidate Genes

RGS5



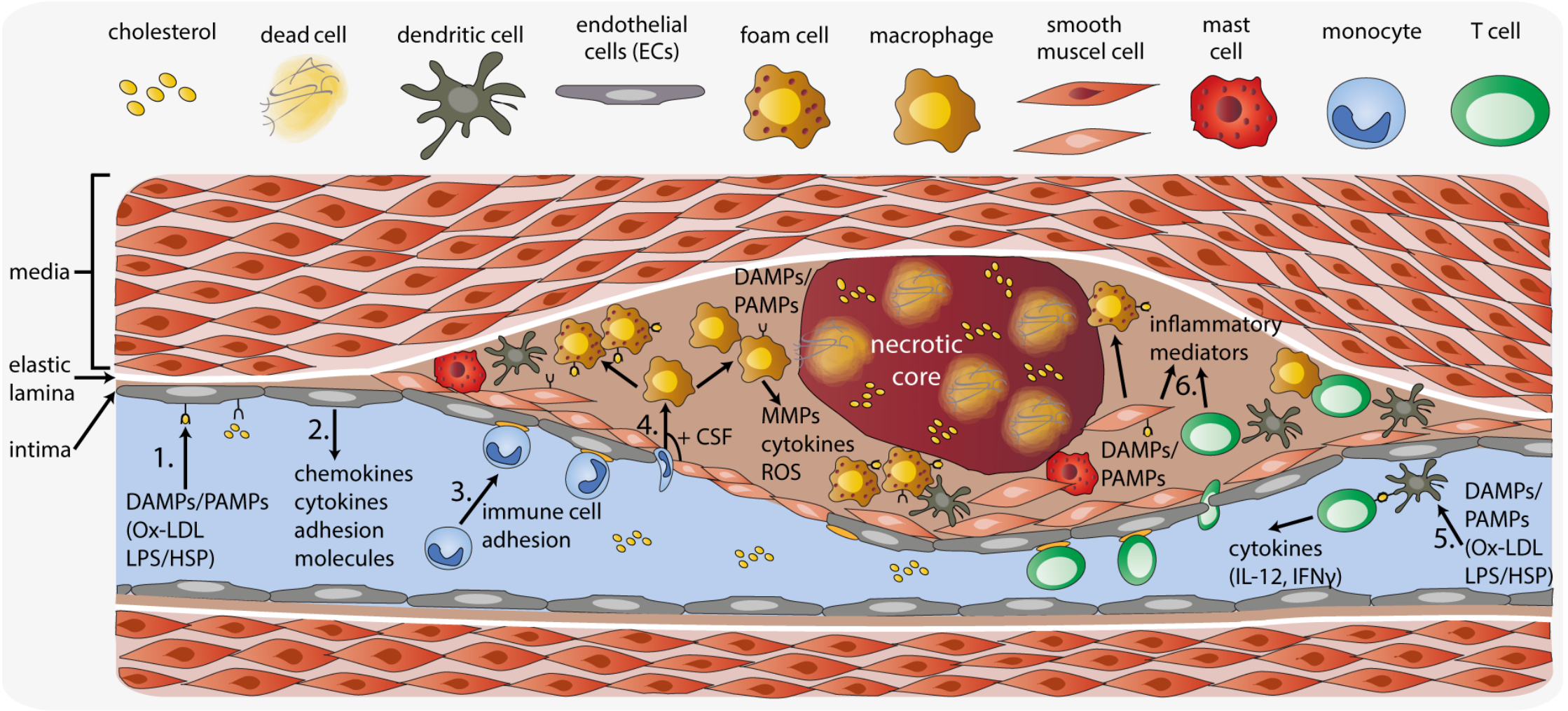
TL1A



Regulator of G protein signaling 5 – SMC vasoconstriction

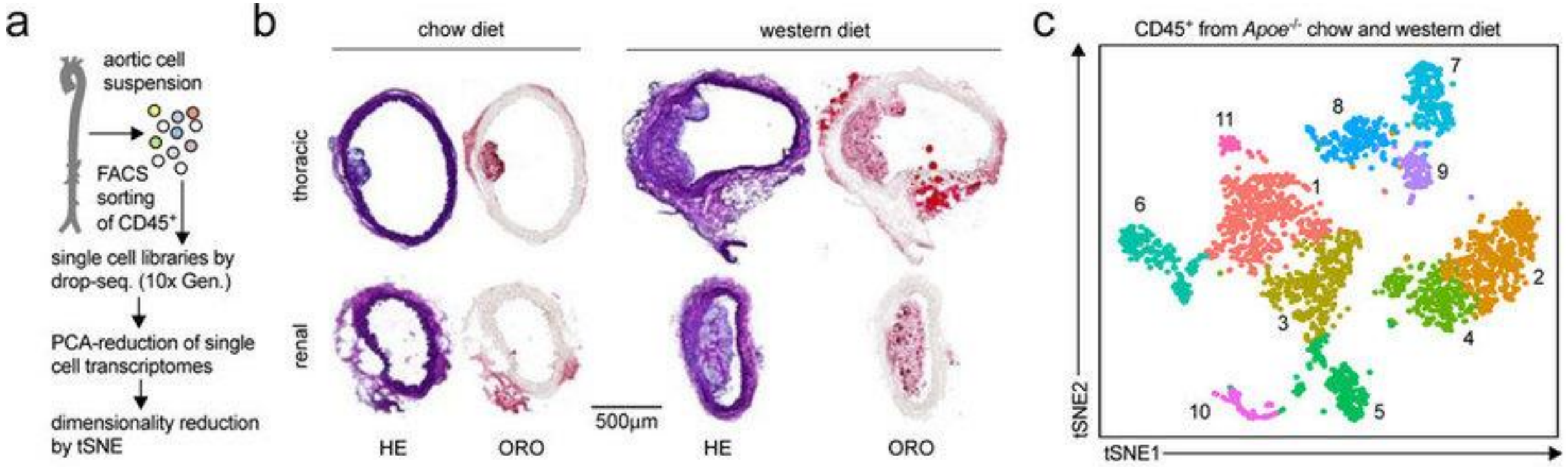
TNF ligand-related molecule 1A (TL1A) is a vascular endothelial growth inhibitor to reduce neovascularization

Atherosclerosis originates from vascular inflammation



Atherosclerotic Plaque: Single Cell sequencing

Figure 1. The single cell transcriptome identifies 11 distinct leukocyte populations in the atherosclerotic aorta



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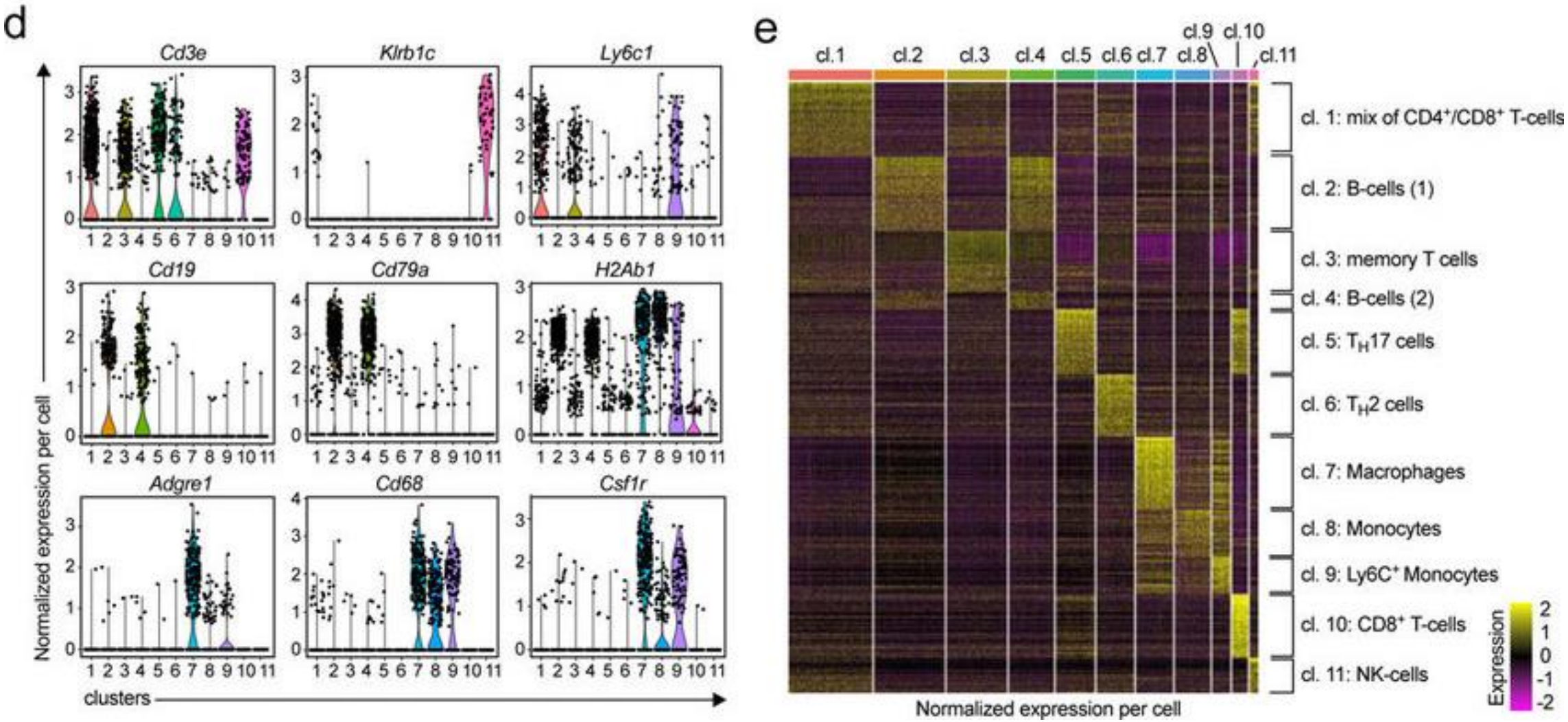

