Decreased vascular expression of endothelin converting enzyme-1 and neprilysin in Inflammatory Bowel Disease

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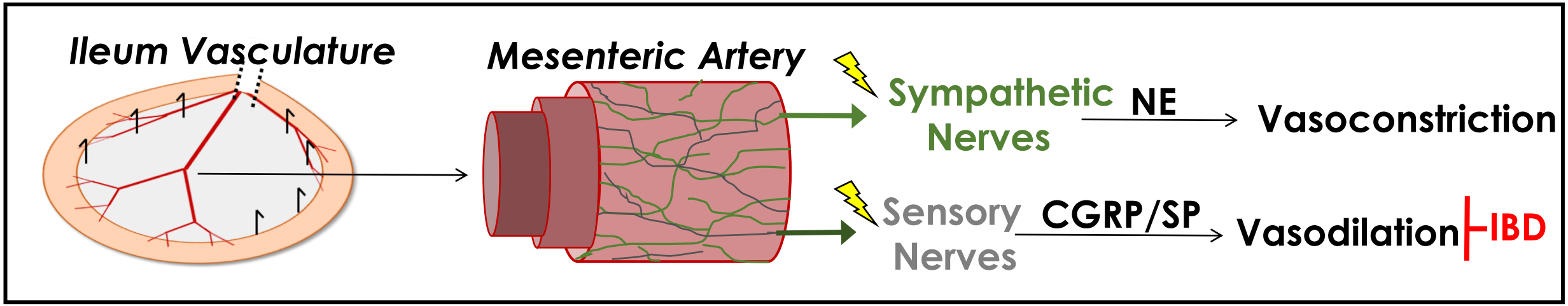
Background

- Inflammatory Bowel Diseases (IBD) are chronic diseases that are diagnosed in around 70,000 Americans each year, and 1.6 million Americans in total.

- Inflammatory Bowel Diseases (IBD) are linked to impaired intestinal blood flow and comorbid with cardiovascular diseases, despite the absence of traditional risk factors

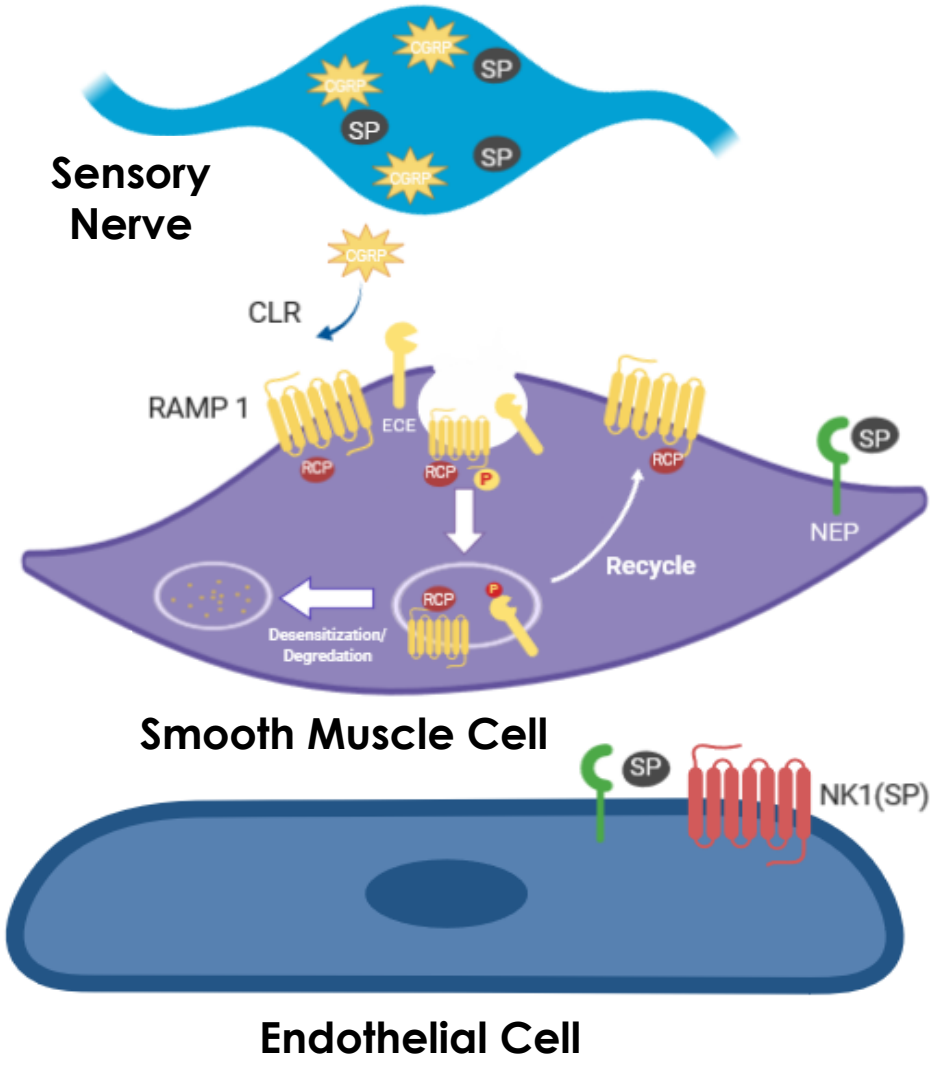
- Perivascular sensory nerves that increase blood flow are impaired with IBD



