

# The Determination of the Subcellular Location of Spermidine Synthase

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Under direction of Dr. Mark Stayton  
Molecular Biology

Funded by INBRE

# Background

- Since 1900, cardiovascular disease has been the leading cause of death in every year except 1918.
- CVD kills more people each year than the next five leading causes of death combined.

# Global Look at Transcriptome of Mouse Heart After AMI

- Microarray study done by Stayton and collaborators (McCormick and Thomas)
- Surgical mouse model used to do a comprehensive study of early responses to acute myocardial infarction (AMI) across the complete mouse transcriptome
- ARG1 was induced 121 fold.

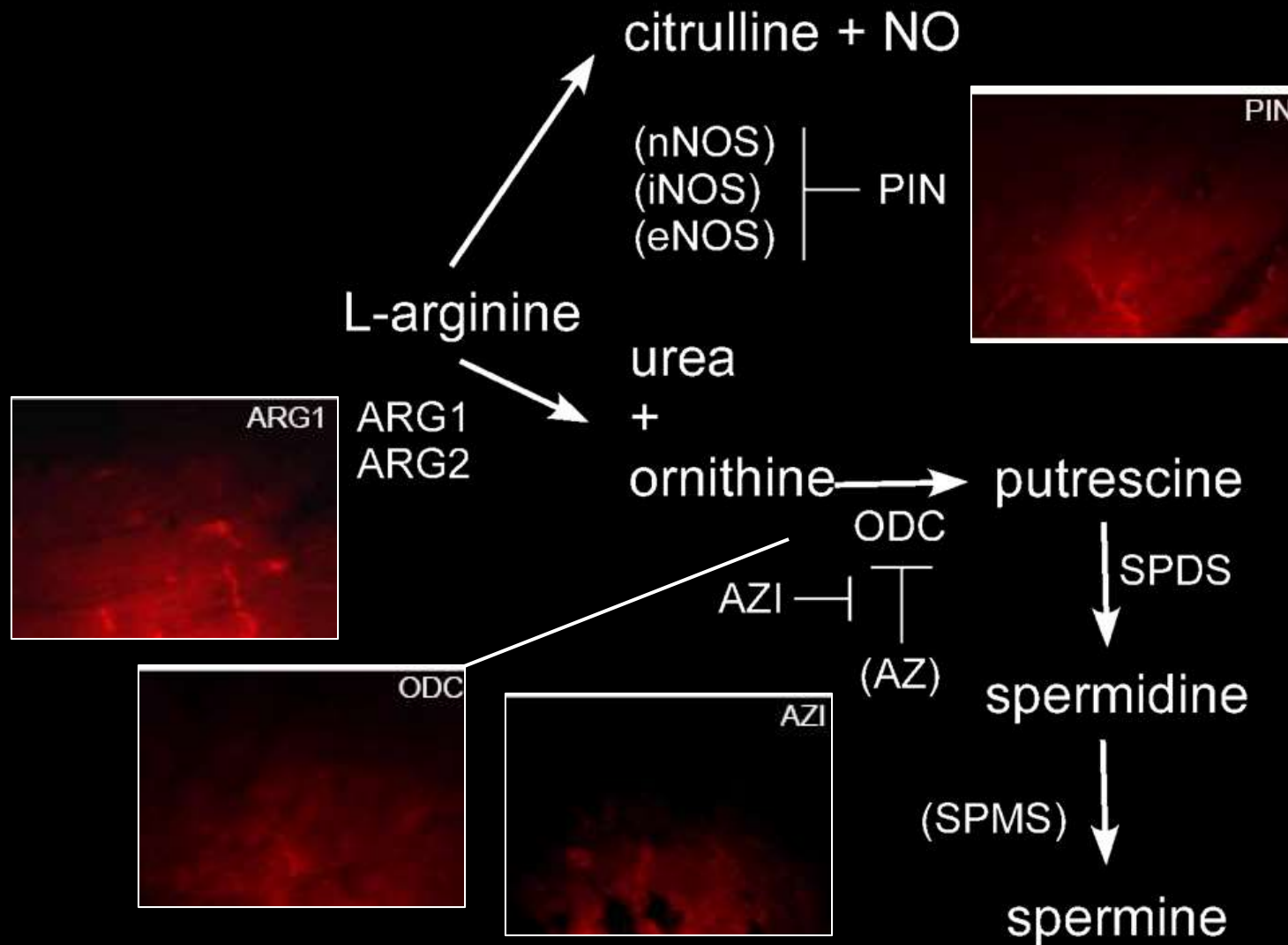


• Earliest changes in the left ventricular transcriptome postmyocardial infarction  
Mark H. Harpster, et al *Mammalian Genome*, 17, 701-715 (2006)