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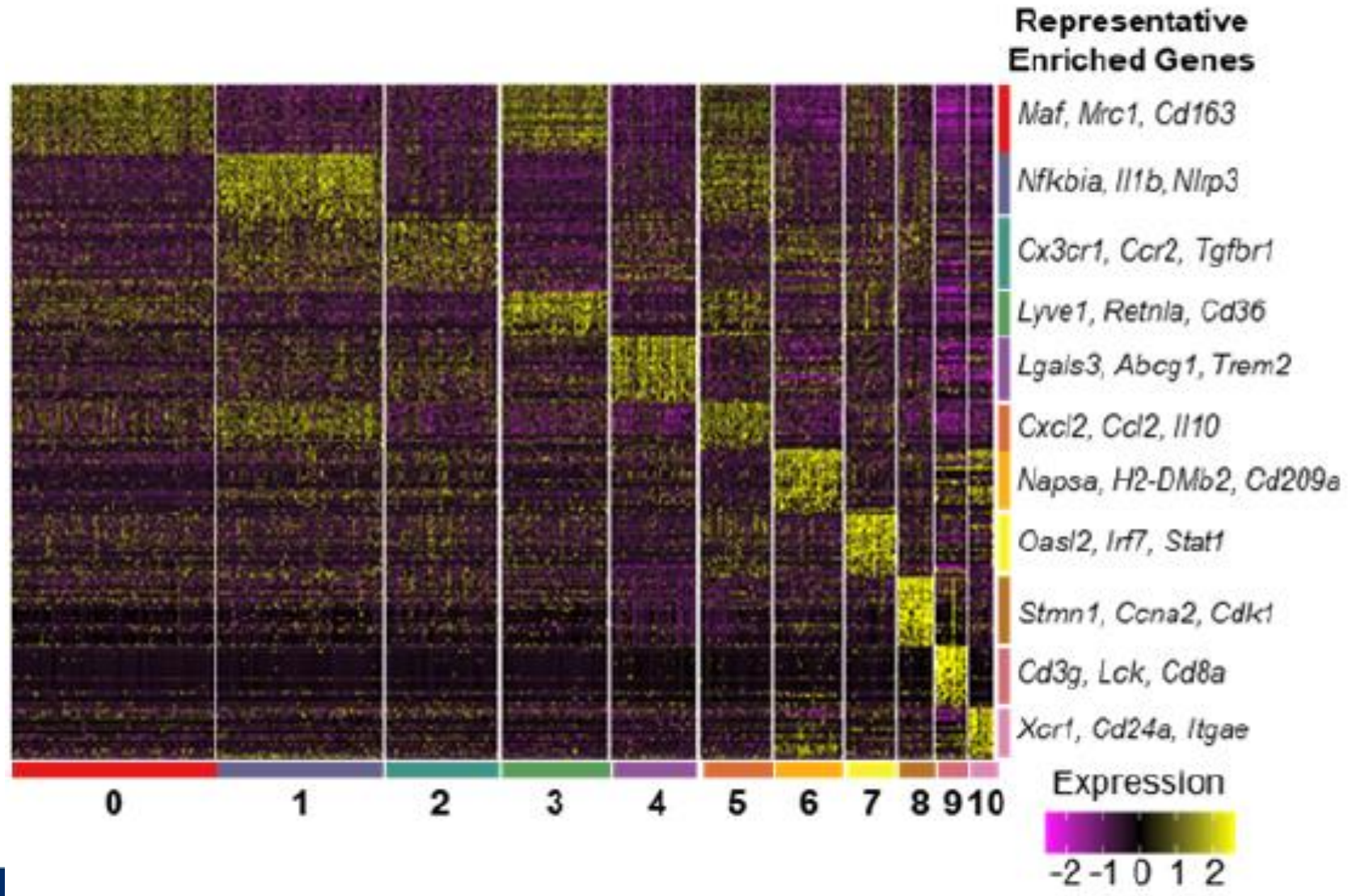
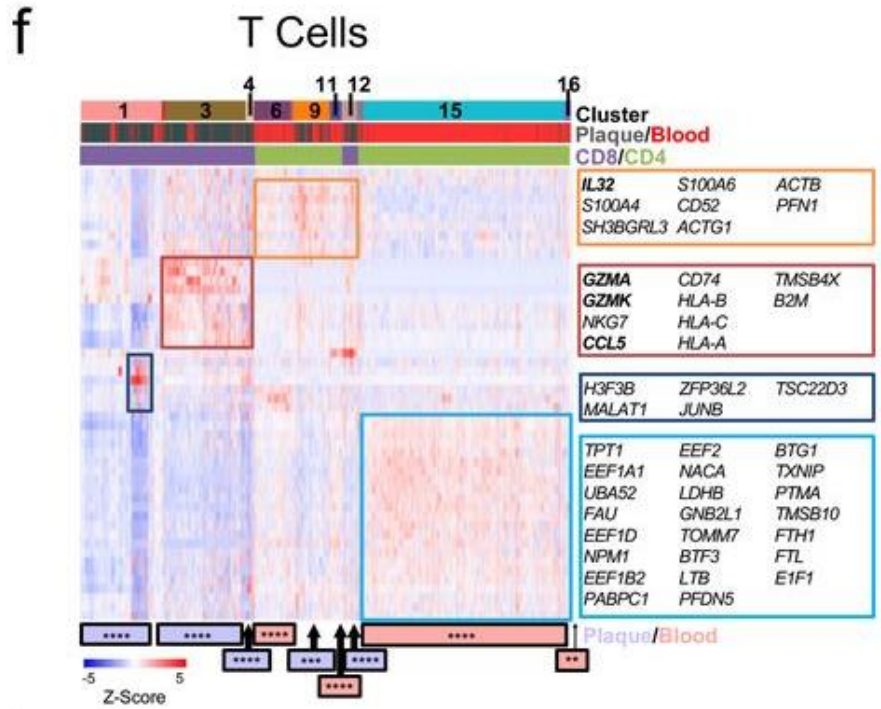
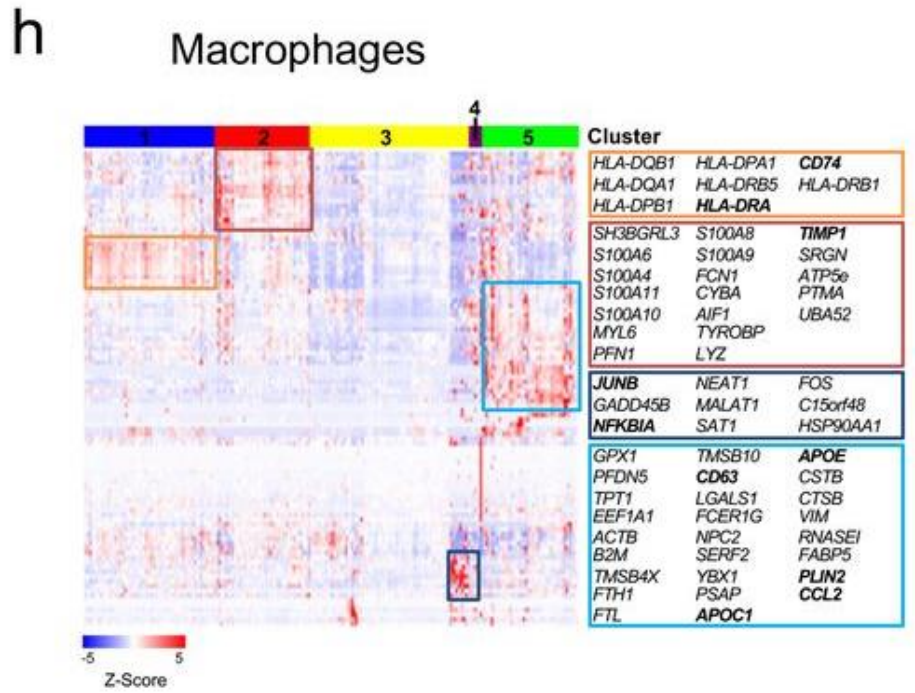
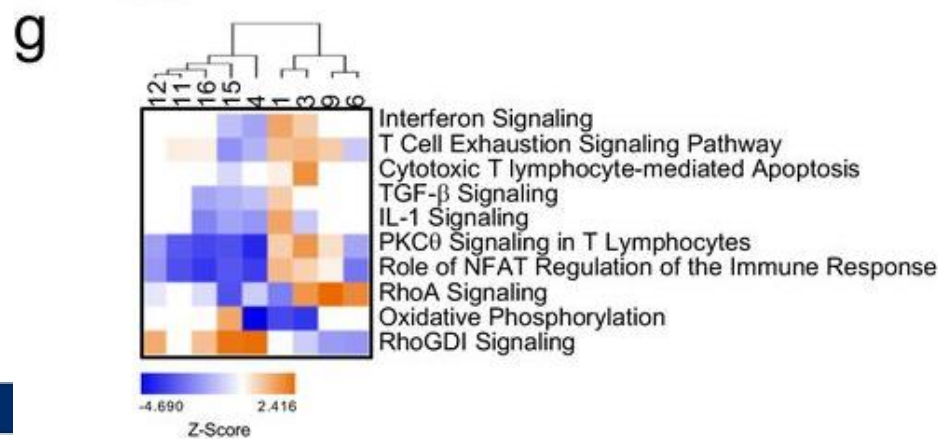
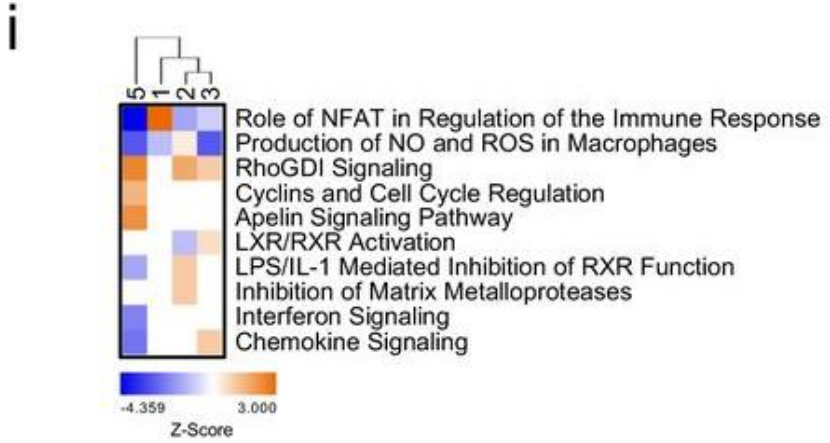


Atherosclerotic Plaque: Single Cell sequencing









Atherosclerotic Plaque: Single Cell sequencing

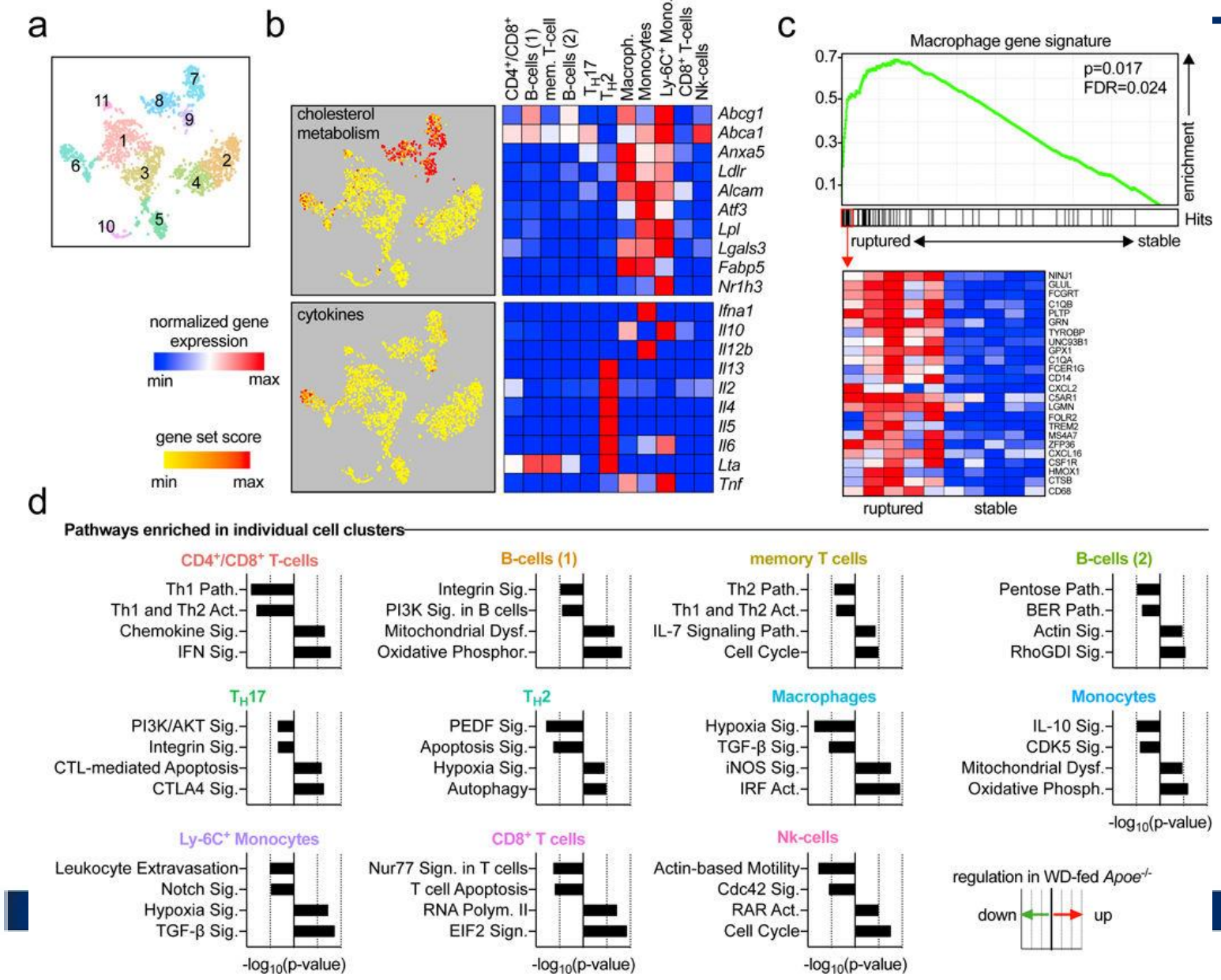


Figure 3. Enrichment of distinct genetic pathways in aortic leukocyte populations Single cell transcriptomes of the eleven identified leukocyte clusters (**a**) were analyzed for the enrichment of specific genes and pathways.

