MAPPING THE CELLULAR ORIGINS OF ATHEROSCLEROTIC PLAQUE

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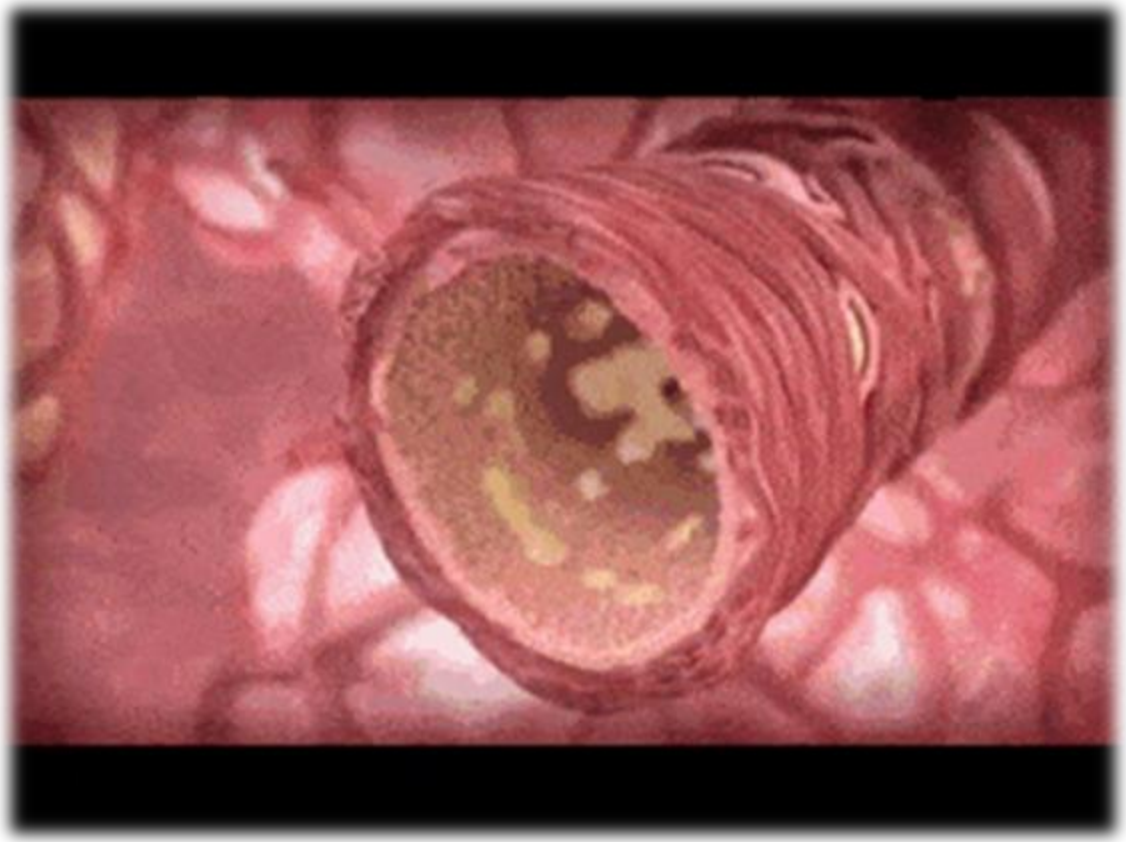
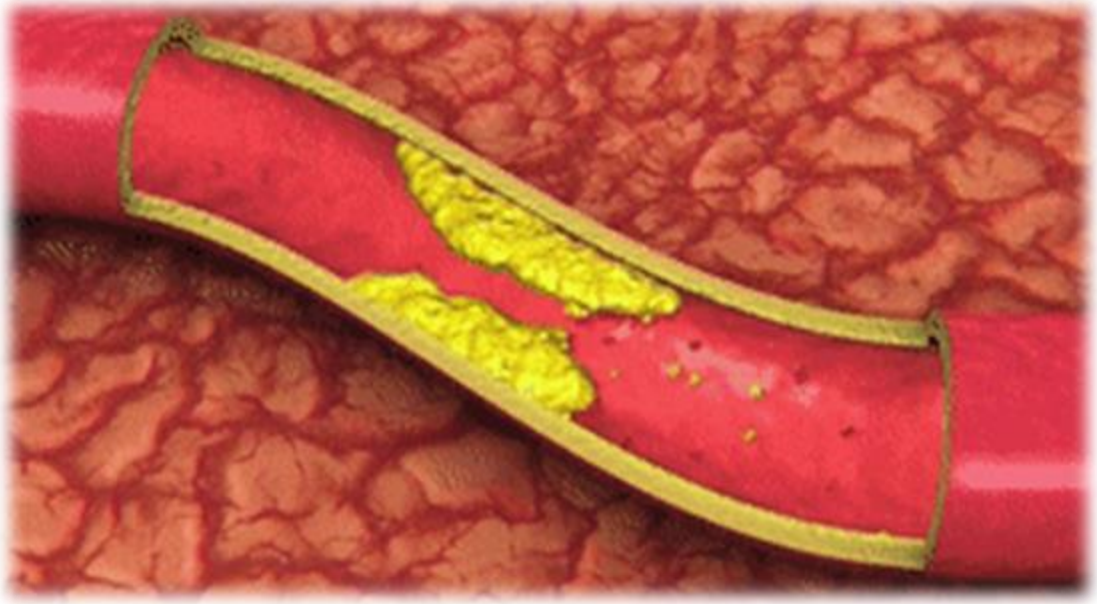
ATHEROSCLEROSIS