# We've chosen to focus on Nitric Oxide (NO) and L-arginine metabolism: Why?

- NO has multiple potent regulatory effects on the cardiovascular system.
- NO synthases and arginase compete for common substrate, L-arginine: Thus, induction of arginase activity may represent an additional mechanism to regulate and deplete NO levels.
- Although widely studied in the vasculature, arginase induction and polyamine production in cardiomyocytes has not previously been reported.

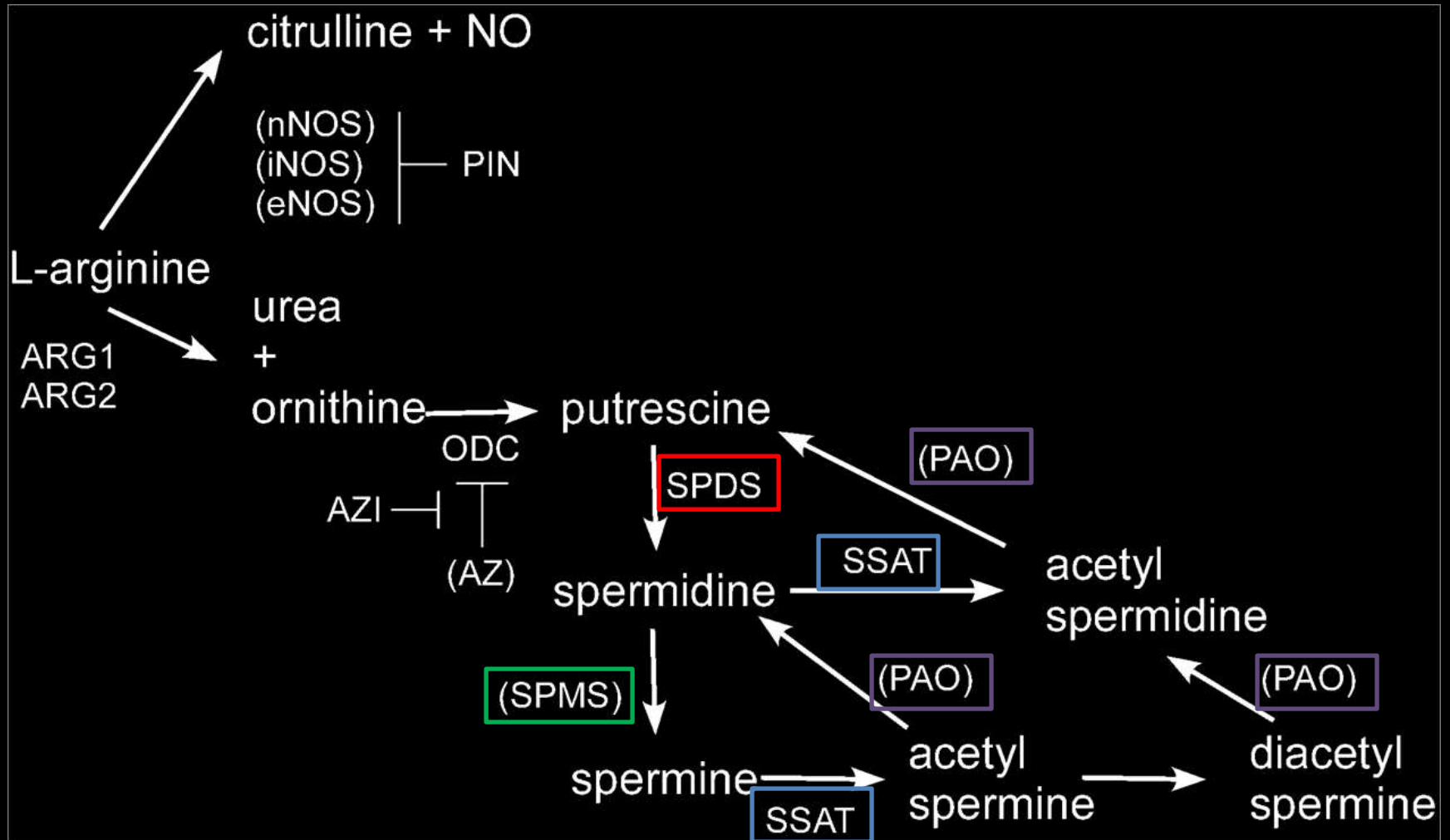
# Previous Work by JA Keele



# Hypothesis

- Given that all up-regulated transcripts are for enzymes localized in the intercalated discs...
- We hypothesized that:
  - SPDS would be found in intercalated discs
  - SPSY and PAO would not be in intercalated discs
  - SSAT ???