Patho-mechanistics

vs

Diagnostic markers

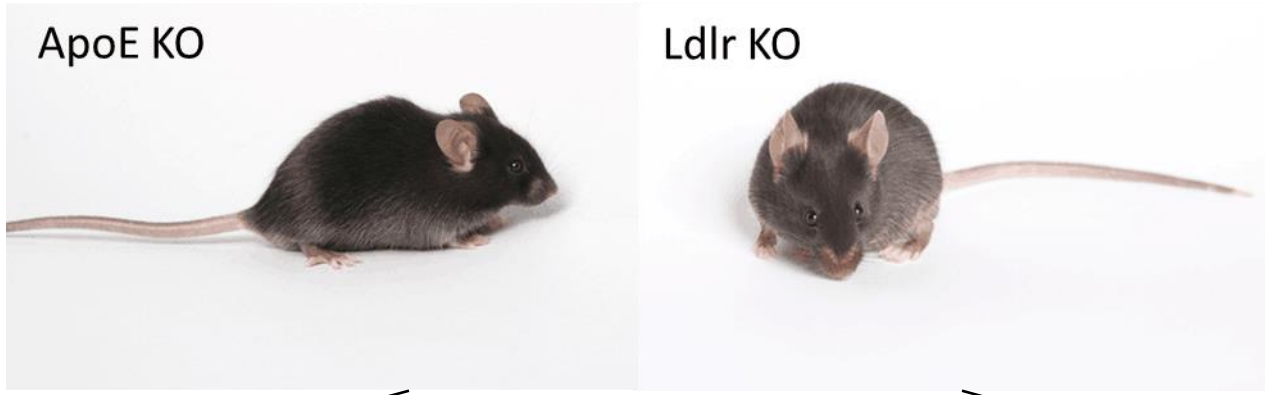
vs

Therapeutic targets

Mouse models of Atherosclerosis: Applications

Patho-mechanism

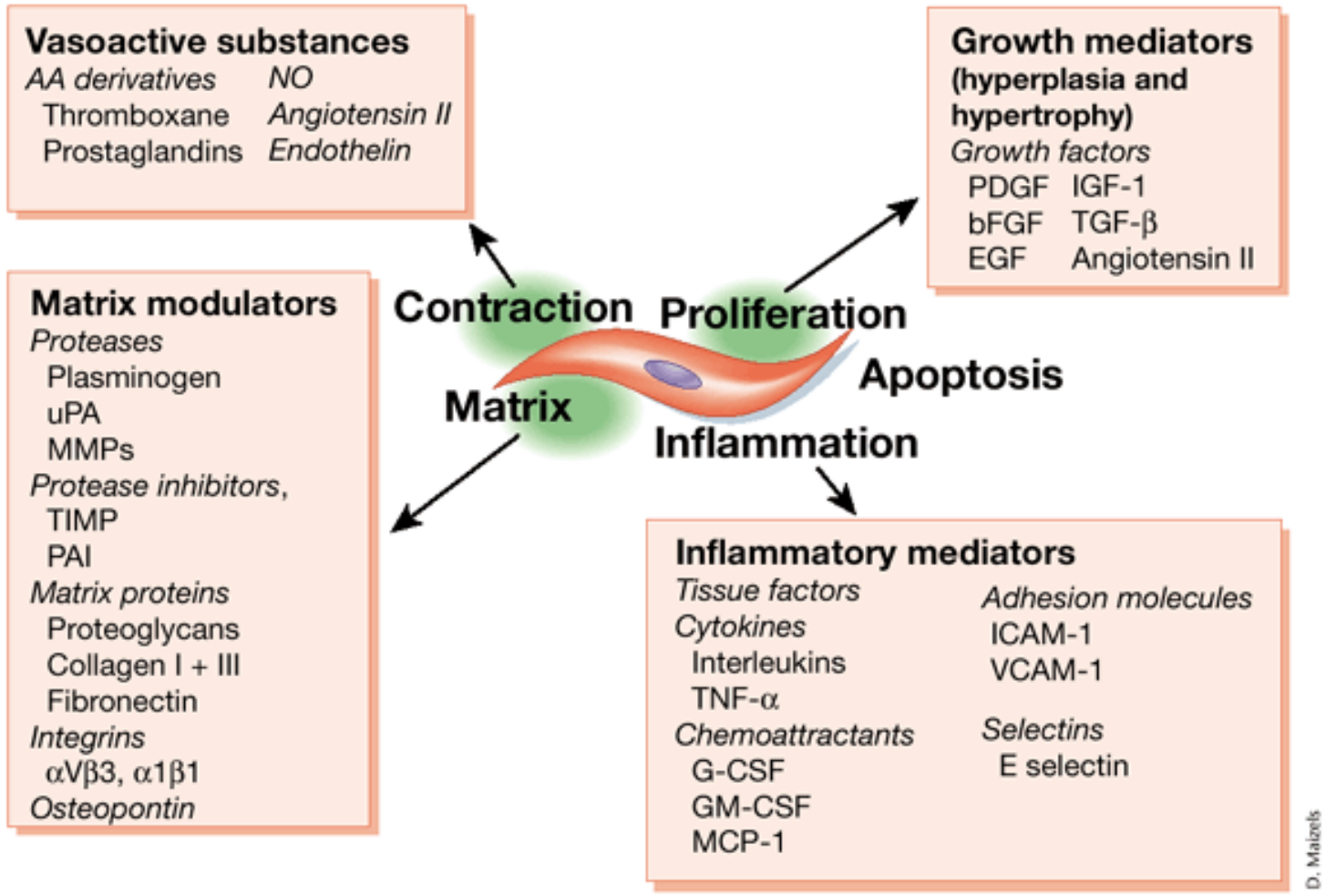
Diagnostics



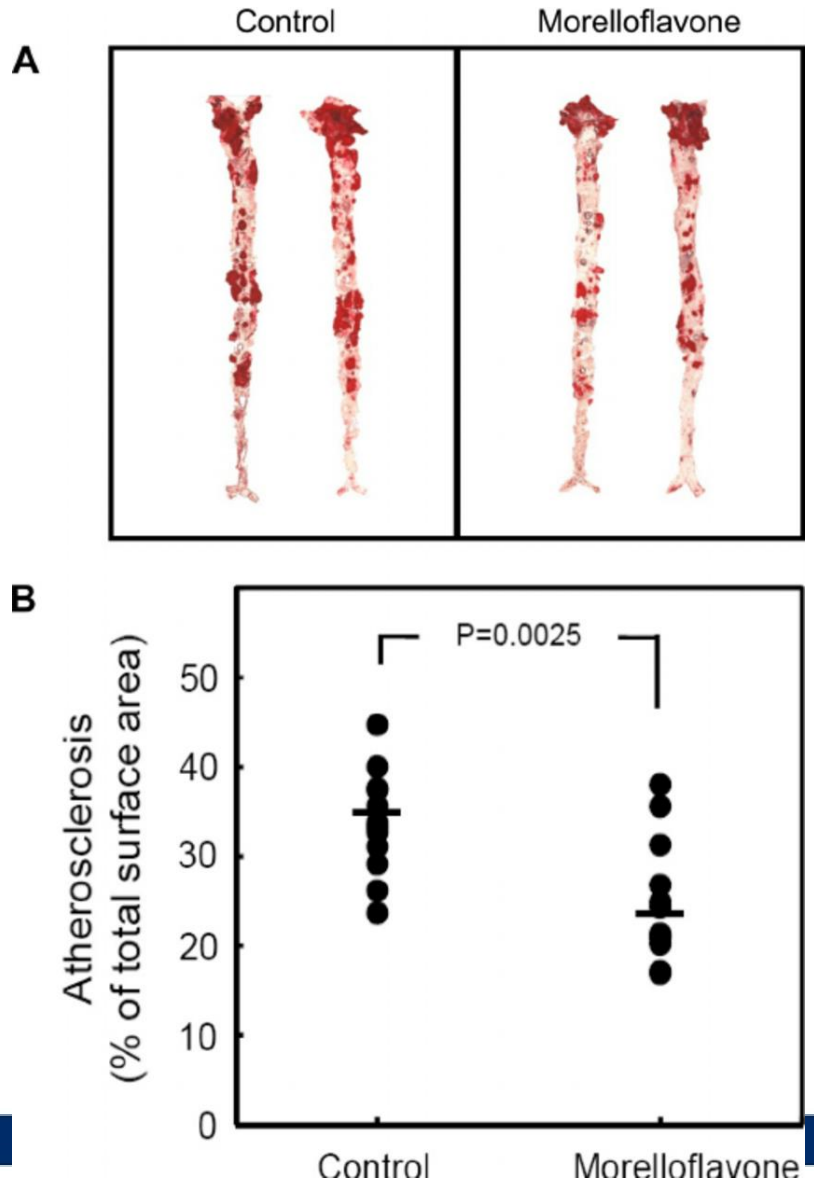
Therapeutics

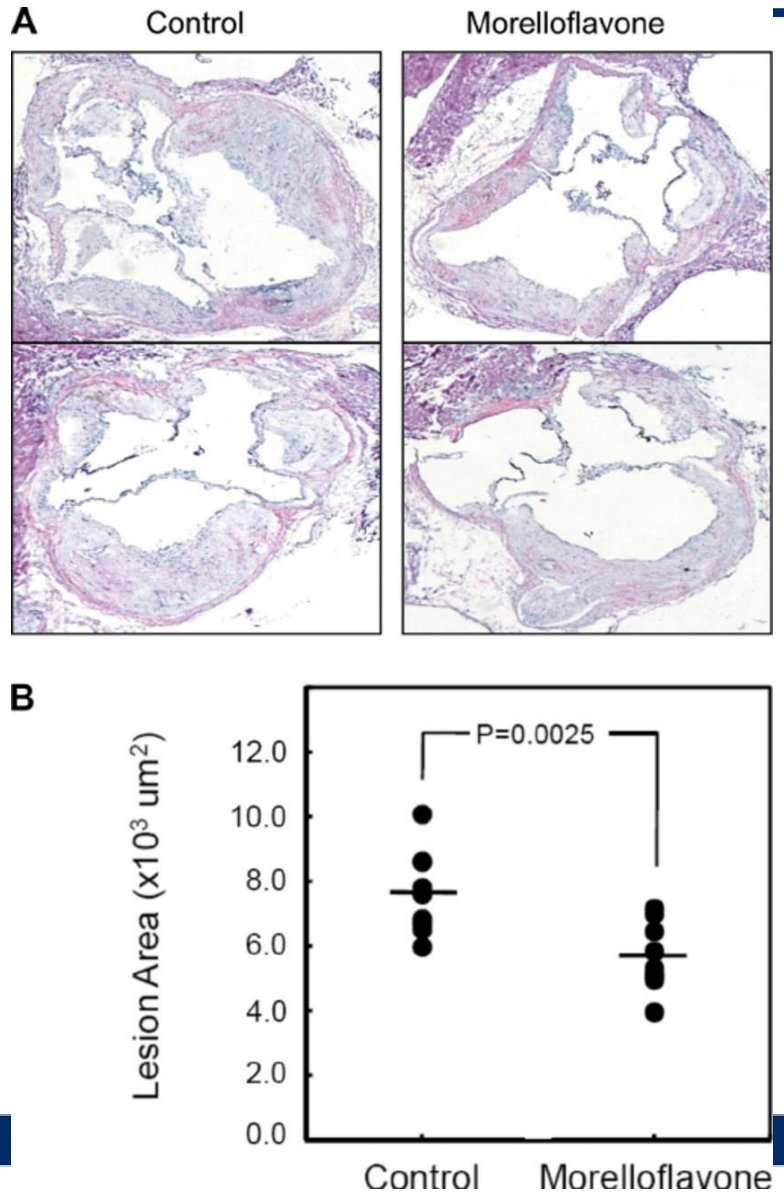
Bio Imaging

Atherosclerosis: Novel treatment strategies



Anti-Atherosclerotic Treatment Strategies



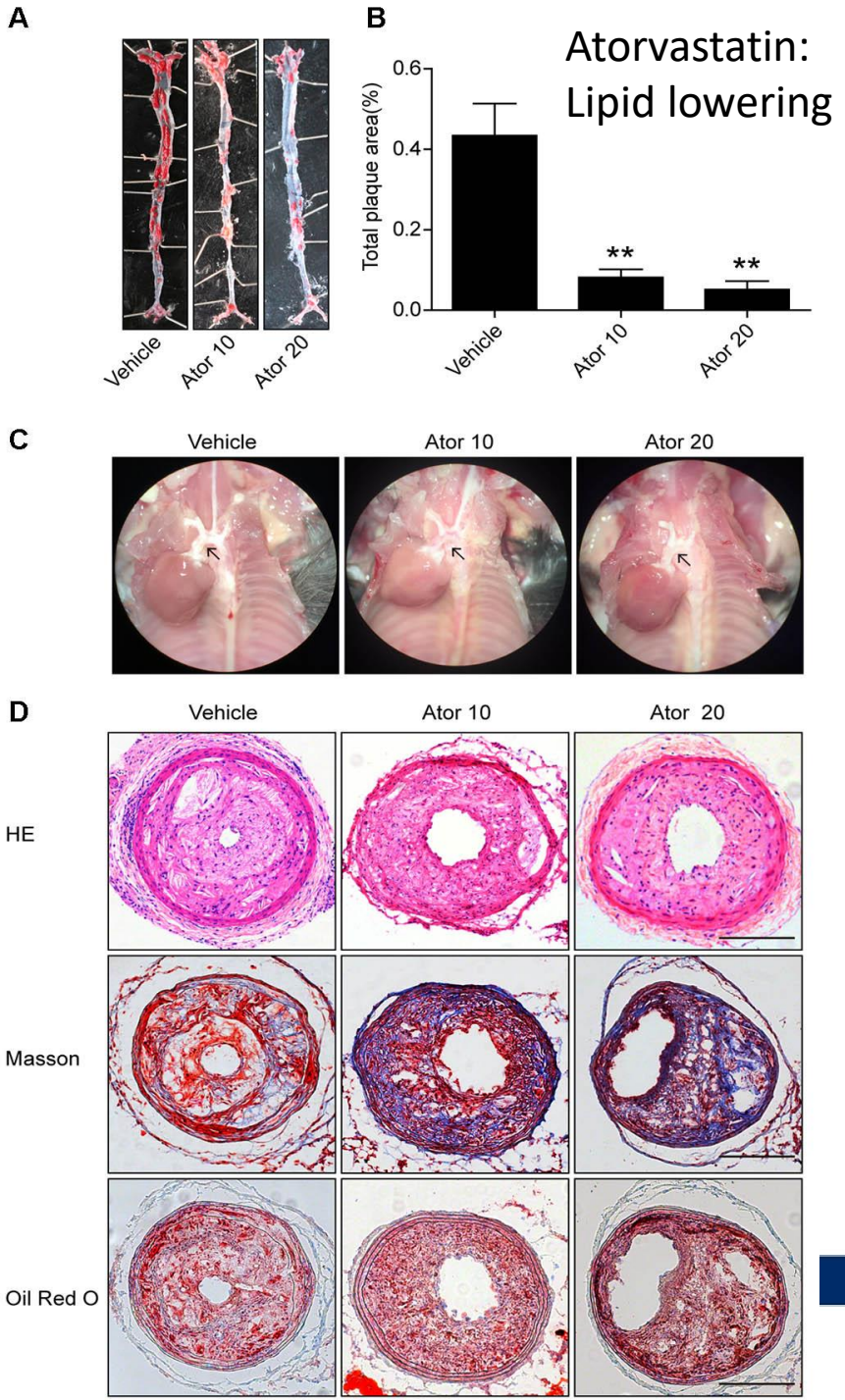


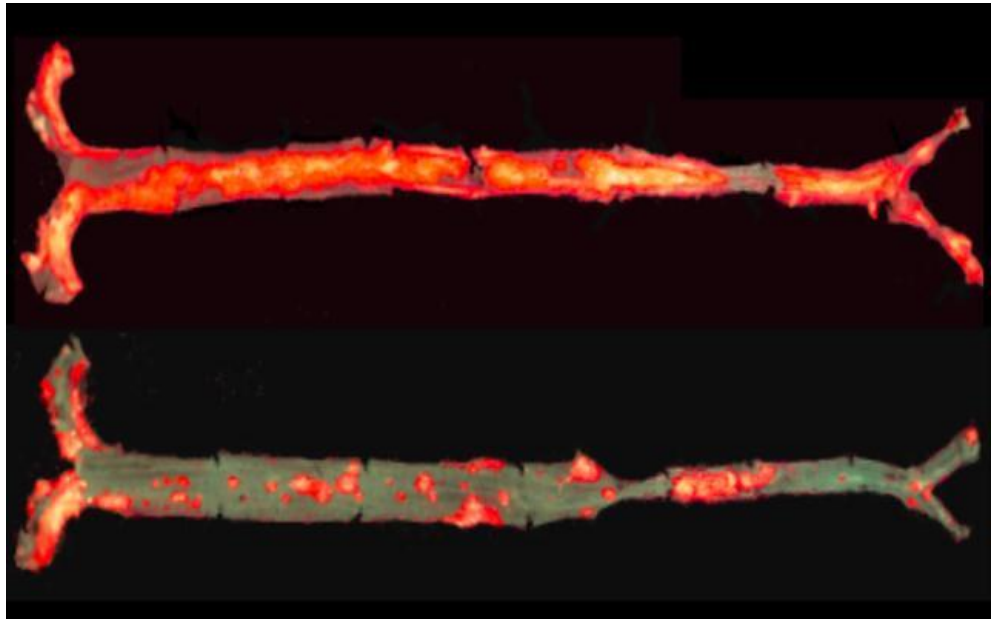
Morelloflavone:

-Belongs to the family of biflavonoids and is an inhibitor of HMG-CoA reductase

-Inhibitor of cell migration

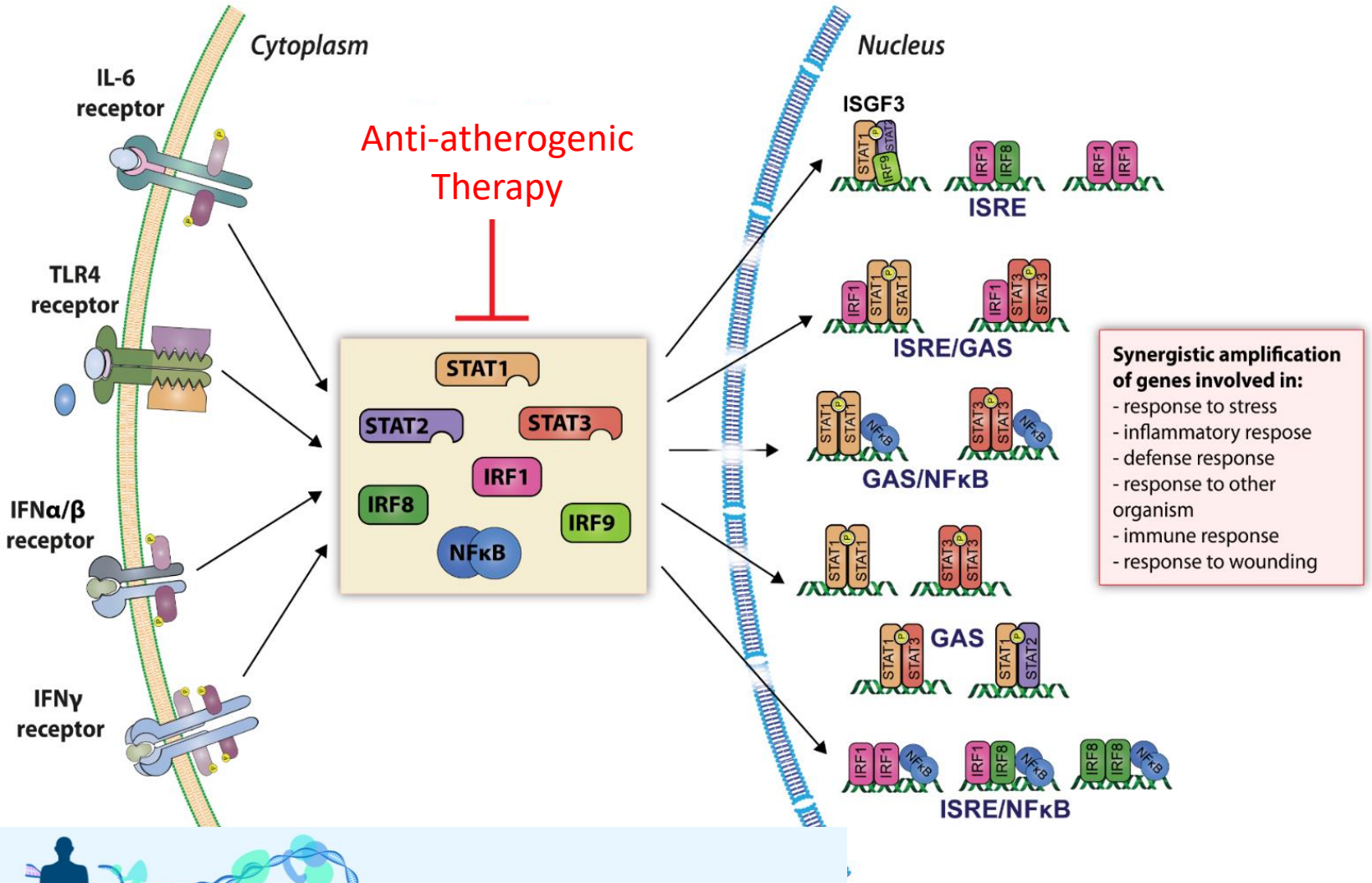
Anti-Atherosclerotic Treatment Strategies





The aorta of a mouse model of atherosclerosis on a high-fat diet for 12 months (top) has significantly more plaques (bright red) than the aorta of the same type of mouse that also produces the anti-inflammatory E06 antibody (bottom).

STATs as Novel Therapeutic Targets in Vascular Inflammation



Synergistic amplification of genes involved in:

- response to stress
- inflammatory respose
- defense response
- response to other organism
- immune response
- response to wounding

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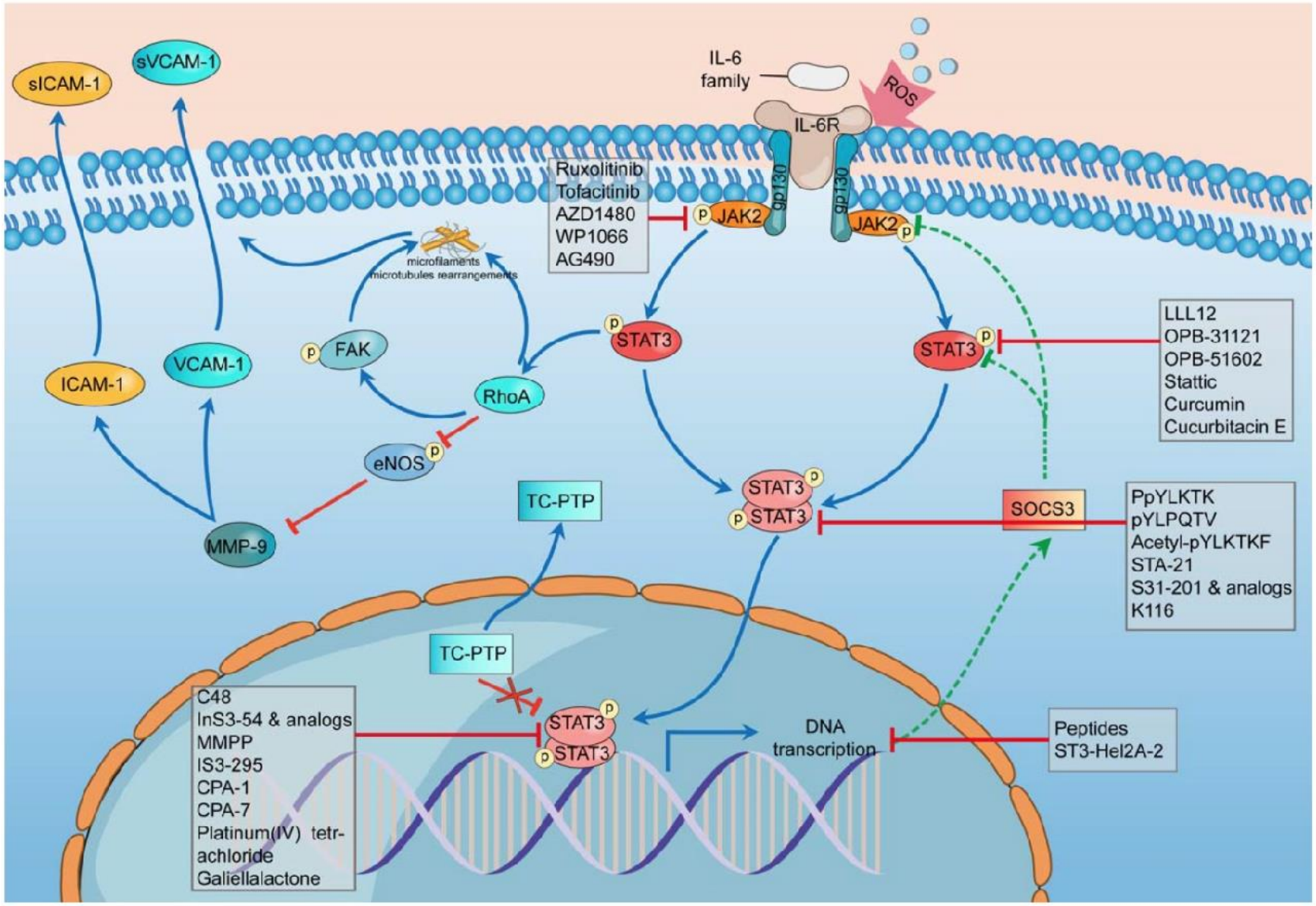
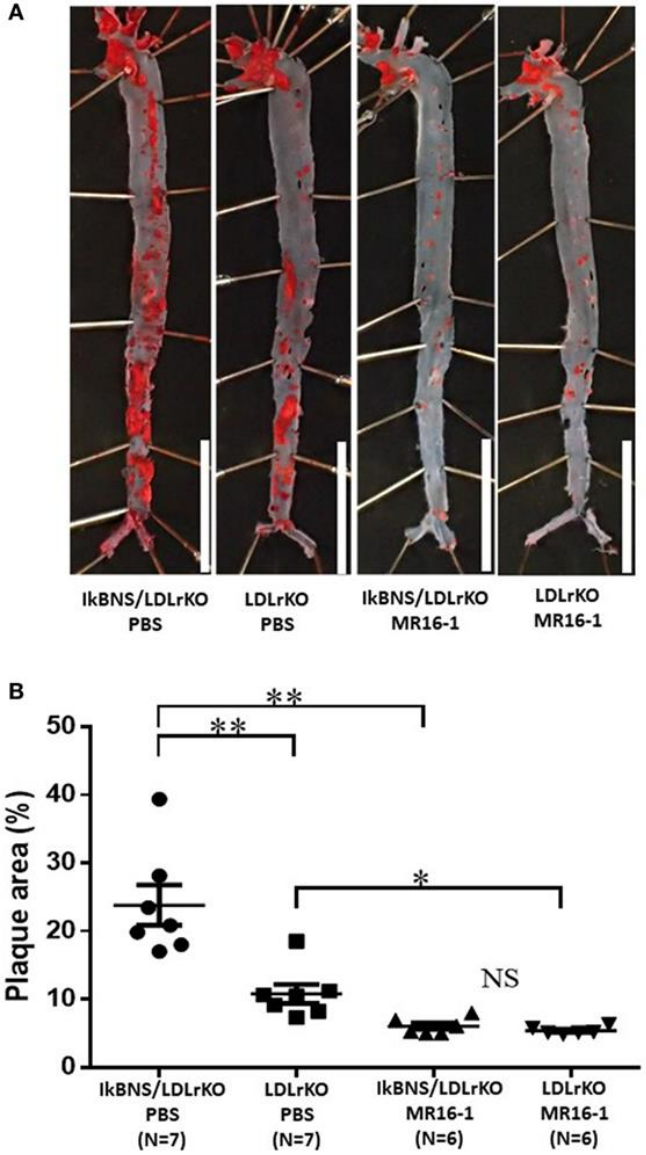
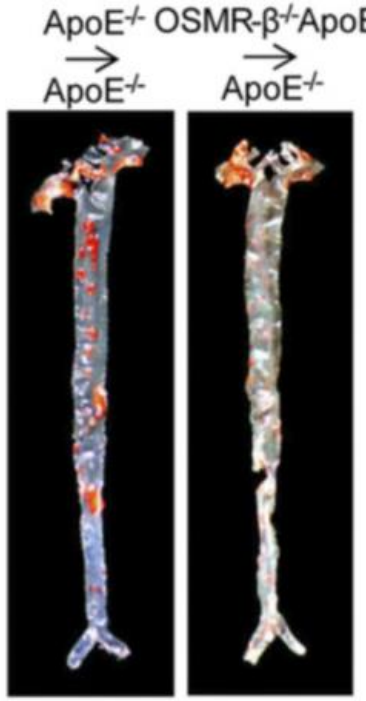
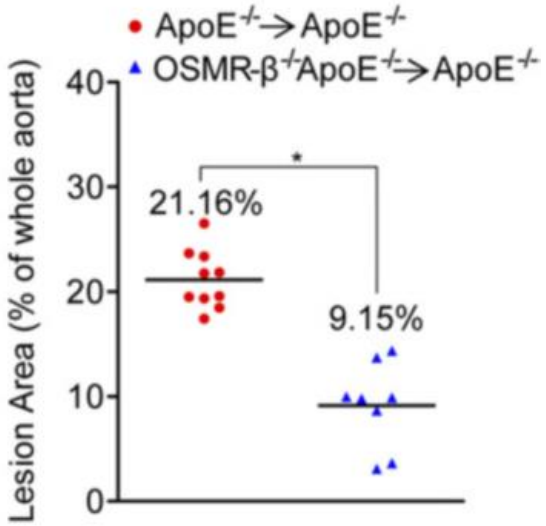
STAT3 Inhibition in Atherosclerosis