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Gifs by gifycat.com

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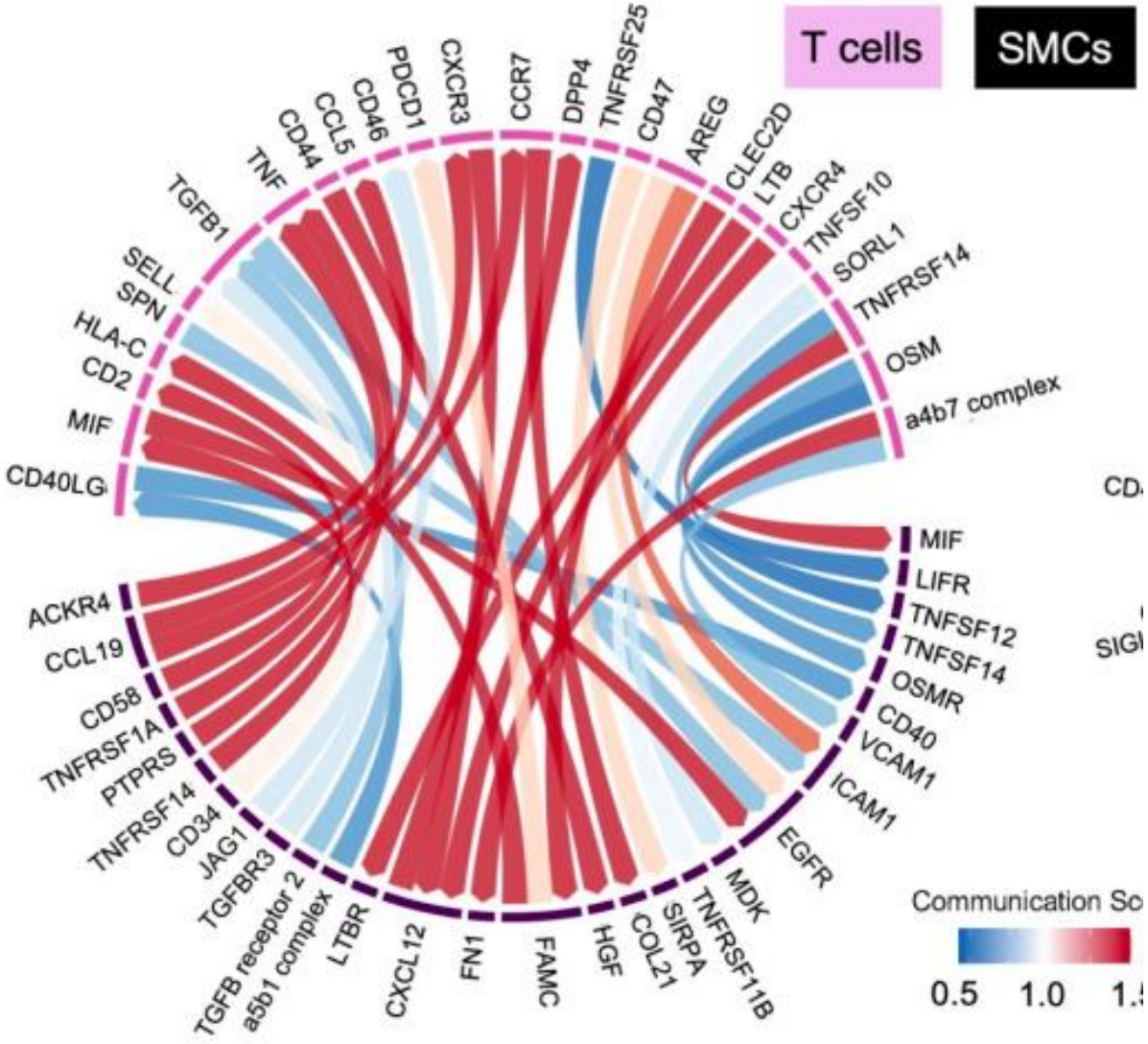
AMPHIREGULIN

AREG: secretion factor involved in fibrotic development

- possible inducer of plaque development
- chosen factor of interest



Chowdhury *et al.* 2022

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STATEMENT OF AIMS

Hypothesis: We hypothesize that immune cells play a role in the transition of smooth muscle cells to myofibroblasts and fibroblasts. We hypothesize that AREG is a prevalent secretion factor in the development of fibrosis.