ECE and NEP

- Sensory nerves release calcitonin gene-related peptide (**CGRP**) and substance P (**SP**) which bind downstream to CGRP (**RAMP1, CLR, RCP**) and SP (**NK1**) receptors

- Endothelin-converting enzyme-1 (**ECE-1**) and neutral endopeptidase (**NEP**) regulate CGRP and SP signaling pathways through peptide degradation and receptor recycling

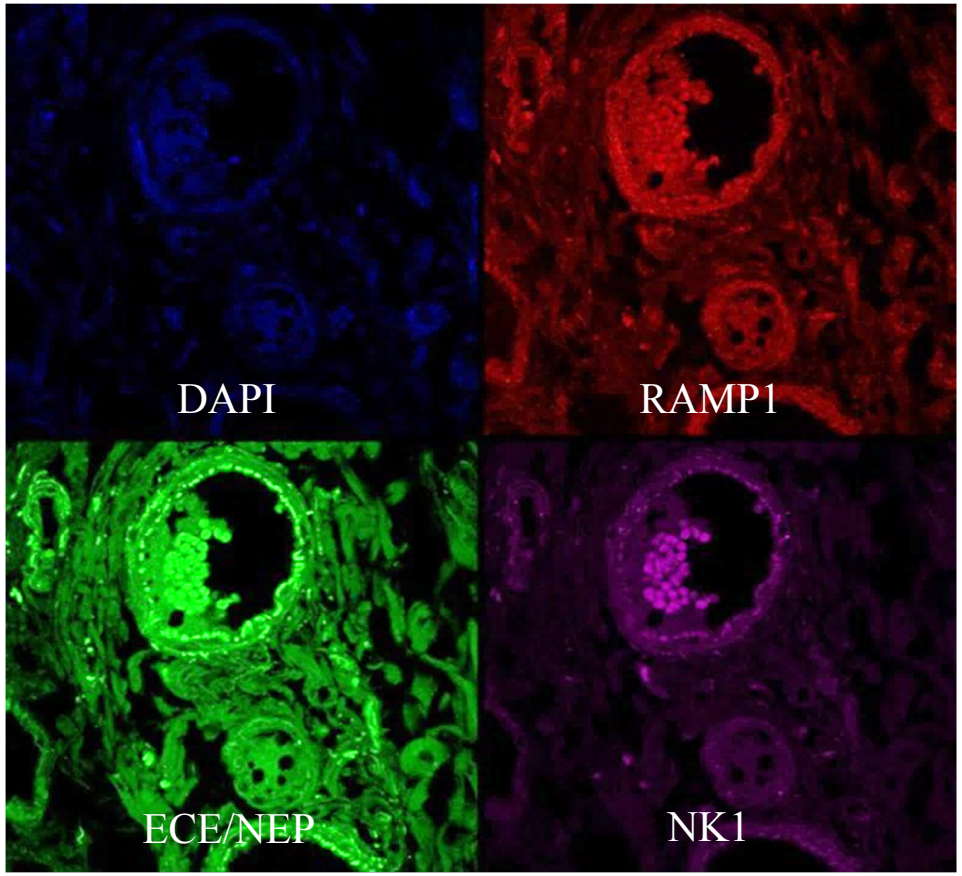


Previous Results and Hypothesis

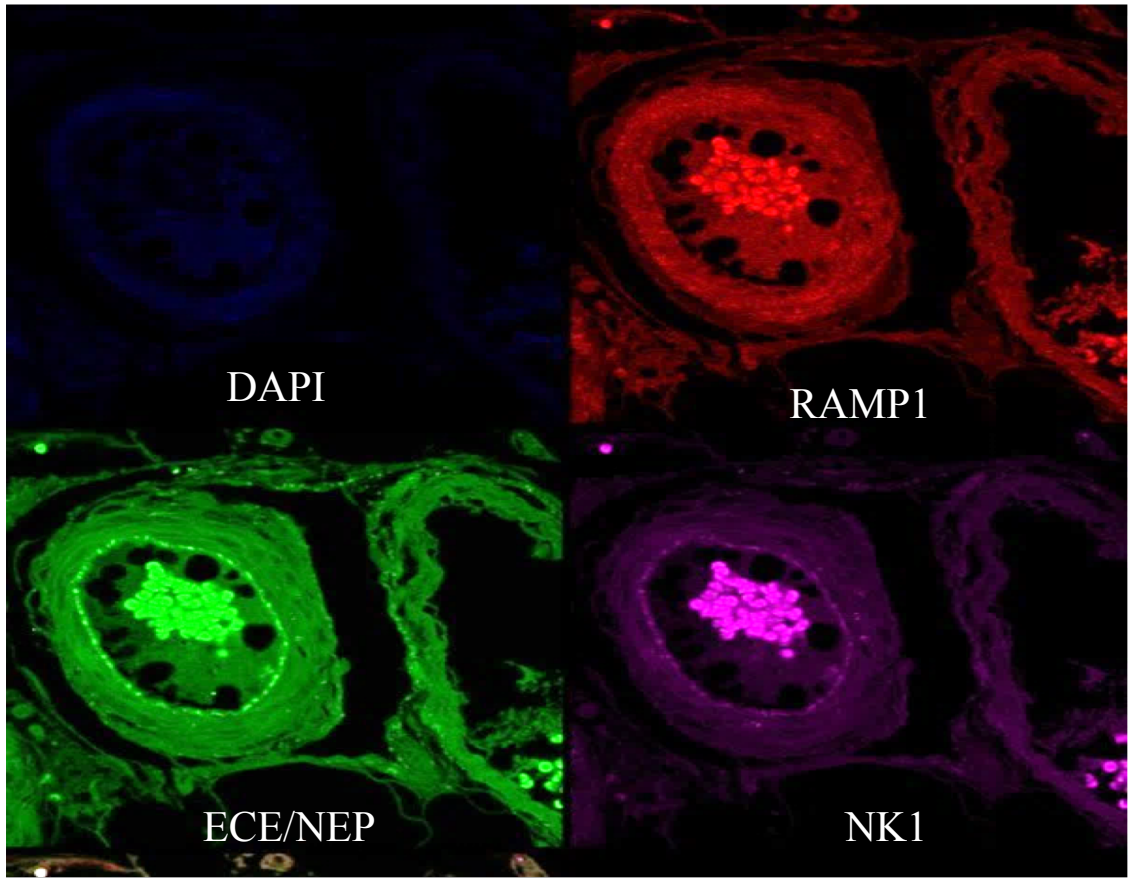