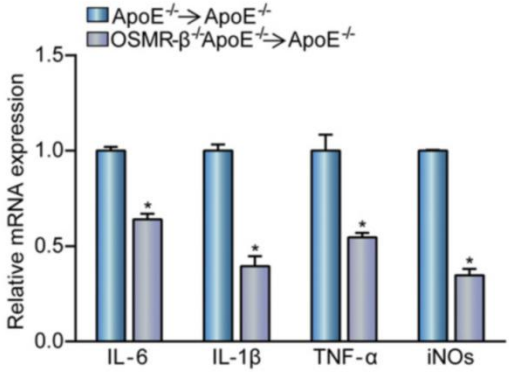


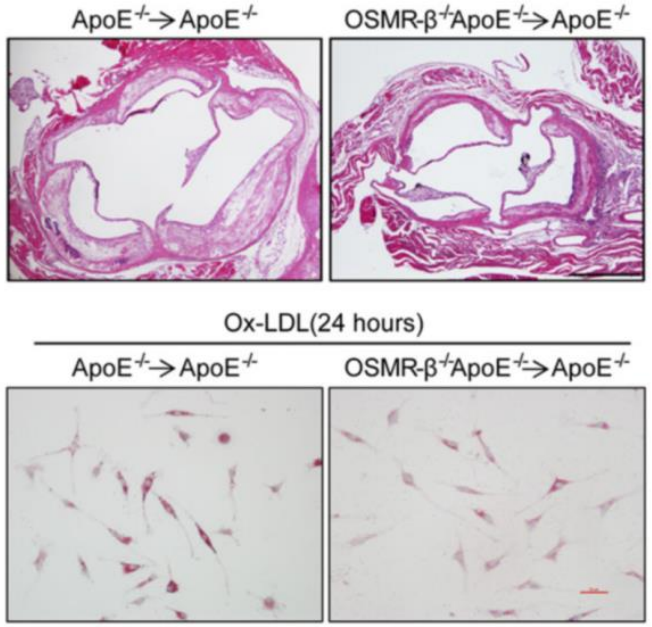


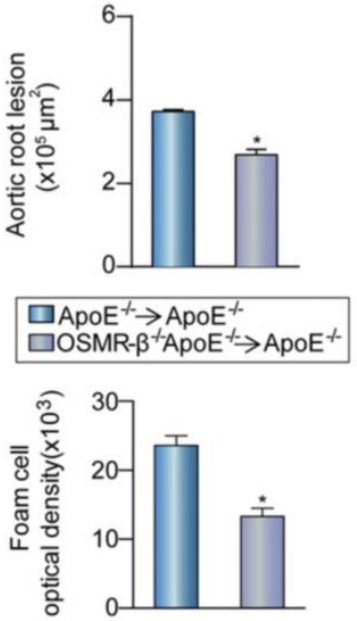
STAT3 Inhibition in Atherosclerosis











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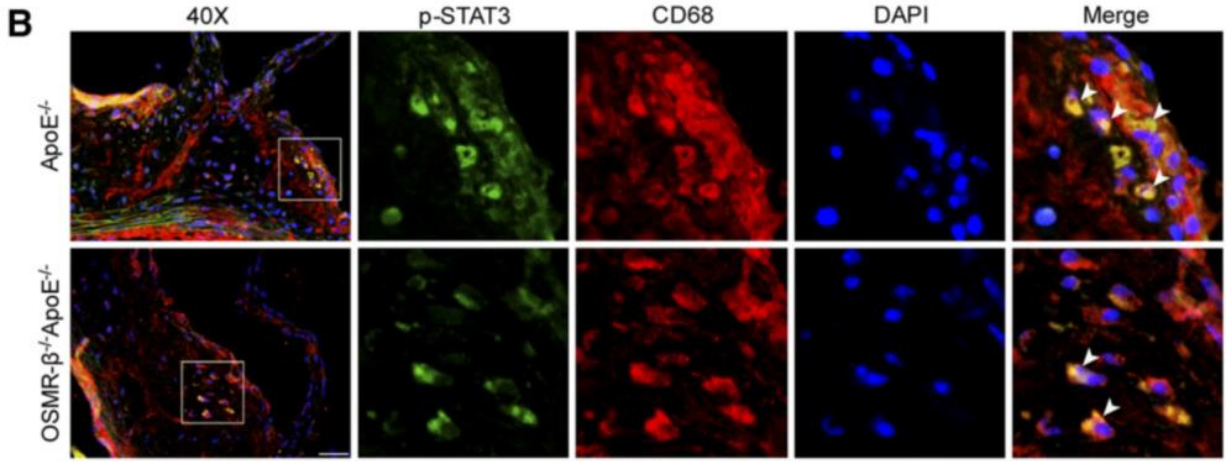
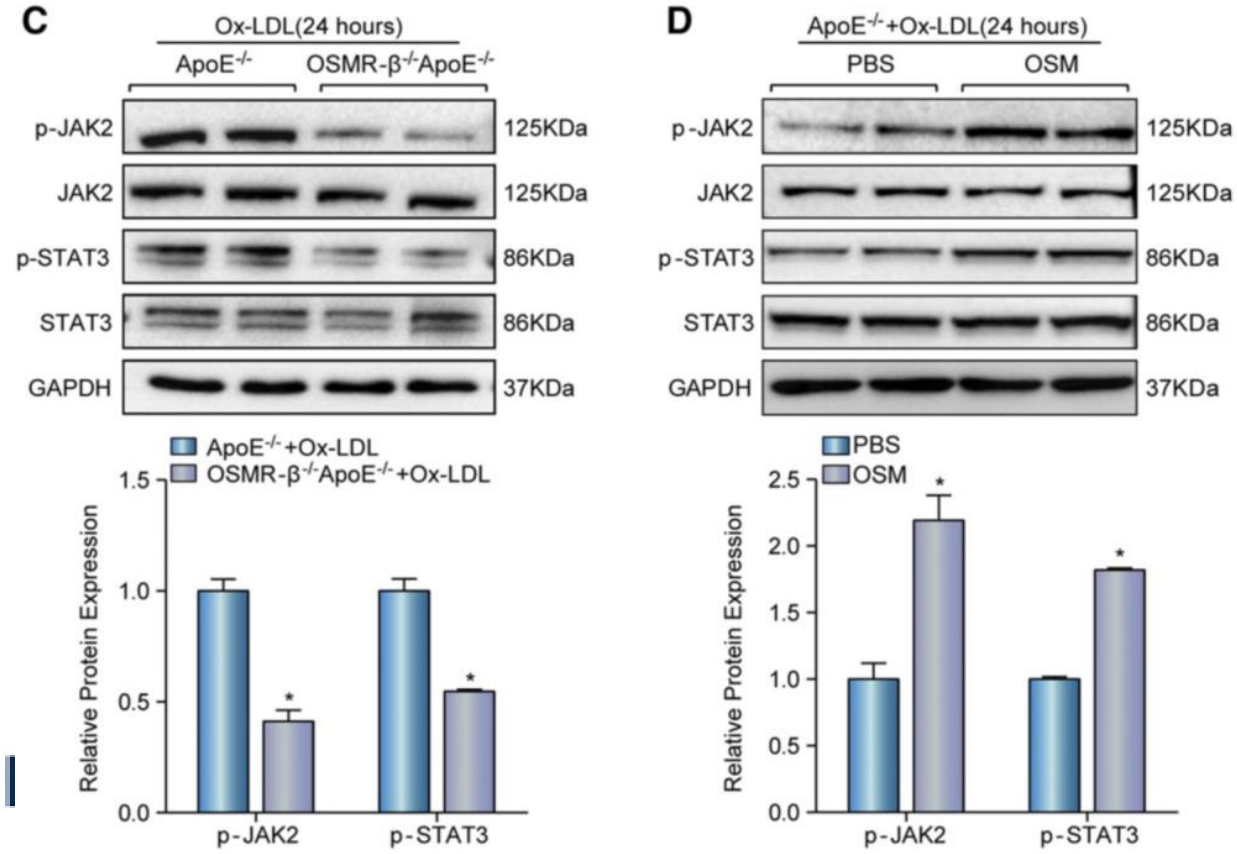
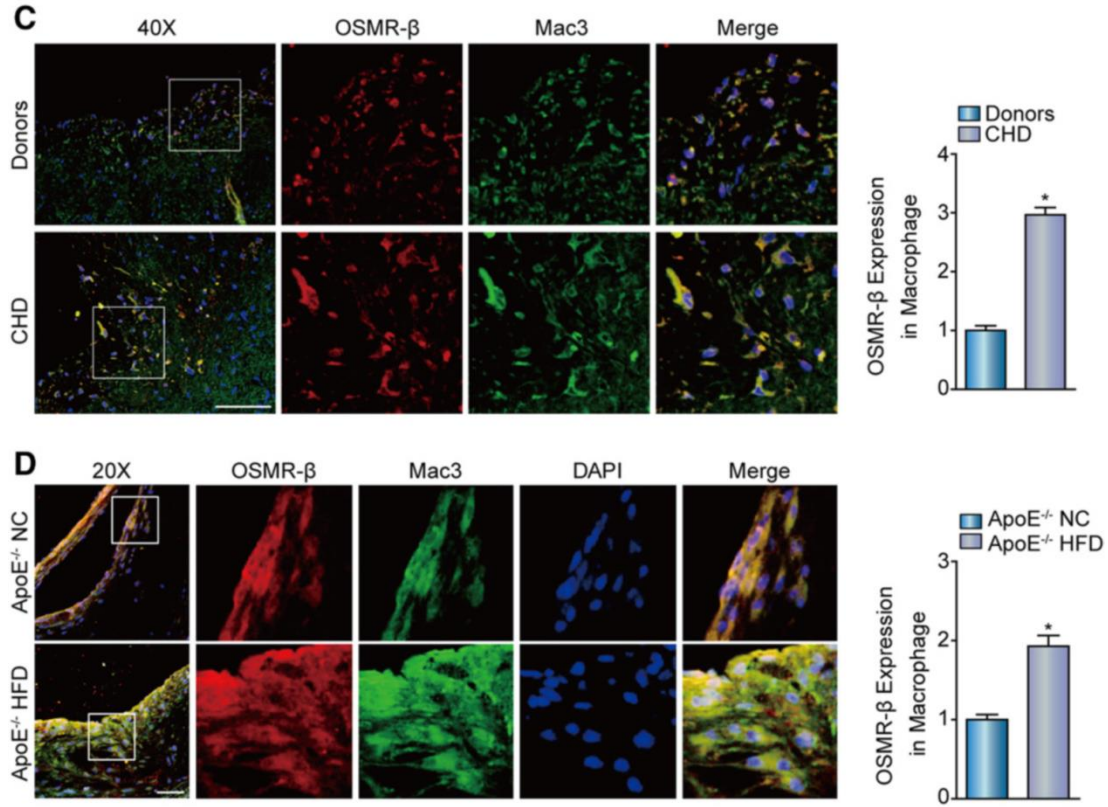
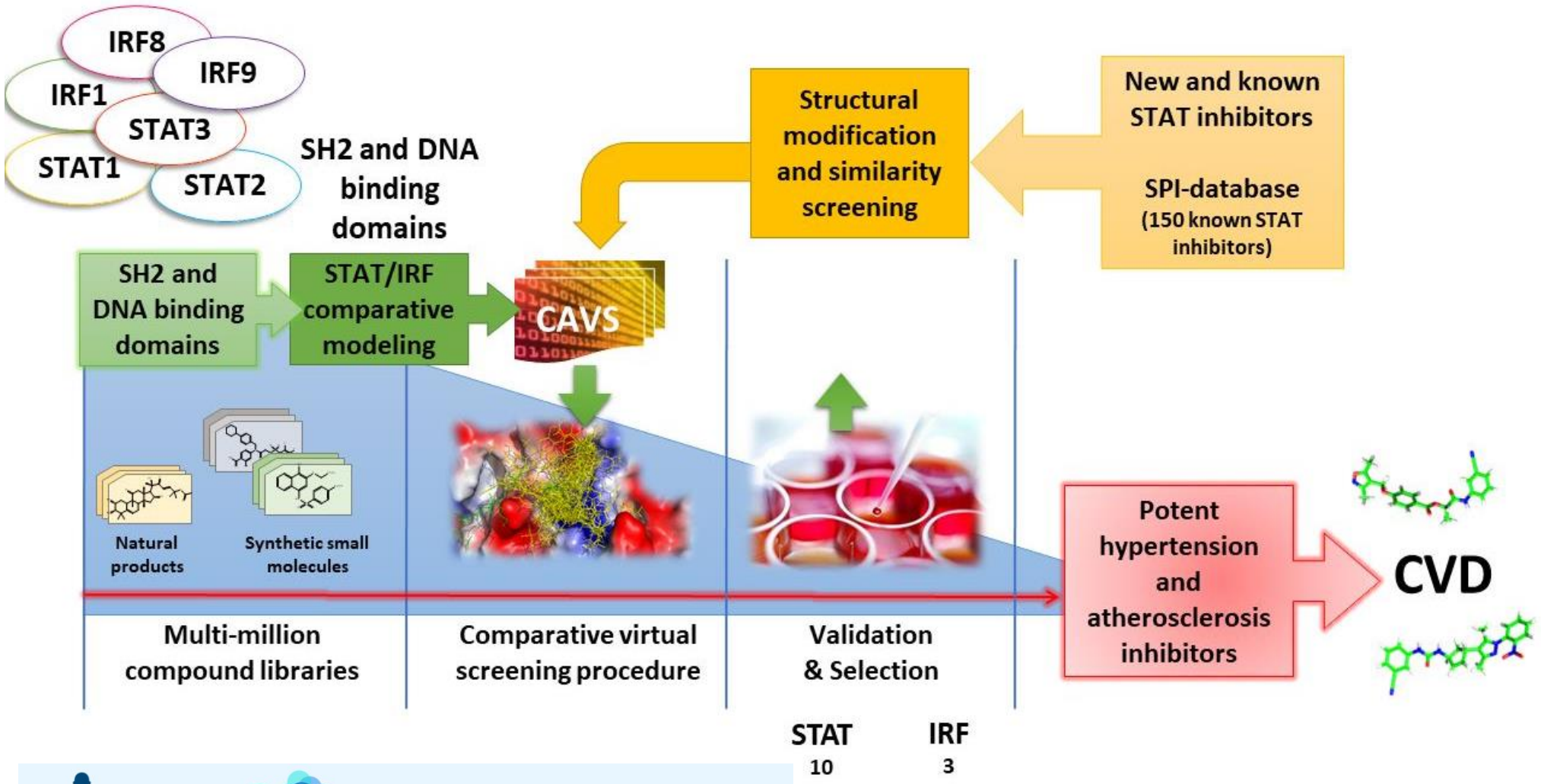






Pipeline approach to identify potent STAT & IRF inhibitors



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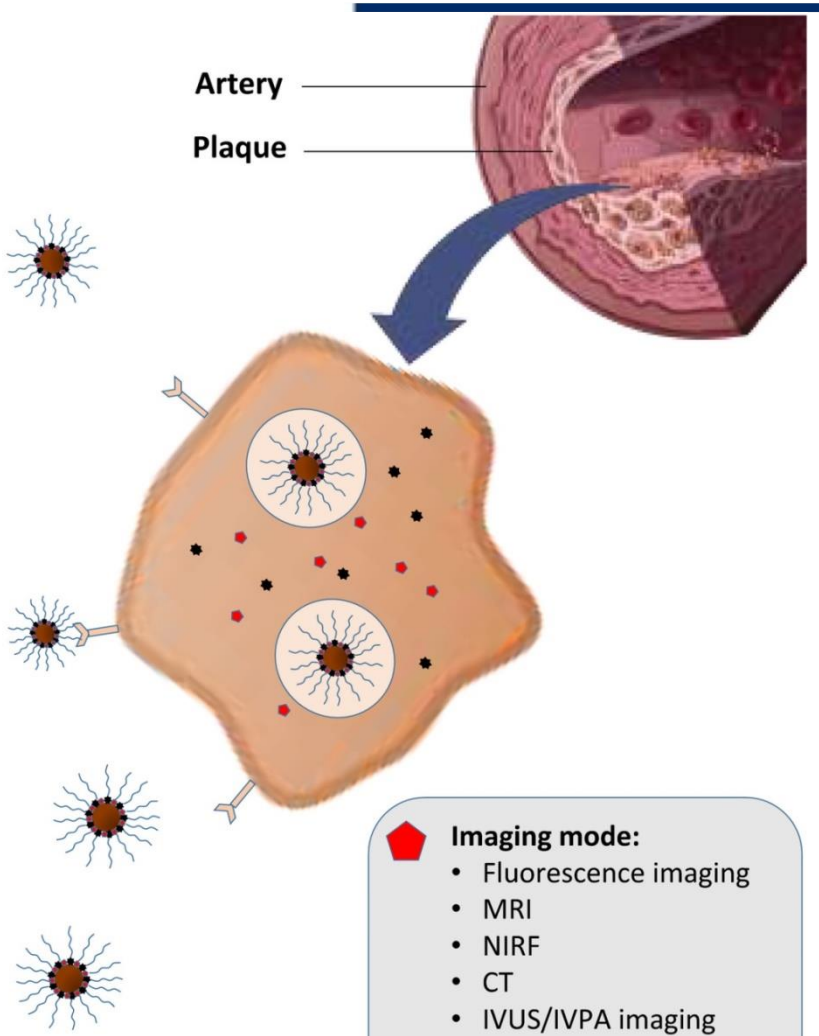
Atherosclerosis: Novel treatment strategies

Target cells or molecules in atherosclerosis:
- Macrophages
- Integrin $\alpha_v\beta_3$
- Annexin V
- Vascular cell adhesion molecule-1 (VCAM-1)

Nanocarrier:
- Lipid-based nanoparticles
- Micelles
- Polymeric nanoparticles
- Dendrimers
- Gel-like nanoparticles
- Magnetic nanoparticles
- Inorganic nanoparticles