Aim 1: To use morphology to identify the plaque structure and environment

Aim 2: To use protein indexing technology (CODEX), spatial transcriptomic analyses (Visium), and targeted RNA visualization (RNAscope) to visualize immune cell role in various stages of plaque and the cellular interactions with AREG

Aim 3: To create a rainbow-mouse model and use PCSK9 gain of function to induce atherosclerotic development in a control and double AREG knockout mouse to then isolate the plaque, compare the morphology, and lineage trace plaque components

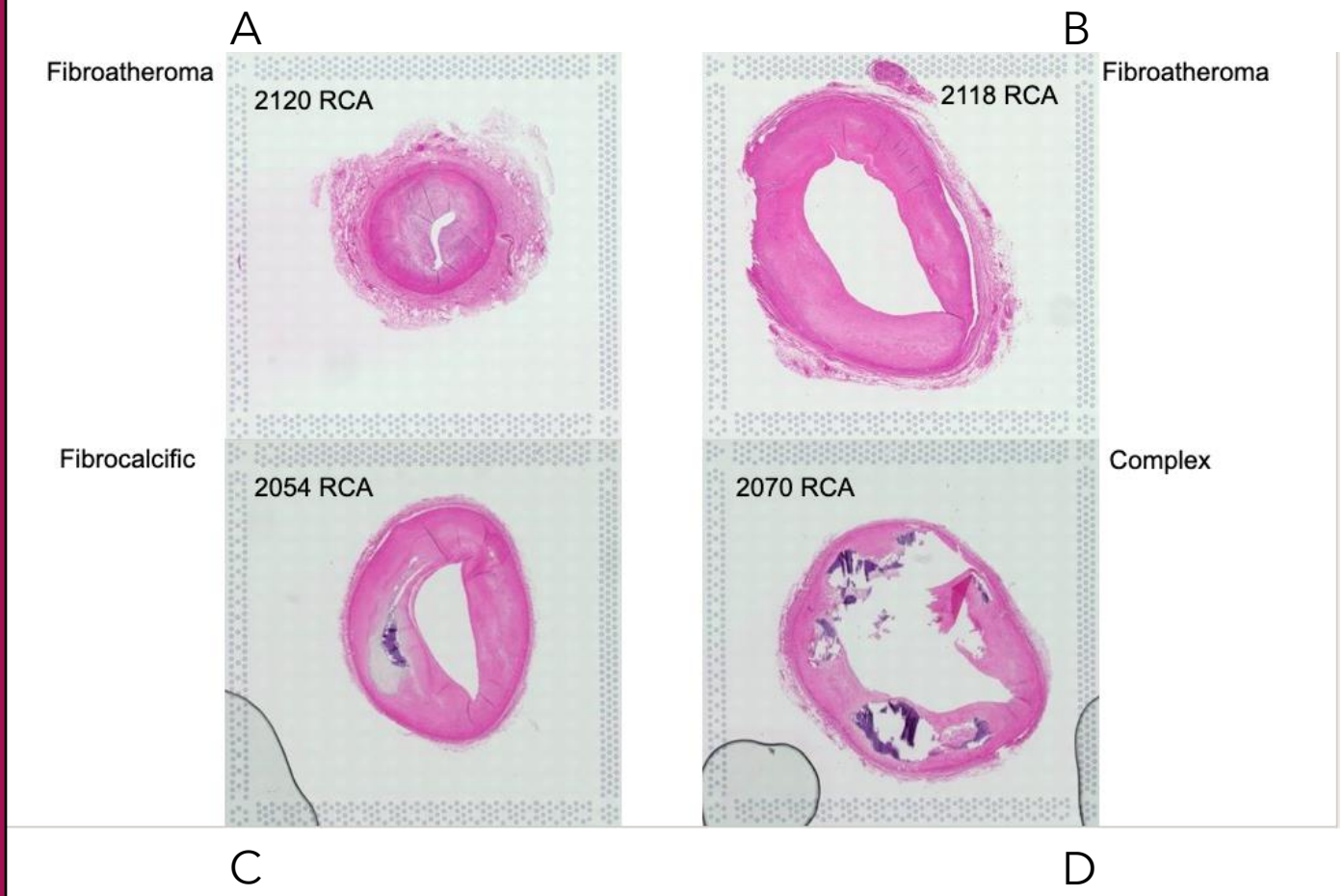
METHODS

- Embedding:
  - Coronary arteries are received from recent heart explants and fixed in NBF or PFA for 24 hours
  - Arteries are then processed and placed in 70% EtOH or PBS (depending on embedding method)
  - Tissues are embedded and sectioned onto slides prior to histology
- Histology
  - Hematoxylin and Eosin: stains for the nuclei (H) and cytoplasm (E)
  - Movat: can visualize the components of connective tissue, including elastic fibers, collagen, proteoglycans, and fibrinoid material
  - Trichrome: can visualize connective tissue, collagen, and smooth muscle fibers
  - Dab: can visualize the location and distribution of specific antigens of interest (AREG antibody)