# Polyamine enzymes



# Materials and Methods

## Immunohistochemistry

1. Cryosectioned heart tissue 5  $\mu\text{m}$  thick
2. Fix with acetone
3. Stained for proteins using antibodies
  - a) Blocked with serum from 2° ab host
  - b) Primary antibody
  - c) Fluorescent secondary antibody
4. Analyze with fluorescent microscope
5. False color and overlay with ImageJ



# Results/Discussion

- SPDS
- SPSY
- PAO
- SSAT

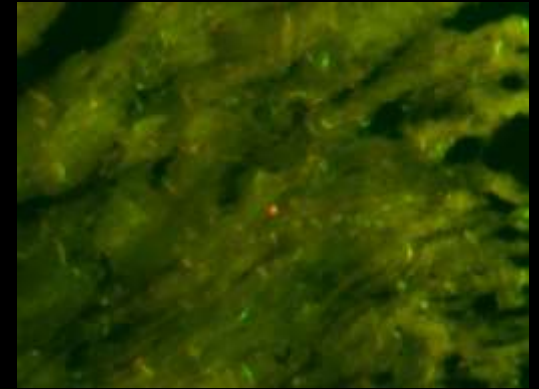
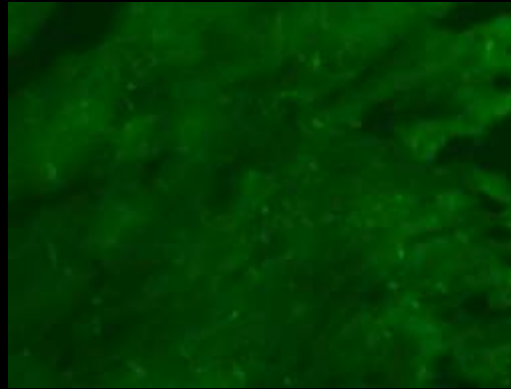
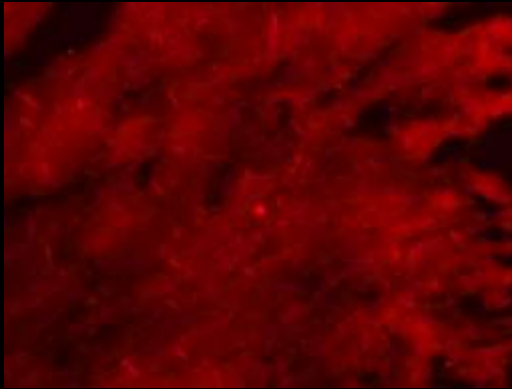
# Spermidine Synthase (SPDS)

SPDS

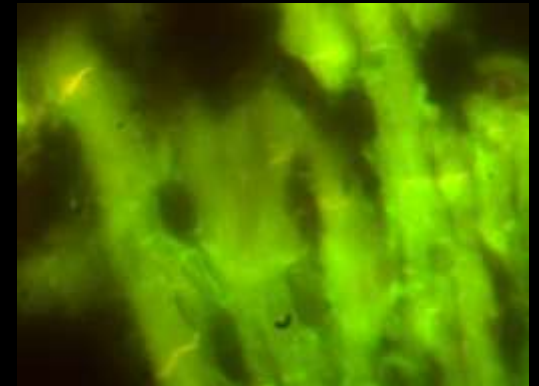
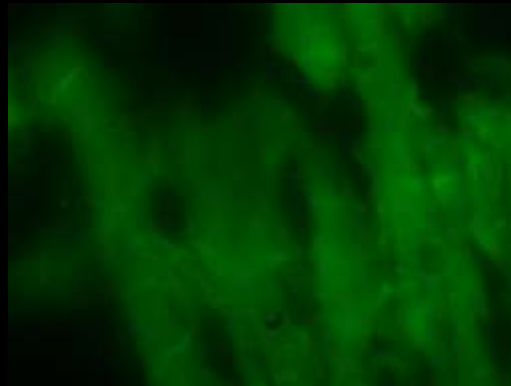
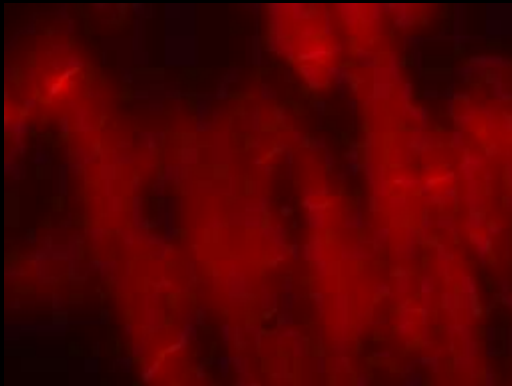
N-cadherin