- Previous ELISA results show decreased NEP and ECE-1 concentration in mesenteric arteries (NEP & ECE), colon (NEP), Aorta (ECE) and perivascular adipose (ECE).
- Thus, we tested the hypothesis that: **IBD alters the expression and/or localization of ECE and NEP in mesenteric arteries.**

Human Sample Images

Control

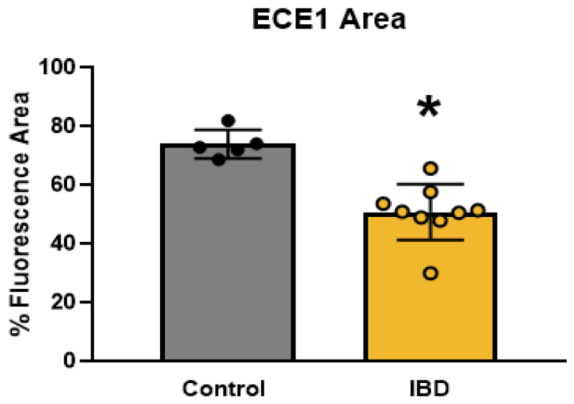


IBD



Human Sample Results

Figure 3: ECE1 Percent Area of Human Colon:
Data are individual values and mean ± SE; (Control: n=5) (IBD: n=9) ($p \leq 0.05$)