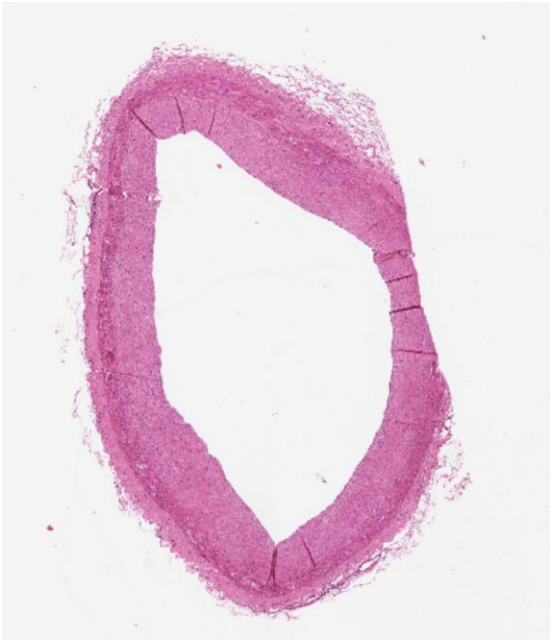
METHODS

- CODEX:
  - Perform slide preparation and antigen retrieval prior to Enable Medicine's CODEX protein marking and imaging (Black *et al.* 2021 and Sanchez-Molina *et al.* 2022)
- Visium:
  - Spatial transcriptomic analysis technology that enables the profiling of gene expression in tissues with spatial resolution (Hudson & Sudmeier 2022)
  - Capture of mRNA from a tissue section on a microarray slide, followed by cDNA synthesis and sequencing
- RNAscope:
  - RNA visualization of target genes using RNAscope chromogenic assay (Zhang *et al.* 2022)
  - Hybridization of probes (positive, negative, target) and amplification of RNA signal
- Rainbow-mouse/PCSK9 gain of function:
  - Inject rainbow-mice control and rainbow-mouse AREG double knockout with PCSK9 vector via tail vein injection (Boone *et al.* 2019)
  - Sacrifice at specific week intervals and compare plaque development and lineage tracing

RESULTS



E



H&E

- Calcification is often seen as dark purple coloration within the tissues and causes increased fragility of artery integrity
- **A:** middle stage of plaque development
- **B:** middle stage of plaque development

- **C:** dark purple stain indicates a region of calcification within the intimal layer
- **D:** an example of the difficulties of processing calcified arteries as the calcium deposits make regions prone to tearing
- **E:** early stage of plaque development (intimal thickening, but closer to 'normal')

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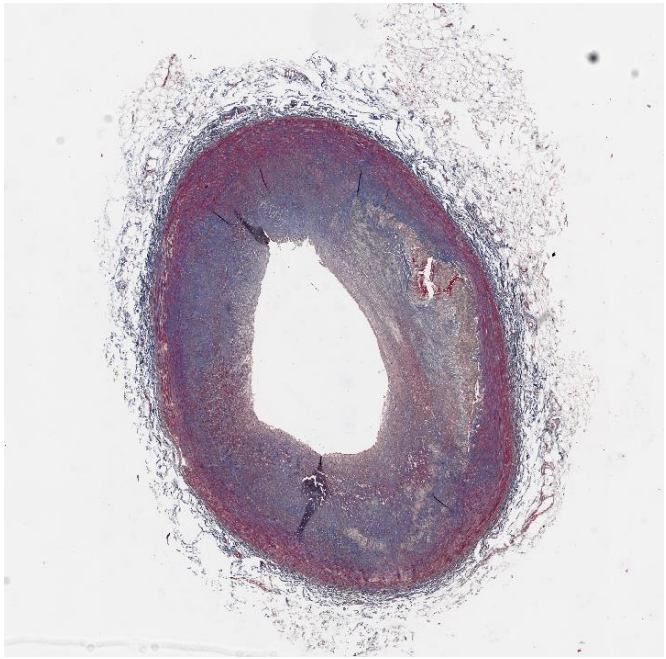
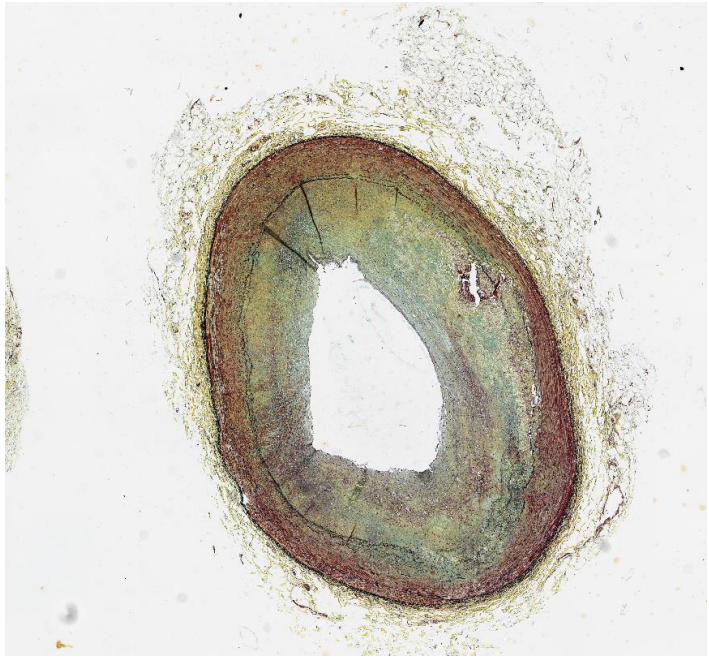
RESULTS