Merged

40x



100x



# Results/Discussion

- SPDS colocalized with the intercalated discs of cardiomyocytes

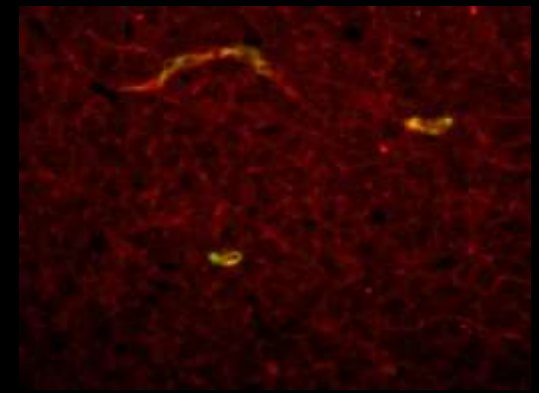
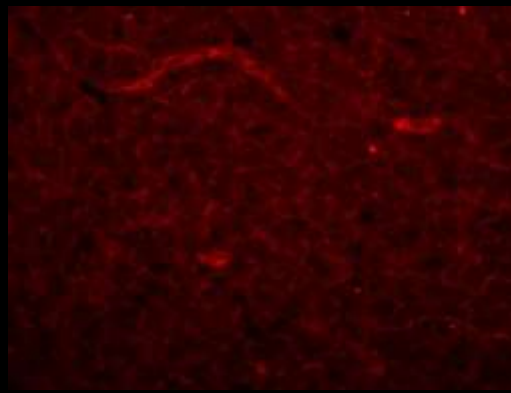
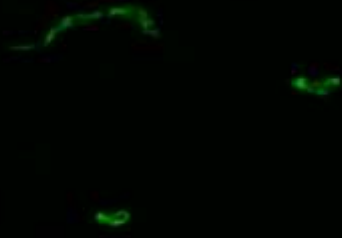
# Spermine Synthase (SPMS)

SPSY

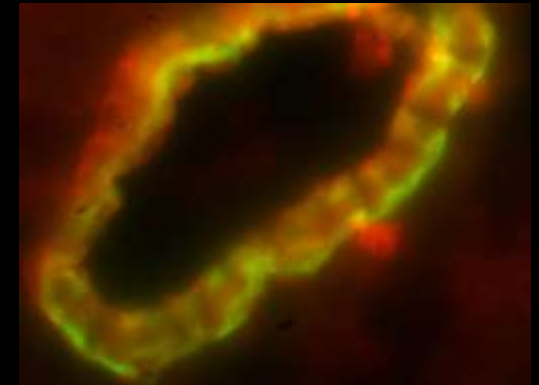
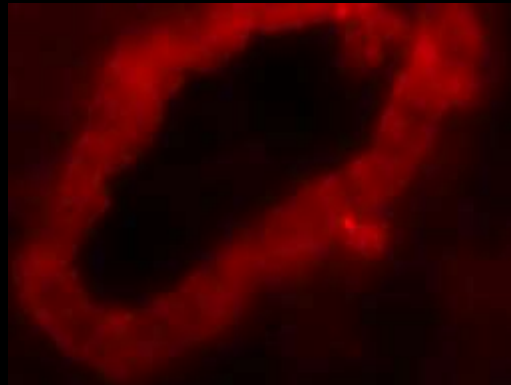
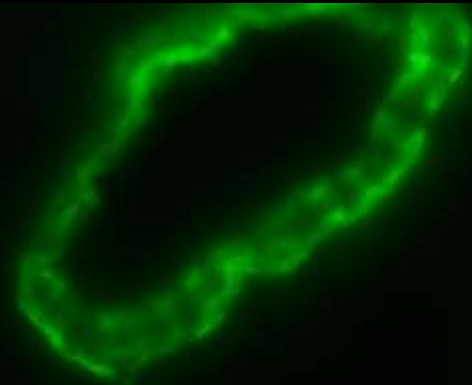
Calponin-3