Therapy agent for atherosclerosis:
- Anti-inflammatory drugs
- Immunomodulation drugs
- Gene (DNA/RNA)
- Antibodies
- Proteins
- Photoabsorbers
- Photosensitisers



Imaging mode:
- Fluorescence imaging
- MRI
- NIRF
- CT
- IVUS/IVPA imaging
- PET
- CMR molecular imaging

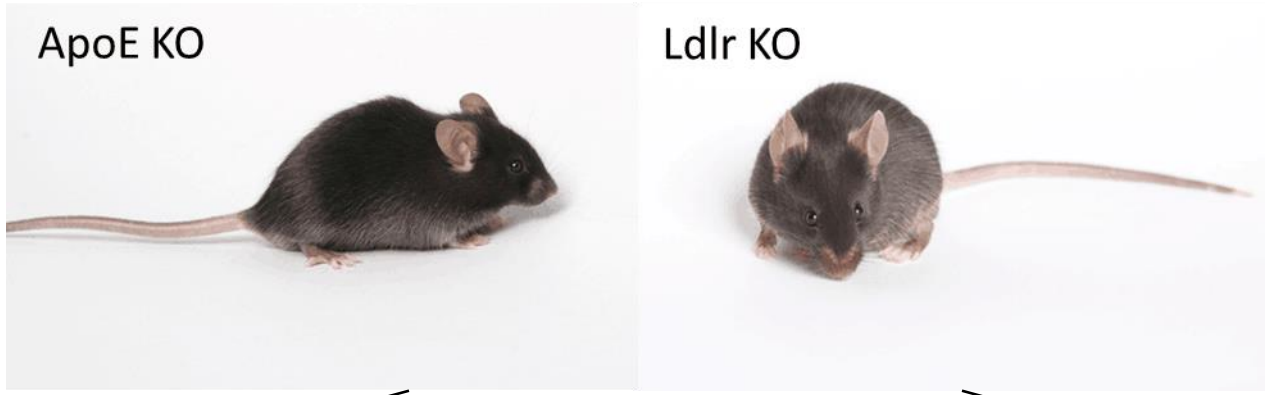
Nanocarrier Therapy agent Imaging agent Binding ligand

Plaque Targeted Therapy

Mouse models of Atherosclerosis: Applications

Patho-mechanism

Diagnostics



Therapeutics

Bio Imaging

Imaging vs VSMC-Biology

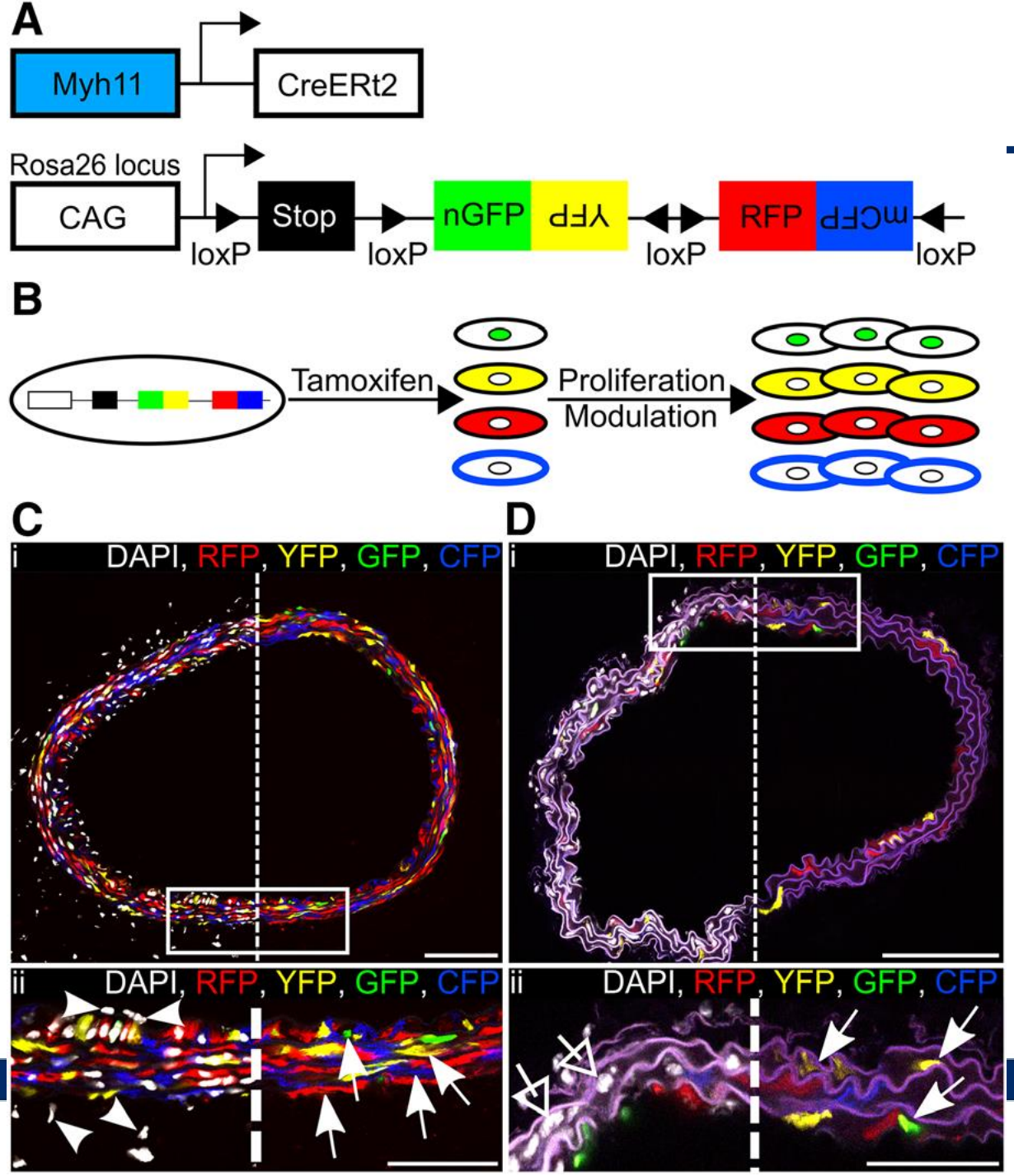


Figure 1. Efficient and specific multicolor vascular smooth muscle cell (VSMC) labeling in Myh11-CreERt2/Rosa26-Confetti animals.

Imaging vs VSMC-Biology

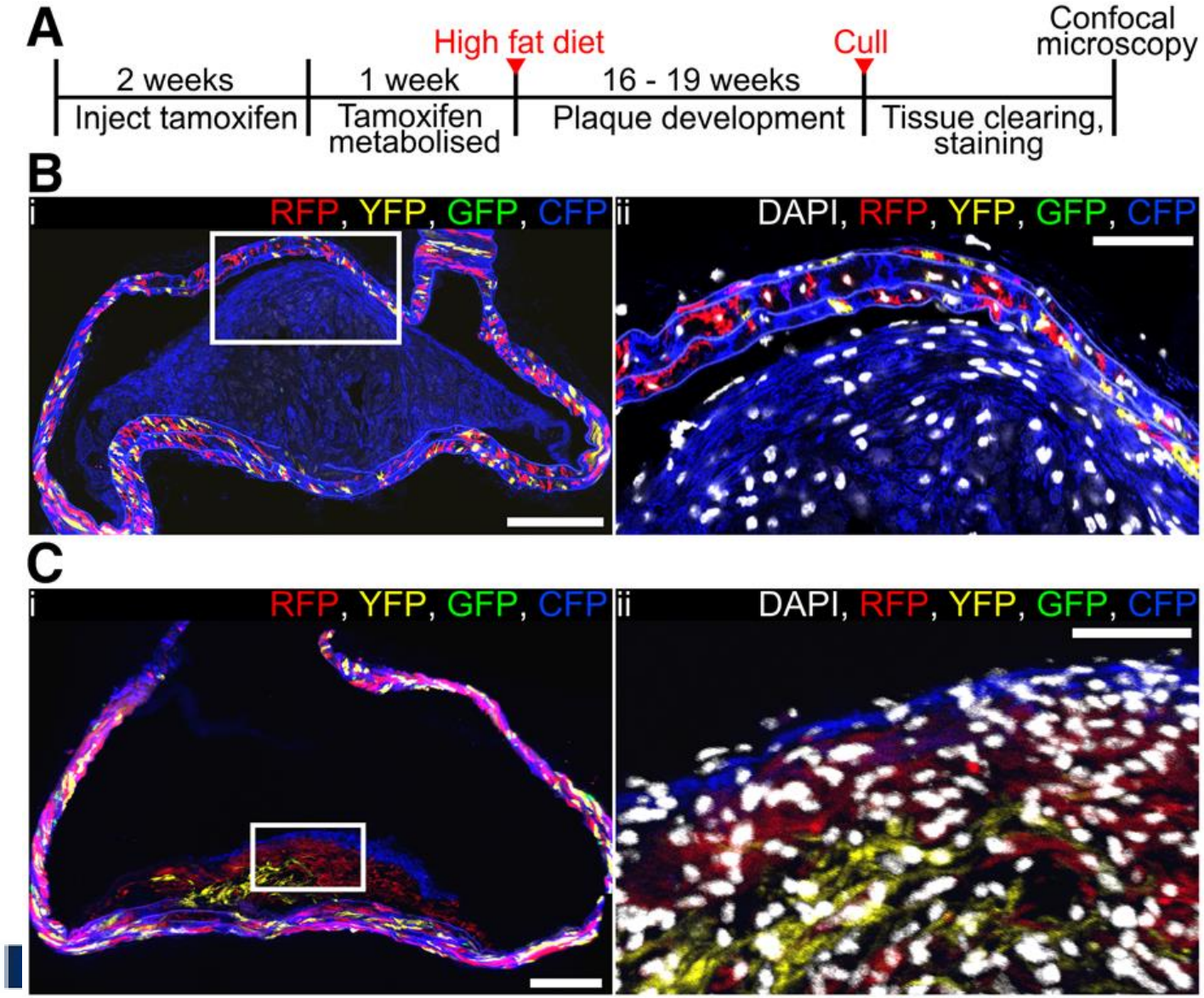


Figure 2. Vascular smooth muscle cell (VSMC)–derived cells generate oligoclonal atherosclerotic plaques.

Imaging vs VSMC-Biology

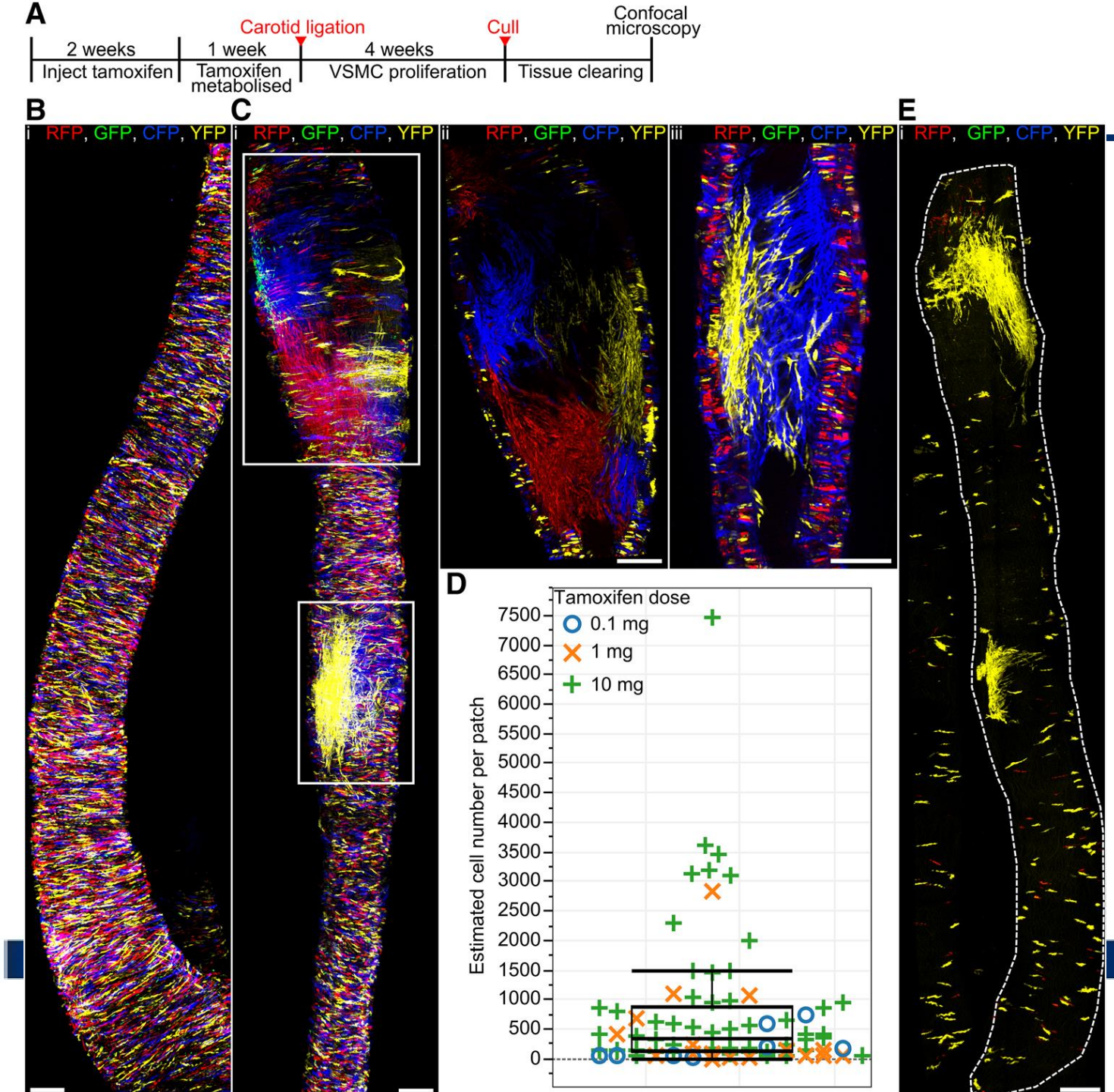
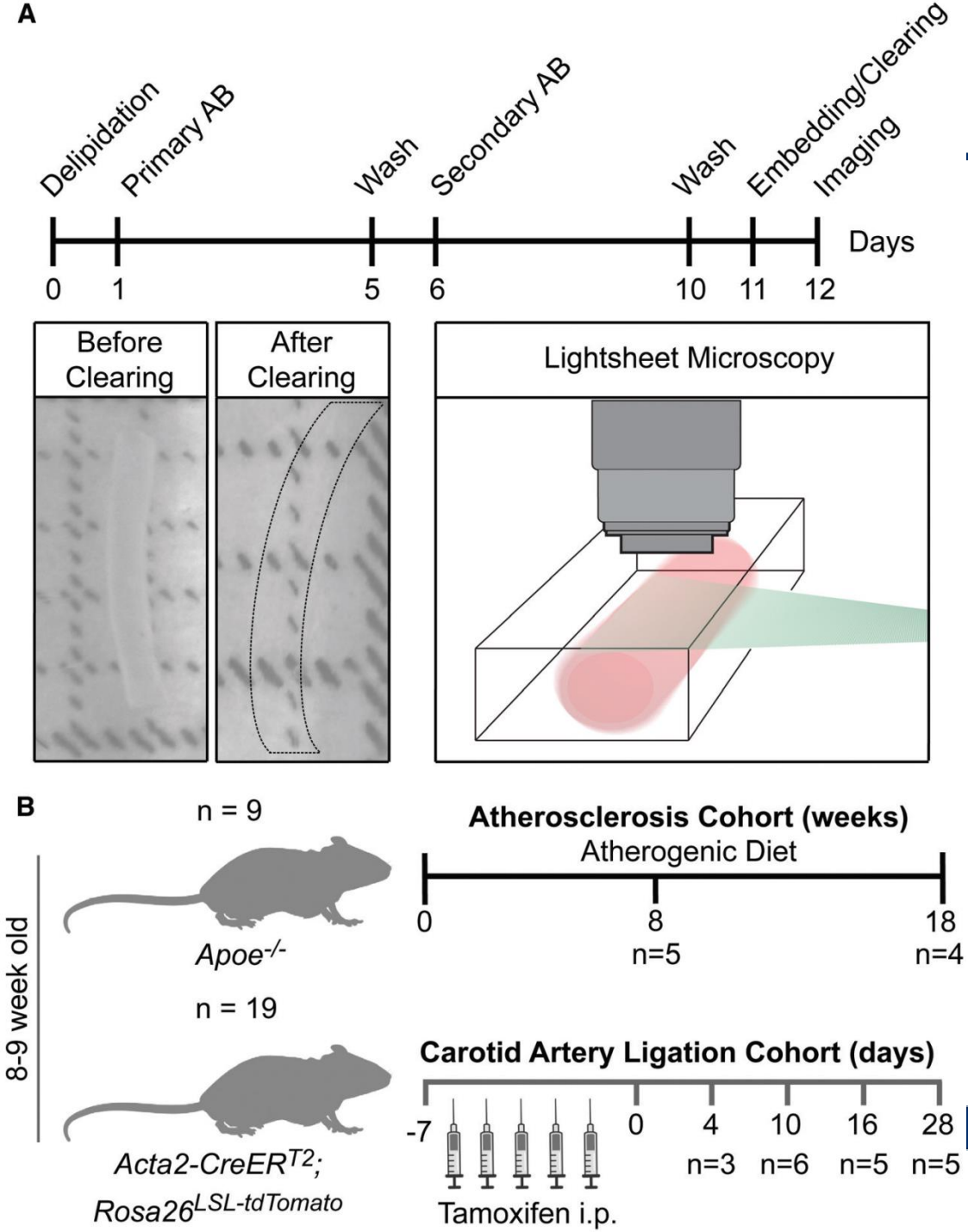