Masson's Trichrome

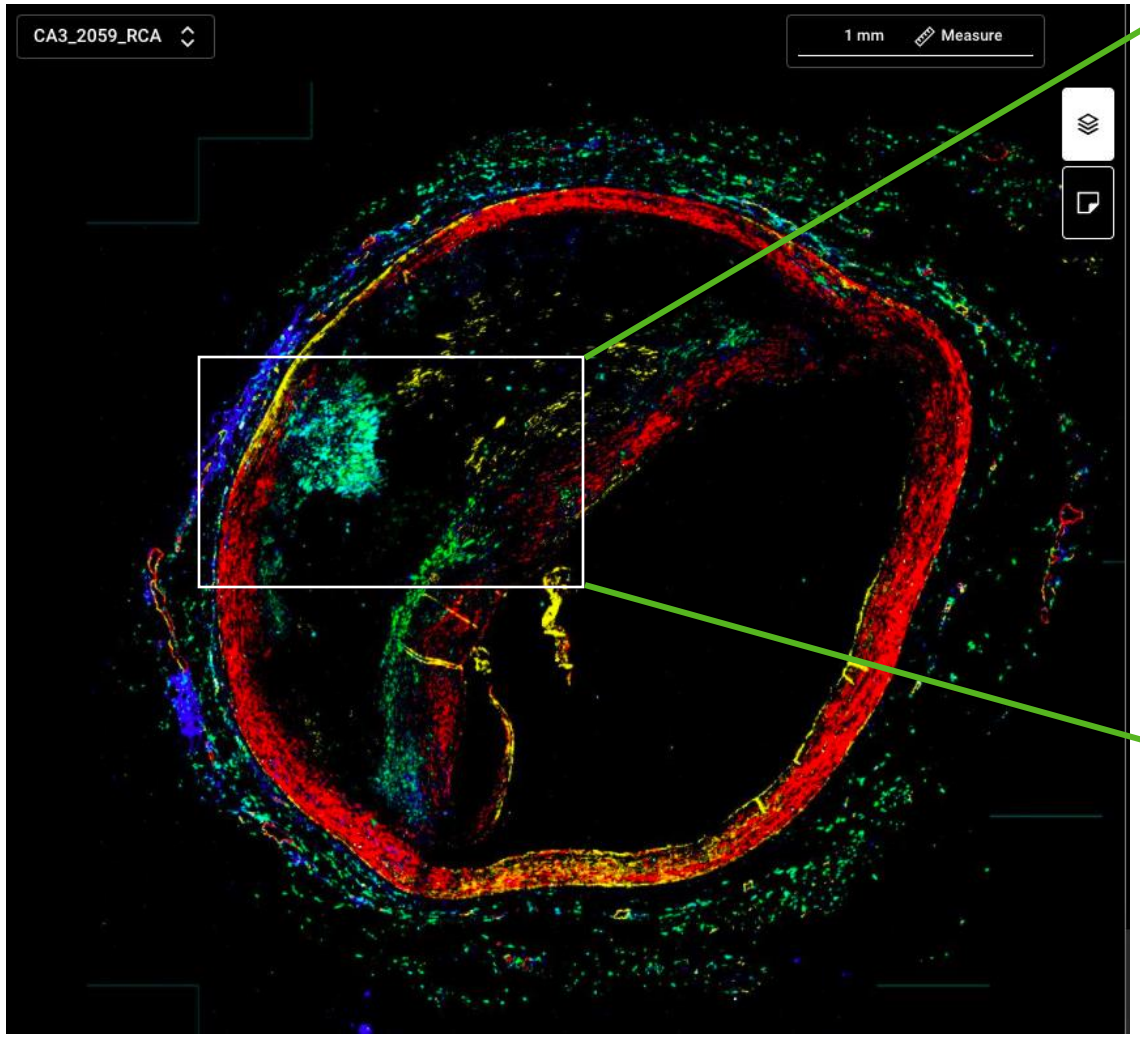
- **Red stain:** smooth muscle layer (media) composed of spindle-like smooth muscle cells
- **Blue stain:** fibrosis and collagen found in the intimal layer during late stage atherosclerosis
- **Pink stain:** cytoplasm
- **Brown stain:** nuclei