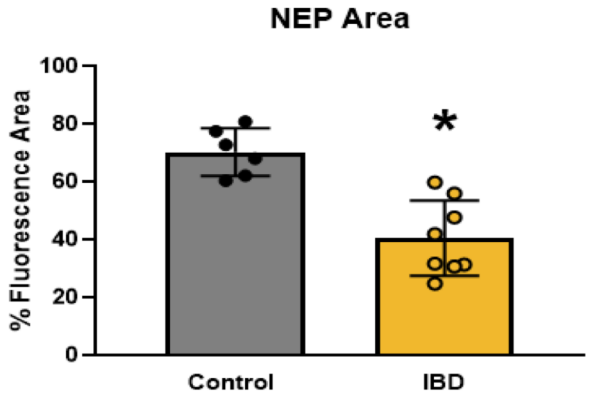
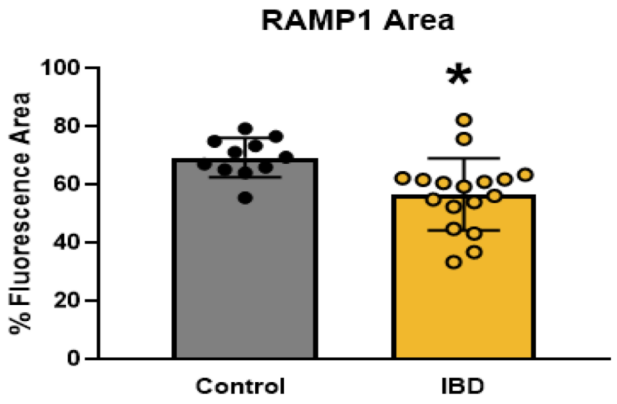


Figure 4: NEP Percent Area of Human Colon:
Data are individual values and mean ± SE; (Control: n=6) (IBD: n=8) ($p \leq 0.05$)

Figure 5: RAMP1 Percent Area of Human Colon:
Data are individual values and mean ± SE; (Control: n=11) (IBD: n=18) ($p \leq 0.05$)



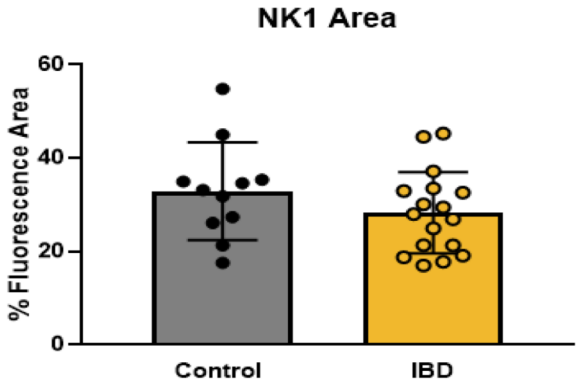


Figure 6: NK1 Percent Area of Human Colon:
Data are individual values and mean ± SE; (Control: n=11) (IBD: n=17)