Figure 6. A subset of vascular smooth muscle cells (VSMCs) proliferate to form the injury-induced neointima.

3D-Imaging vs Plaque build



3D-Imaging vs Plaque build

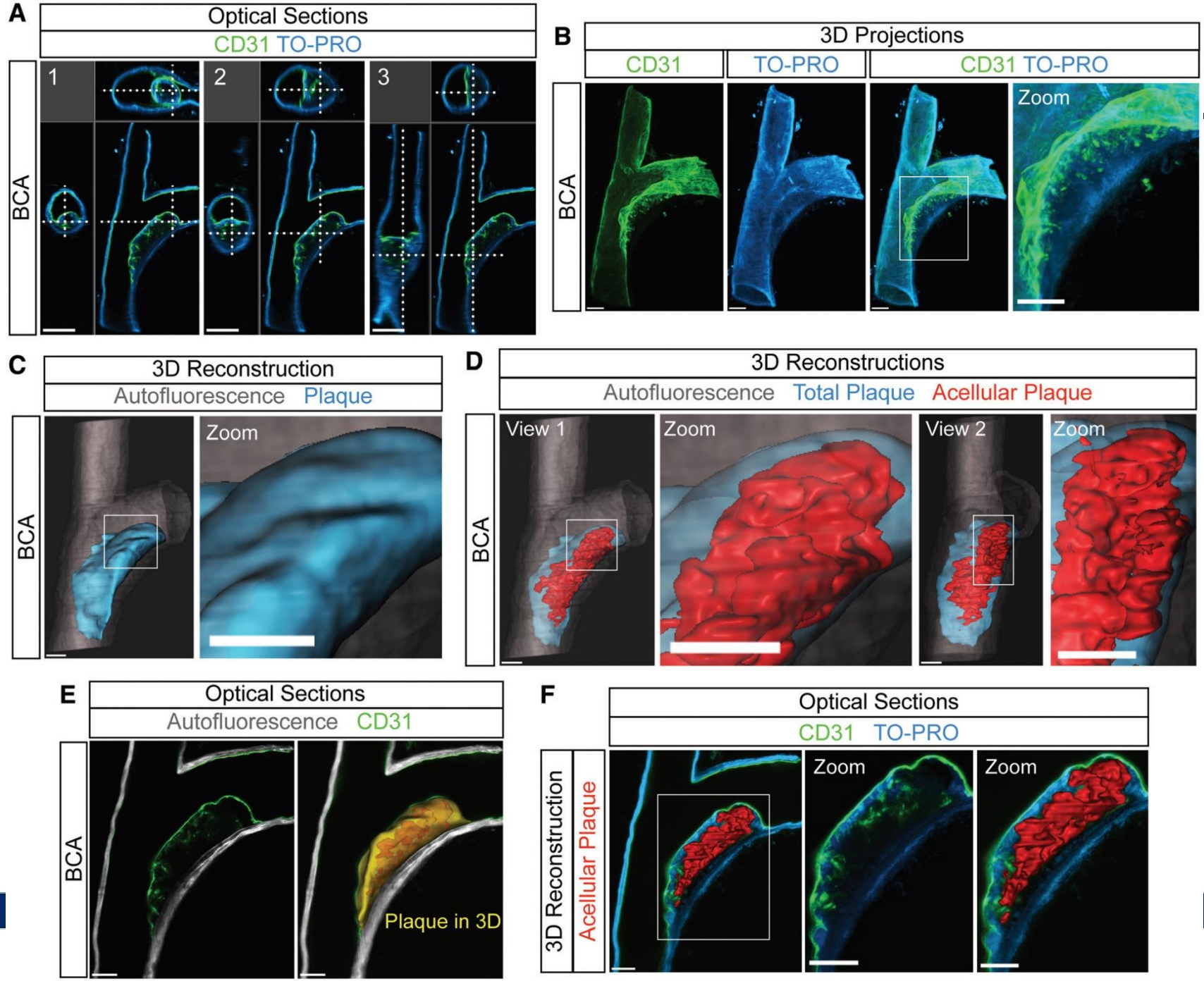
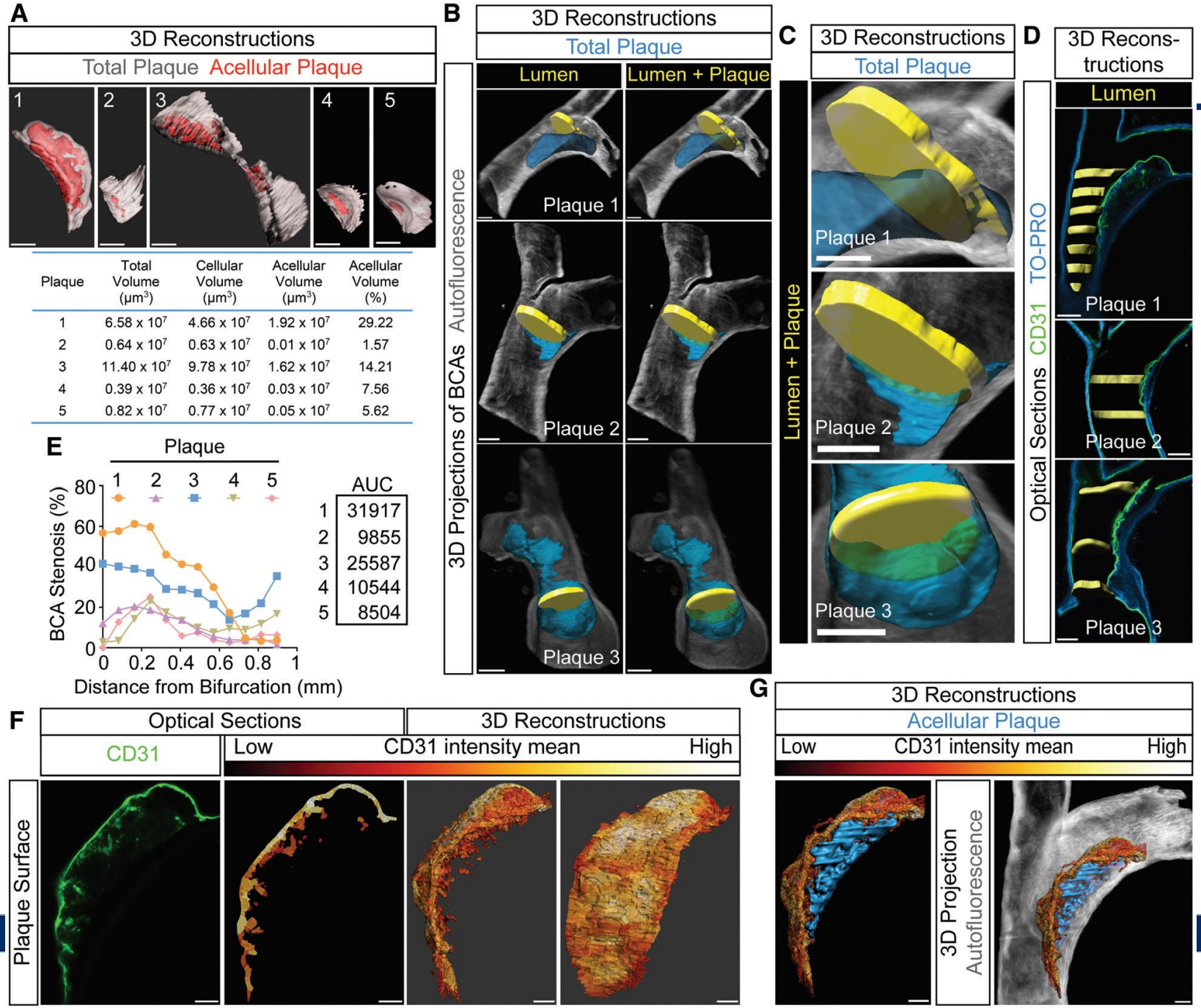


Figure 5. Three-dimensional (3D) evaluation of atherosclerotic plaques by Adipo-Clear and light-sheet microscopy.

CD31: PCAM-1



Plaque	Total Volume (μm^3)	Cellular Volume (μm^3)	Acellular Volume (μm^3)	Acellular Volume (%)
1	6.58×10^7	4.66×10^7	1.92×10^7	29.22
2	0.64×10^7	0.63×10^7	0.01×10^7	1.57
3	11.40×10^7	9.78×10^7	1.62×10^7	14.21
4	0.39×10^7	0.36×10^7	0.03×10^7	7.56
5	0.82×10^7	0.77×10^7	0.05×10^7	5.62

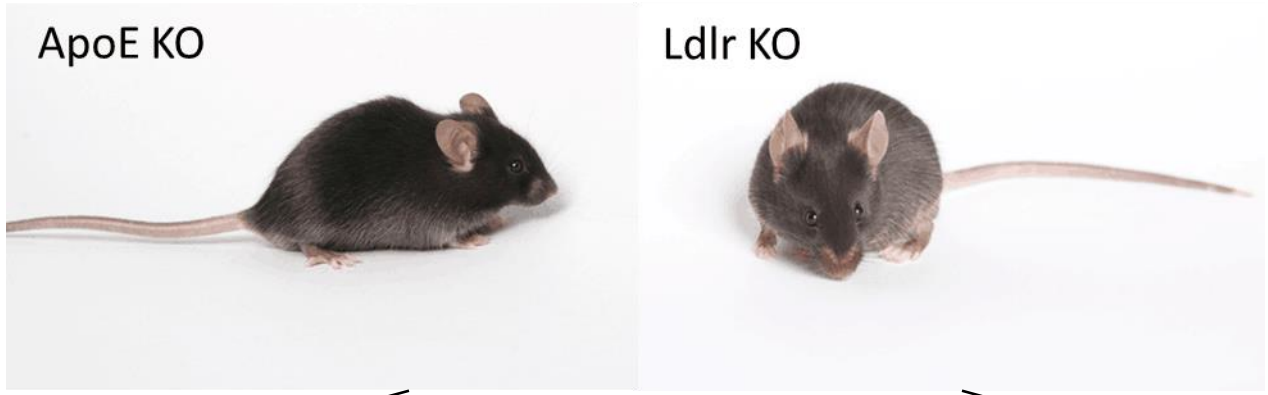
3D-Imaging vs Plaque build

Figure 6. Volumetric analysis of atherosclerosis in the brachiocephalic artery and 3-dimensional (3D) reconstruction of the endothelial lining.

Mouse models of Atherosclerosis: Applications

Patho-mechanism

Diagnostics



Therapeutics

Bio Imaging

Non-invasive Molecular Imaging

Table 2. Noninvasive molecular imaging in mouse models of vulnerable atherosclerotic plaques.