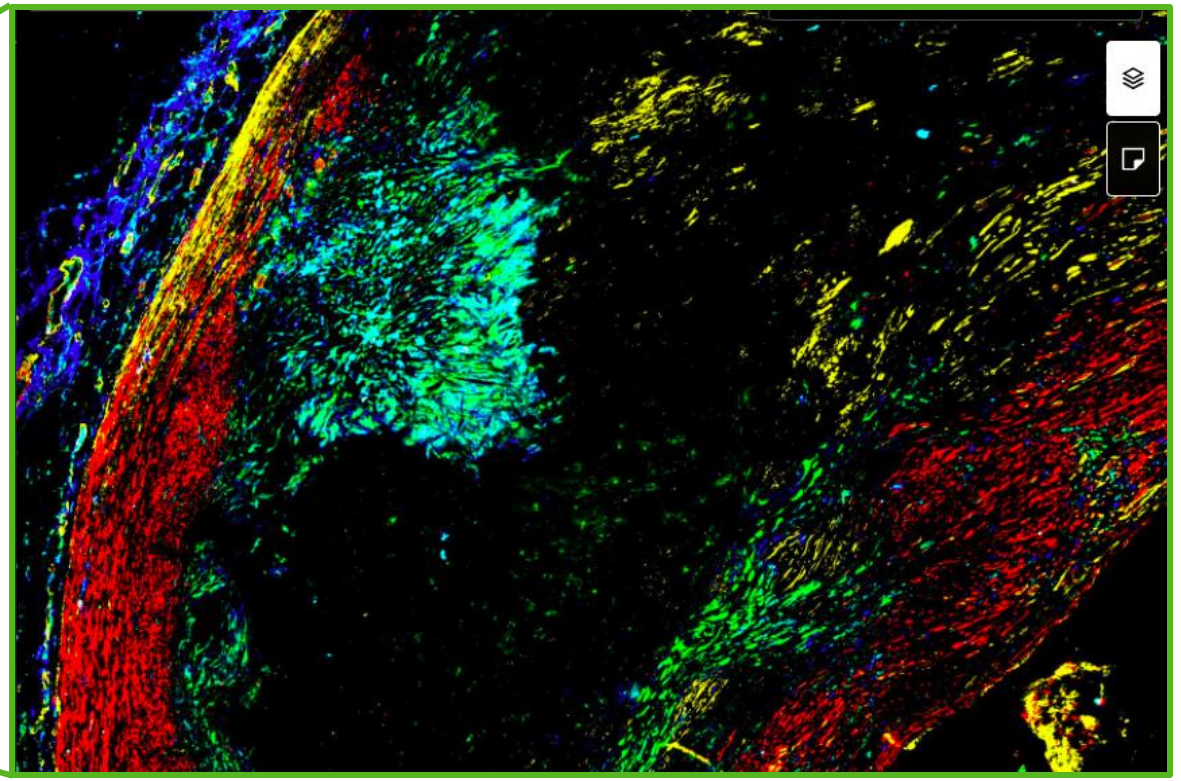


Movat Pentachrome

- **Red stain:** smooth muscle layer (media) composed of spindle-like smooth muscle cells
- **Yellow stain:** fibrosis and collagen found in the intimal layer during late-stage atherosclerosis
- **Purple stain:** elastic fibers that define each layer of the artery
- **Black stain:** nuclei
- **Blue stain:** proteoglycans