3D Confocal Microscopy Images

Control

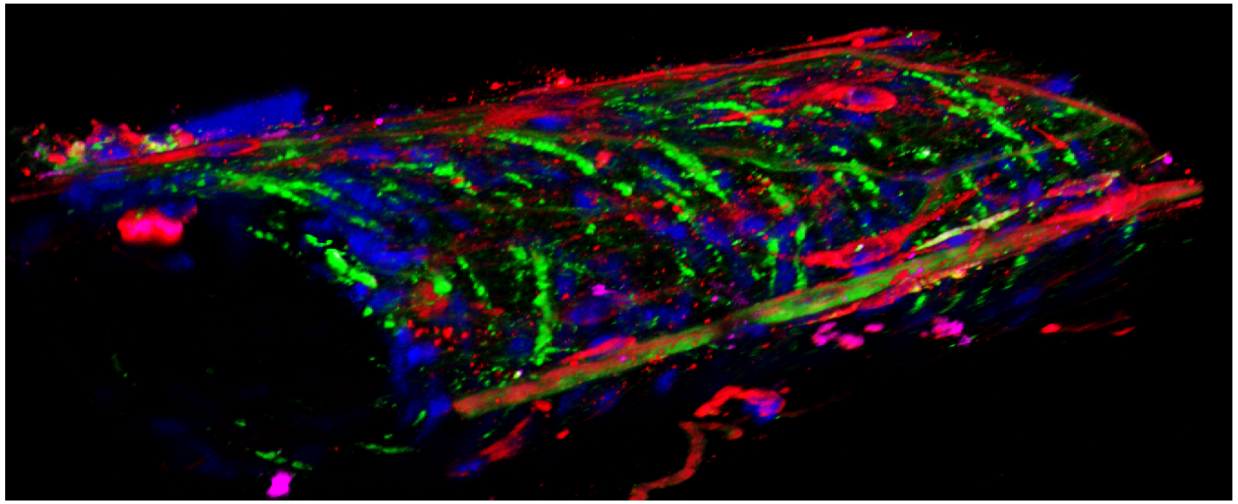


Figure 7: Confocal Imaging of a cannulated Control mesenteric artery.
Green staining labels for NEP/ECE-1. Red staining labels for RAMP1 (CGRP receptors). Magenta labels for NKI (SP receptors). Blue stain labels for DAPI (nuclei). Figure 7 shows a representative image.

IBD

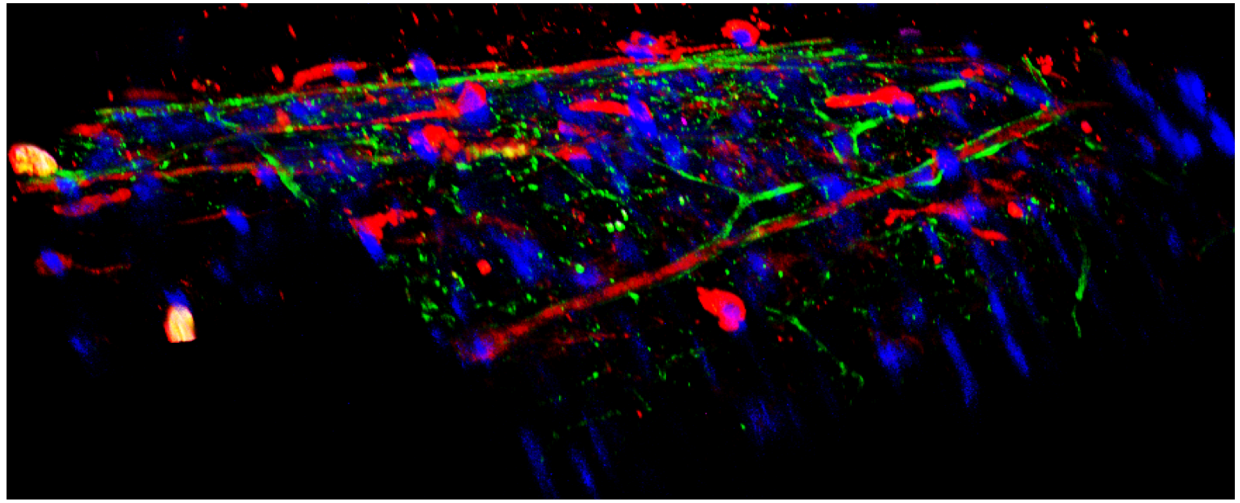


Figure 8: Confocal Imaging of a cannulated IBD mesenteric artery.
Green staining labels for NEP/ECE-1. Red staining labels for RAMP1 (CGRP Receptors). Magenta labels for NKI (SP Receptors). Blue stain labels for DAPI (nuclei). Figure 8 shows a representative image.

3D Confocal Analysis

- We are now beginning to take the head-on sliced images from the 3D confocal microscopy to be analyzed.
- Sliced are analyzed via Image J for percent area as well as the ratio of one staining to another; statistical tests will be run via GraphPad to find any significance.
- Analysis of the 3D images will provide further information, alongside the human sample analysis, to show what effect IBD has on ECE and NEP.