Imaging Modality	Spatial Resolution	Sensitivity (mol/L)	Contrast Agent	Probe Concentration	Advantages	Limits
Ultrasound	50–500 µm	Not well characterized yet	Microbubbles	µM to nM	Real-time Low cost High temporal resolution (0.1–100 s) No ionizing radiation	Operator-dependent
Magnetic Resonance	10–100 µm	10^{-3}–10^{-5}	Gadolinium-based contrast agents Iron oxide and other superparamagnetic nanoparticles (USPIO, SPIO)	mM to nM	High tissue contrast and functional parameters No ionizing radiation	High cost Operator-dependent
Nuclear imaging	PET 1–2 mm SPECT 0.5–2 mm	10^{-11}–10^{-12} 10^{-10}–10^{-11}	Positron or gamma ray emitting radionuclides (^{18}F, ^{64}Cu, ^{99m}Tc tracers)	pM	Molecular and functional parameters High sensitivity	Ionizing radiation Limited spatial resolution (mm) High-medium cost
X-ray computed tomography	30–400 µm	10^{-2}–10^{-3}	Iodinated particles Gold nanorods	mM to nM	Fast acquisition time High temporal resolution (1–300 s) Provides molecular and structural information	Ionizing radiation Low soft tissue contrast resolution Medium cost
Fluorescence tomographic imaging	1–2 mm	10^{-10}–10^{-11}	NIR Fluorophores	nM to pM	High sensitivity No ionizing radiation Low cost	Limited depth of penetration (1–20 mm) Limited spatial resolution (mm)
Photoacoustic imaging	<100 µm	$<10^{-12}$	NIR Fluorophores	nM to pM	High sensitivity No ionizing radiation High depth of penetration (<5 cm) Low cost	Data post-processing and acquisition procedures still being optimized

Non-invasive Molecular Imaging

Table 3. Summary of the major targets for molecular imaging of atherosclerosis recently evaluated in mouse models with features of vulnerability.

Molecular Target	Biological Events	Imaging Techniques	Imaging Probes
VCAM1-R; ICAM1-R; P-selectin	Vascular inflammation	UBM, MRI, PET, SPECT, PAI	Targeted microbubbles, targeted USPIO, ^{18}F-, ^{99m}Tc-labeled VCAM1 antibodies, NIR Fluorophores
Phosphatidylserine	Apoptosis, vulnerable plaque, atherothrombosis	MRI, SPECT, FMT	Targeted USPIO, ^{99m}Tc-labeled annexin 5 or other tracers, NIR dyes conjugated with annexin 5
$\alpha_v\beta_3$	Neoangiogenesis	MRI, PET, FMT	Gadolinium-labeled RGD probes, ^{18}F-labeled RGD or other tracers, NIR dyes conjugated with RGD or other probes
GPVI-R	Platelet adhesion, atherothrombosis	UBM, PET	Targeted microbubbles, ^{64}Cu-labeled GPVI fragment
GP IIb/IIIa-R	Platelet adhesion, atherothrombosis	UBM	Targeted microbubbles
Fibrin-fibronectin complex	Atherothrombosis	MRI, SPECT	Gadolinium-labeled CLT1 peptide or other agents, ^{99m}Tc-labeled antibodies
Von Willebrand factor	Atherothrombosis	MRI, SPECT	Targeted microbubbles,
LOX-1	Macrophagic lipid uptake	MRI, SPECT	Targeted USPIO, ^{99m}Tc-labeled antibodies
TSPO	Activated macrophages	SPECT	[^{125}I]iodo-DPA-713
Cathepsins and metalloproteinases	Macrophagic proteinases activity	FMT	NIR dyes
Macrophages infiltration	Macrophage-rich, rupture-prone plaques	CT, MRI, PET, FMT, PAI	Liposomal-iodine formulations, PEGylated gold nanoparticles, gold-coated iron oxide nanoparticles targeted for CD163 receptor antibody, trimodality ^{64}Cu- iron oxide-NIR dye nanoparticle targeted for CD68, ^{18}F-LyP-1 targeted for p32, NIR Fluorophores

Non-invasive Molecular Imaging

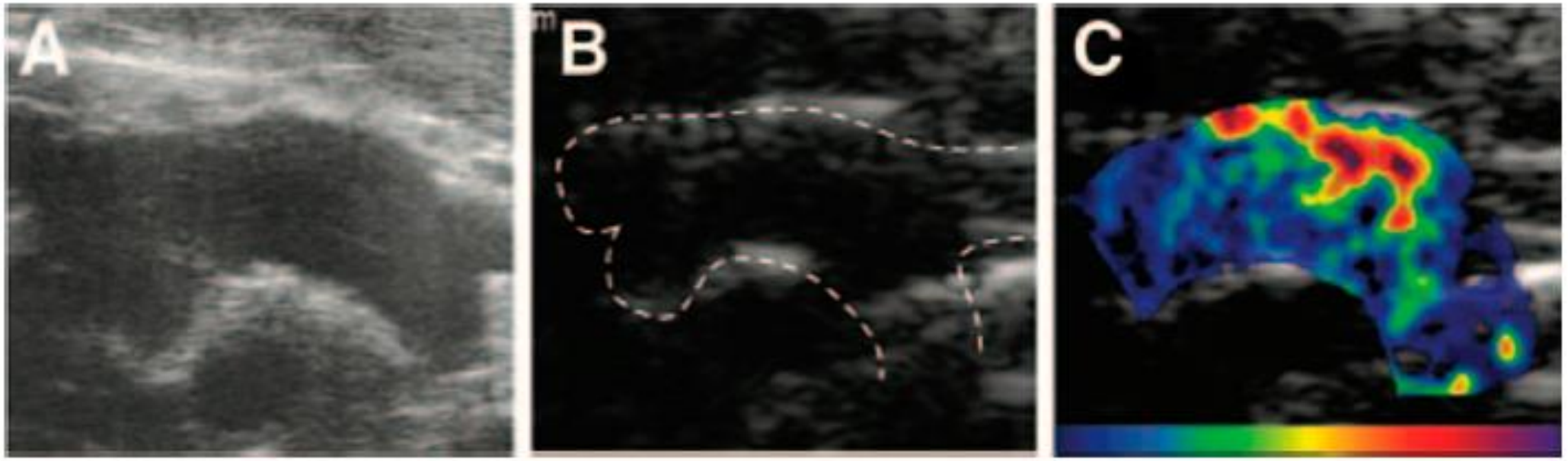


Figure 5. Illustration of spatial matching between morphology and targeted signal enhancement. (**A**) High-frequency ultrasound (40 MHz) image at the level of the aortic arch in a 10-week-old DKO animal; (**B**) Lower frequency multipulse contrast-specific imaging of the aorta at baseline, with the aorta defined by dashed lines, before contrast administration and (**C**) 10 min after administration of P-selectin–targeted microbubbles after background subtraction and color-coding (color scale at bottom). (Reprinted from Reference [220]. Copyright with permission from © 2010, Wolters Kluwer Health.)

Non-invasive Molecular Imaging

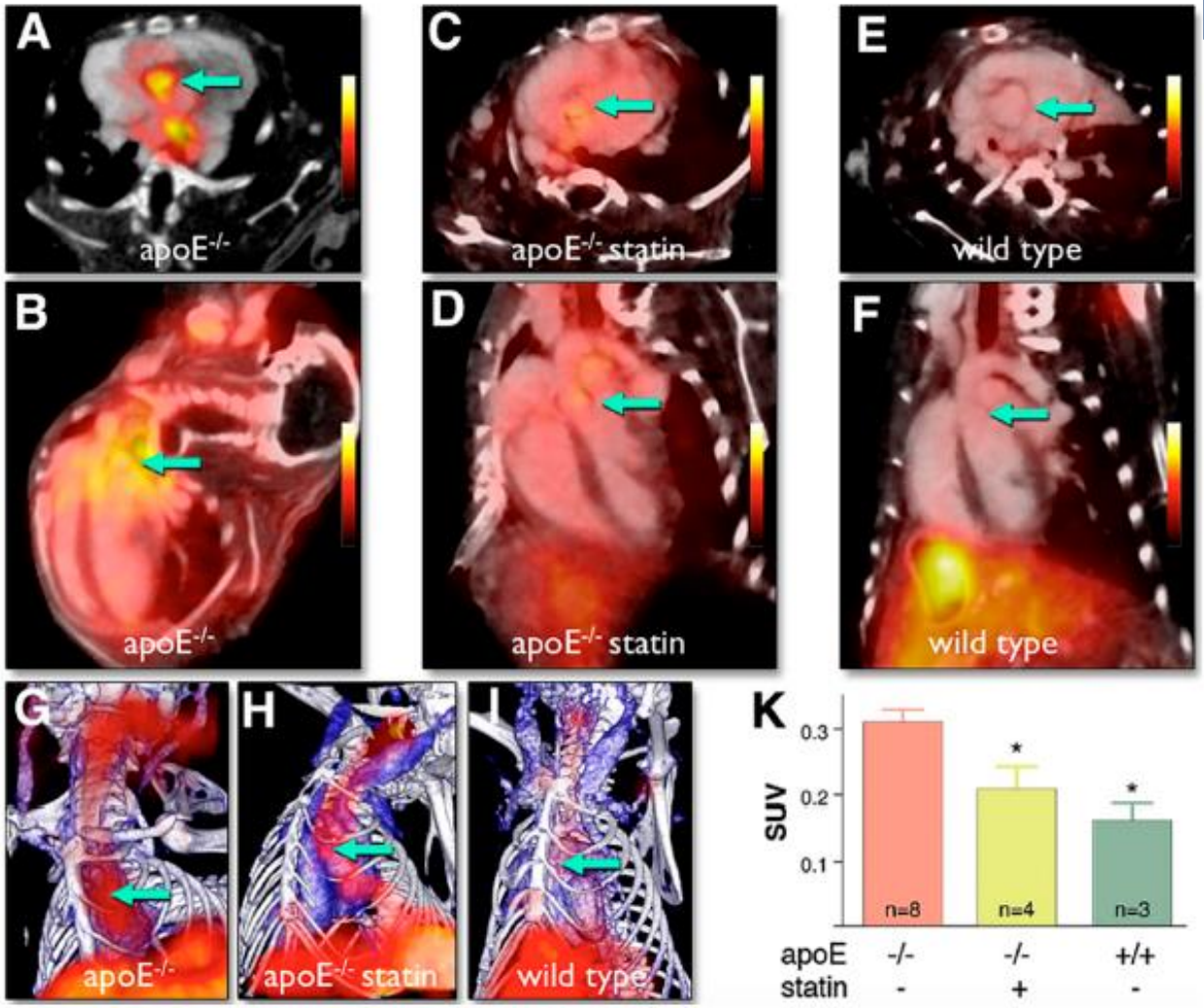


Figure 7. PET-CT in ApoE⁻/⁻ and statin-treated mice. PET-CT imaging shows uptake of 18F-4V in the aortic root (arrows) and arch of atherosclerotic mice. Uptake is lower in statin-treated and in wild-type mice.

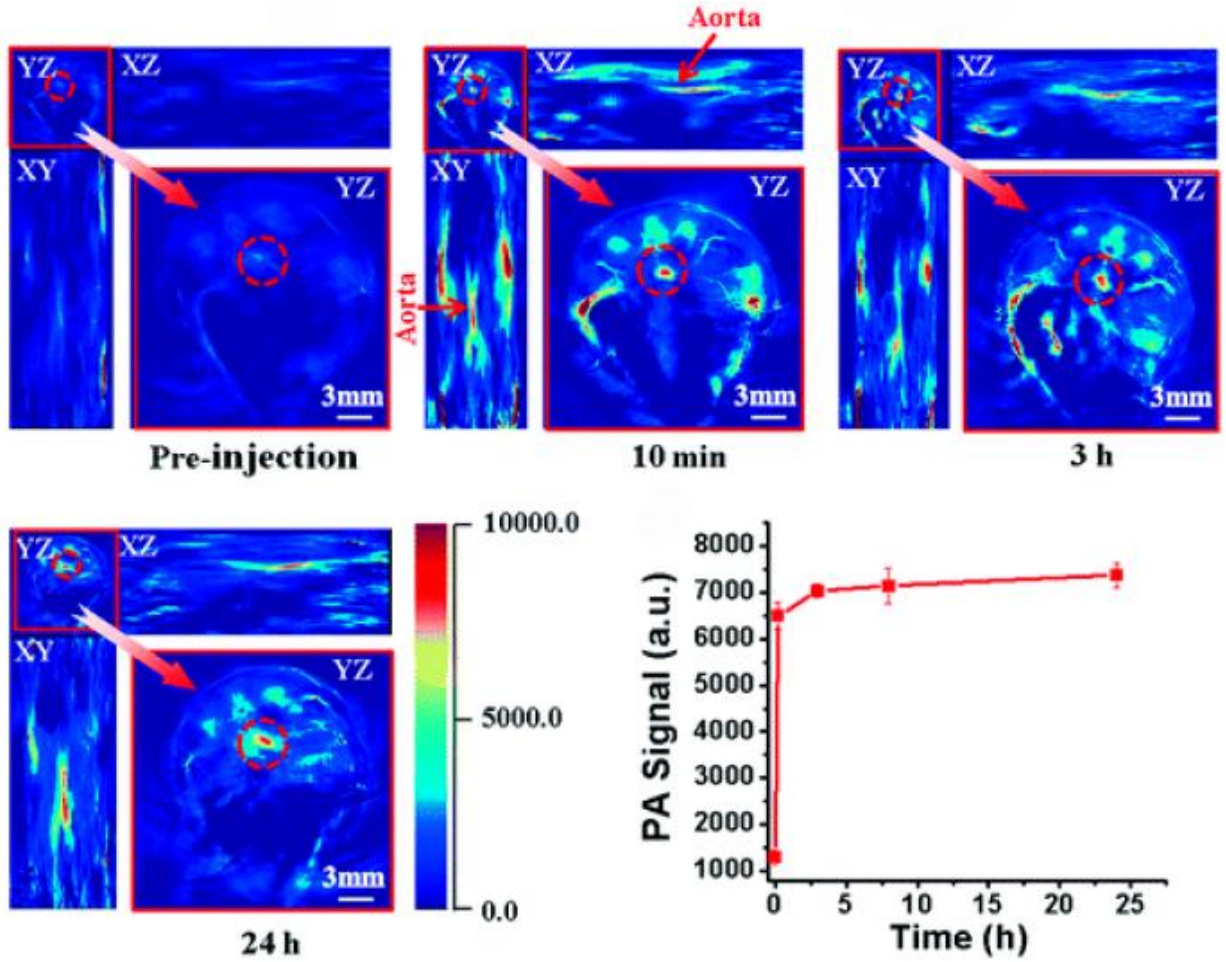


Figure 9. Representative PA imaging of abdominal aorta in an ApoE⁻/⁻ mouse after intravenous injection of ICG@PEG-Ag2S (longitudinal and transverse view): a low contrast in the whole body of the mouse is evident, while a remarkable enhancement of the PA intensity in the region of the aorta (as indicated by red arrows and red circles) was observed over time.