RESULTS

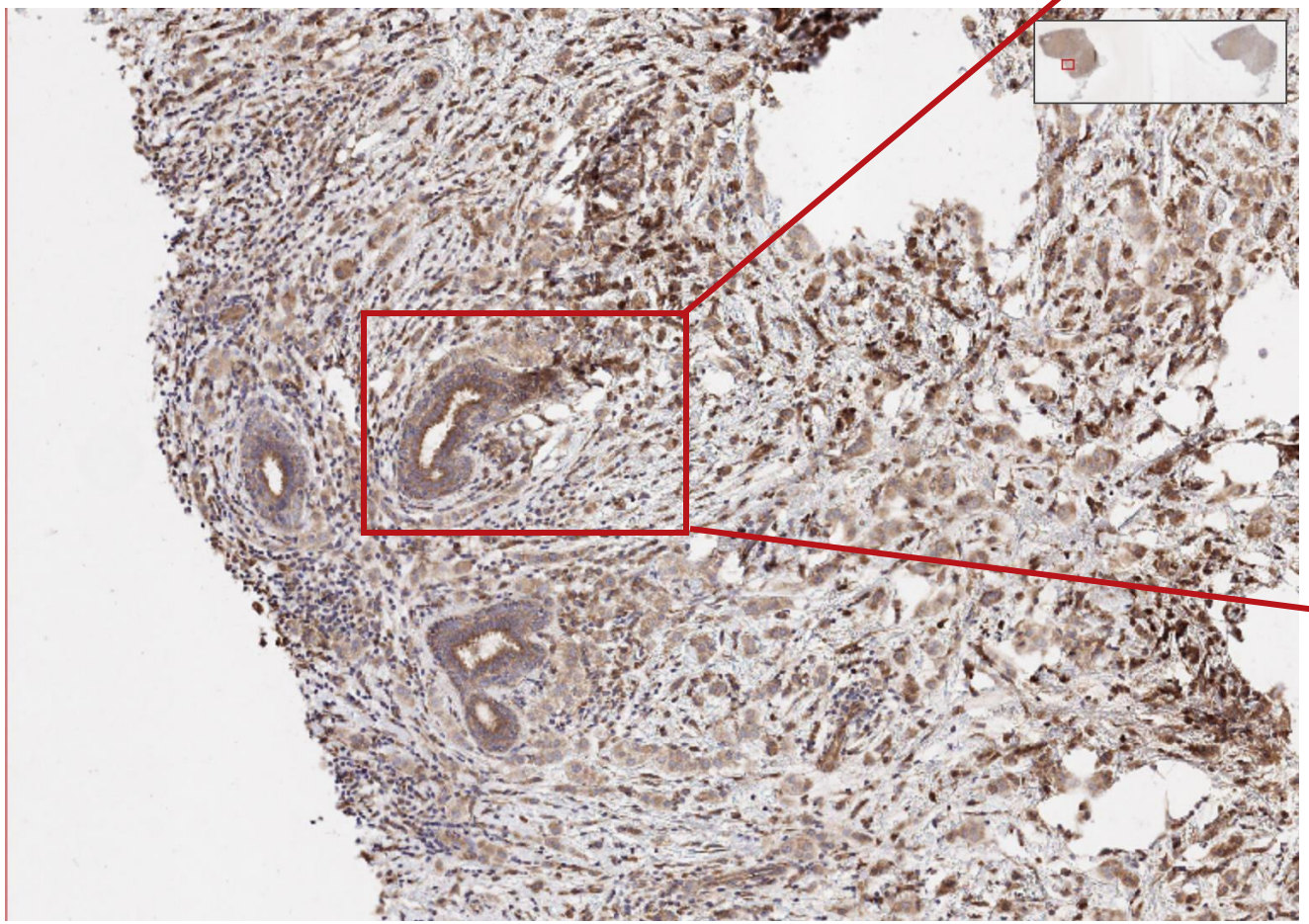


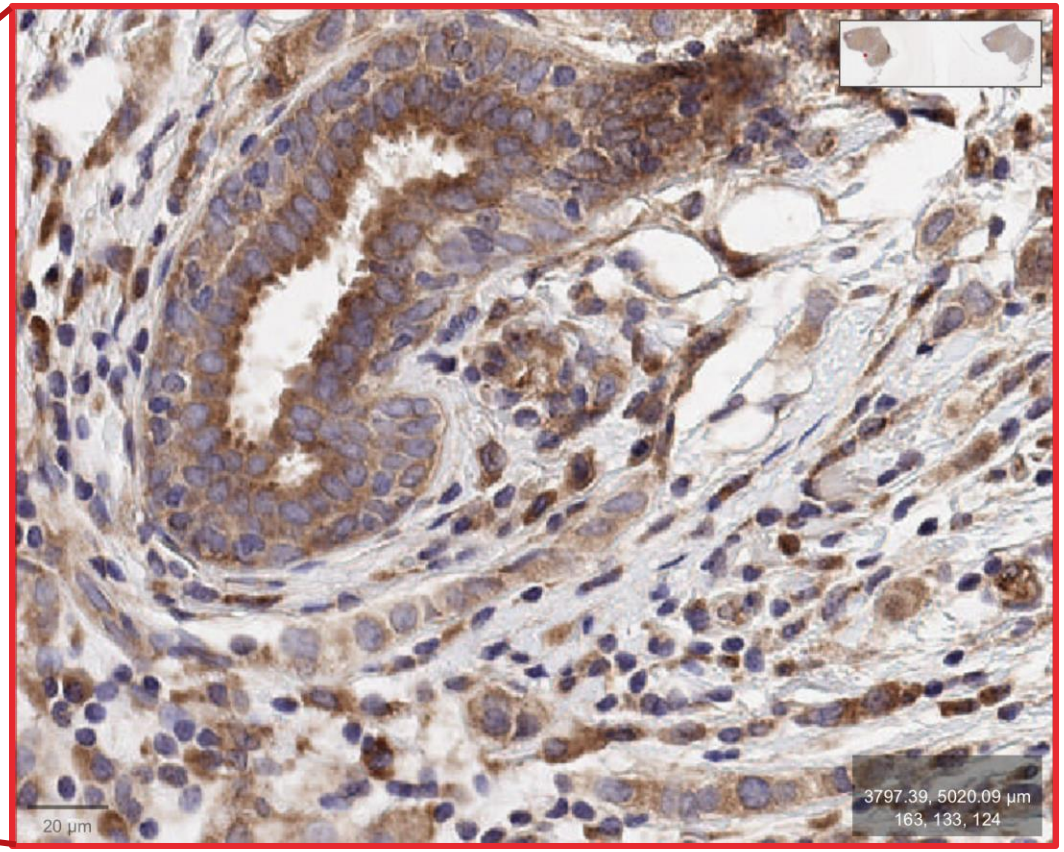


CODEX

- **CD68** (green): macrophages
- **CD8** (light blue): T cells
- **αSMA** (red): smooth muscle cells
- **CD45** (dark blue): leukocytes – general marker
- **CollagenIV** (yellow): collagen (indicative of fibrosis)