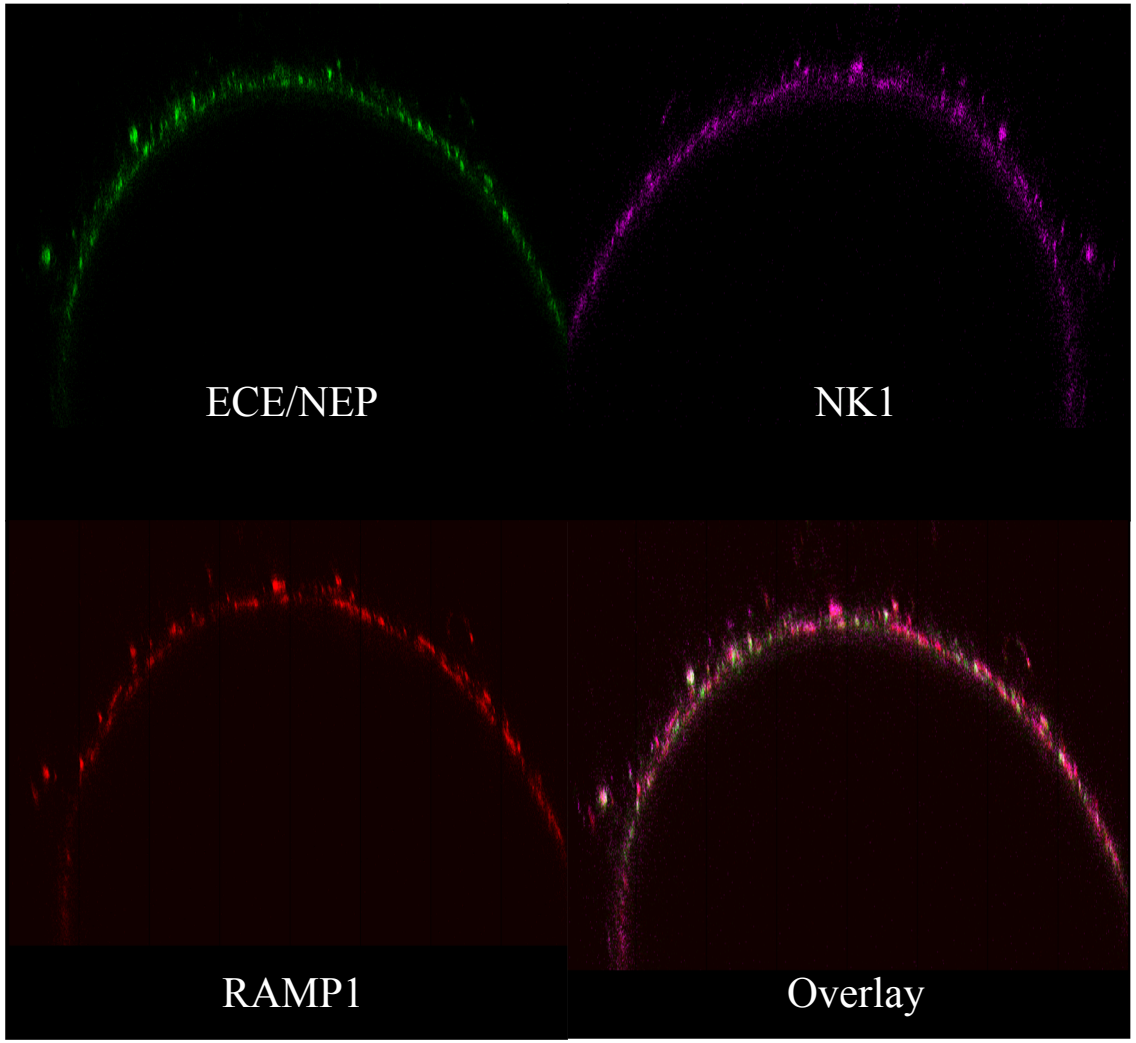


Figure 9: Confocal Imaging (sliced view) of a cannulated IBD mesenteric artery.
Green staining labels for NEP/ECE-1. Red staining labels for RAMP1 (CGRP Receptors). Magenta labels for NKI (SP Receptors). Overlay shown. Figure 9 shows a representative image.

Results

- **ELISA results have shown decreased NEP and ECE expression in multiple tissues associated with vasculature (mesenteric arteries, aorta, perivascular adipose) and the colon.**

- **Confocal imaging of human samples of colon showed a decrease in percent area of ECE1, NEP and RAMP1 in IBD patients.**

- **Current work is being done to examine the vascular expression of ECE1, NEP, RAMP1 and NK1 in cannulated, incubated mouse arteries.**

- **Further analysis from mouse mesenteric arteries will provide more information about how IBD affects CGRP and SP receptor trafficking and degradation as they relate to blood flow through mesenteric arteries.**

Thank You for Listening!