Merged

40x



100x

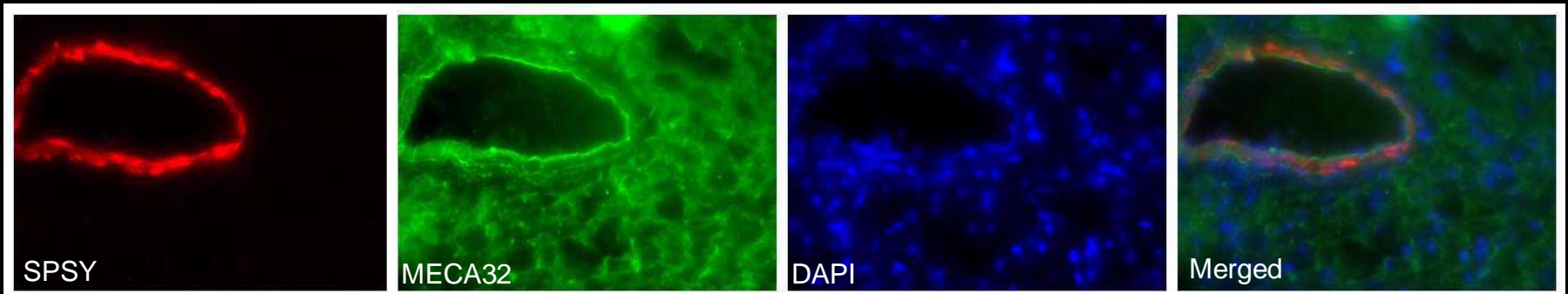
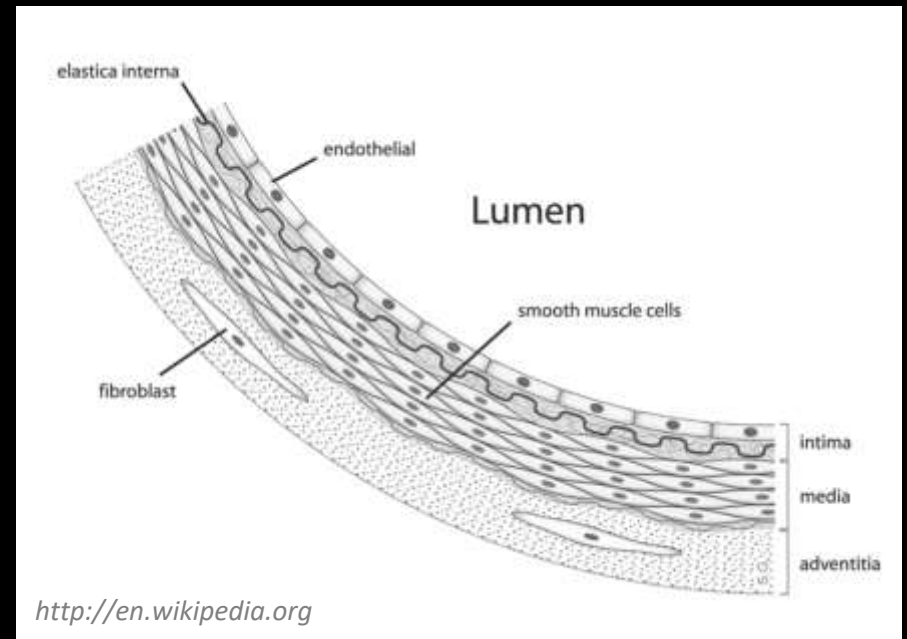