RESULTS





DAB – breast tissue

- **Brown:** the presence of AREG + background staining
- **Blue:** nuclei
- AREG is a cytoplasmic stain
- AREG seen in positive tissue contributing to fibrotic development, we should see in coronary arteries

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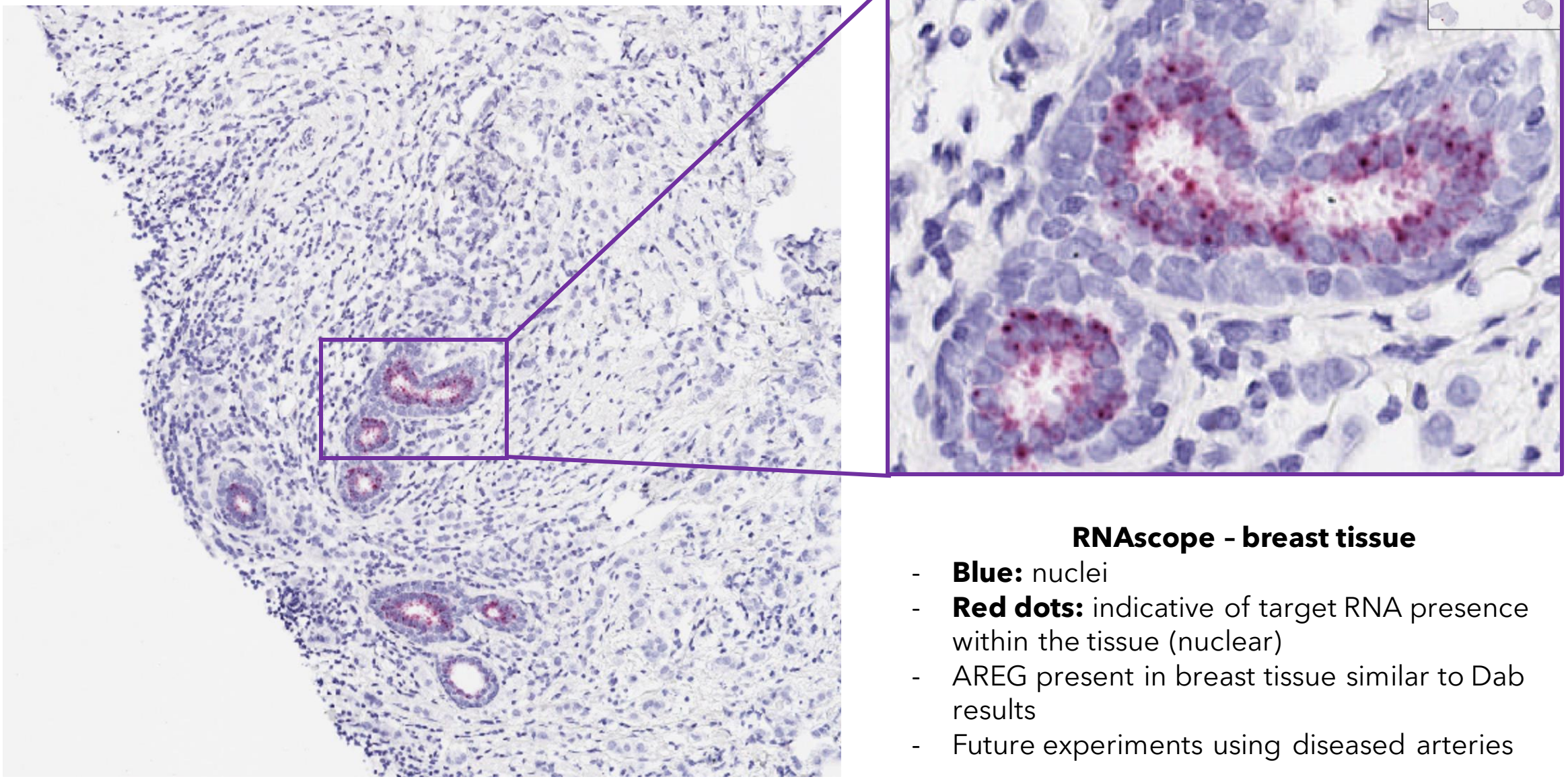
RESULTS



RNAscope – breast tissue

- **Blue:** nuclei
- **Red dots:** indicative of target RNA presence within the tissue (nuclear)
- AREG present in breast tissue similar to Dab results
- Future experiments using diseased arteries

CONCLUSIONS

- Continuing to identify evidence of smooth muscle cell interaction within the plaque
- Amphiregulin is found in many different fibrotic tissues (role in plaque development?)
- Aim to lineage trace rainbow-mice
- Learning more about the plaque niche and cellular components of the plaque itself
- Further experimentation to continue gaining plaque environment knowledge

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