# Results/Discussion

- SPSY:
  - seemed to colocalize with the smooth muscle layer of the vasculature

# Results/Discussion

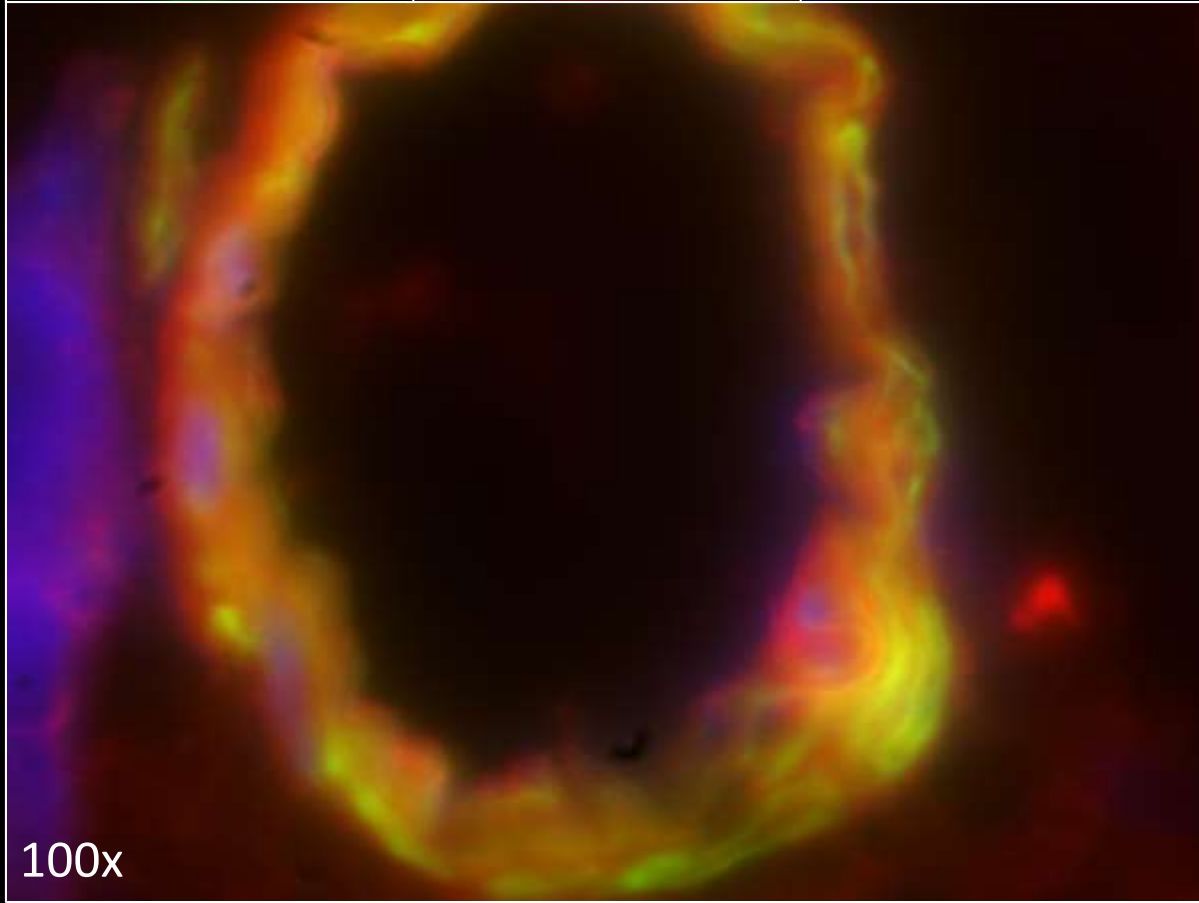
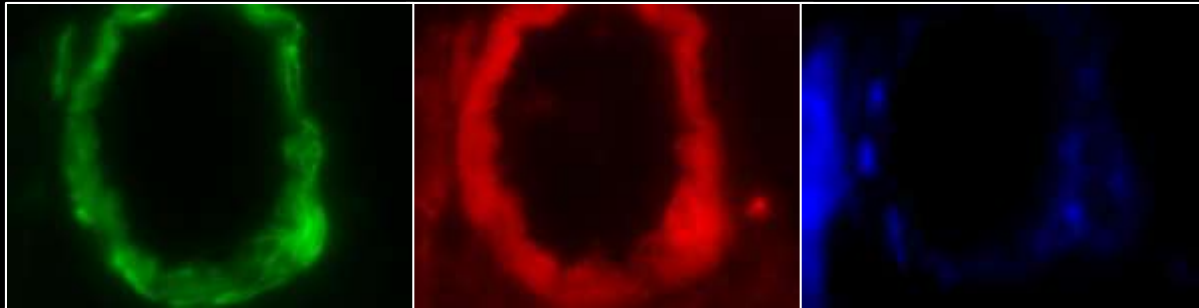
- SPSY did not colocalize with all of the vasculature (not in endothelial cells)



SPSY

Calponin-3

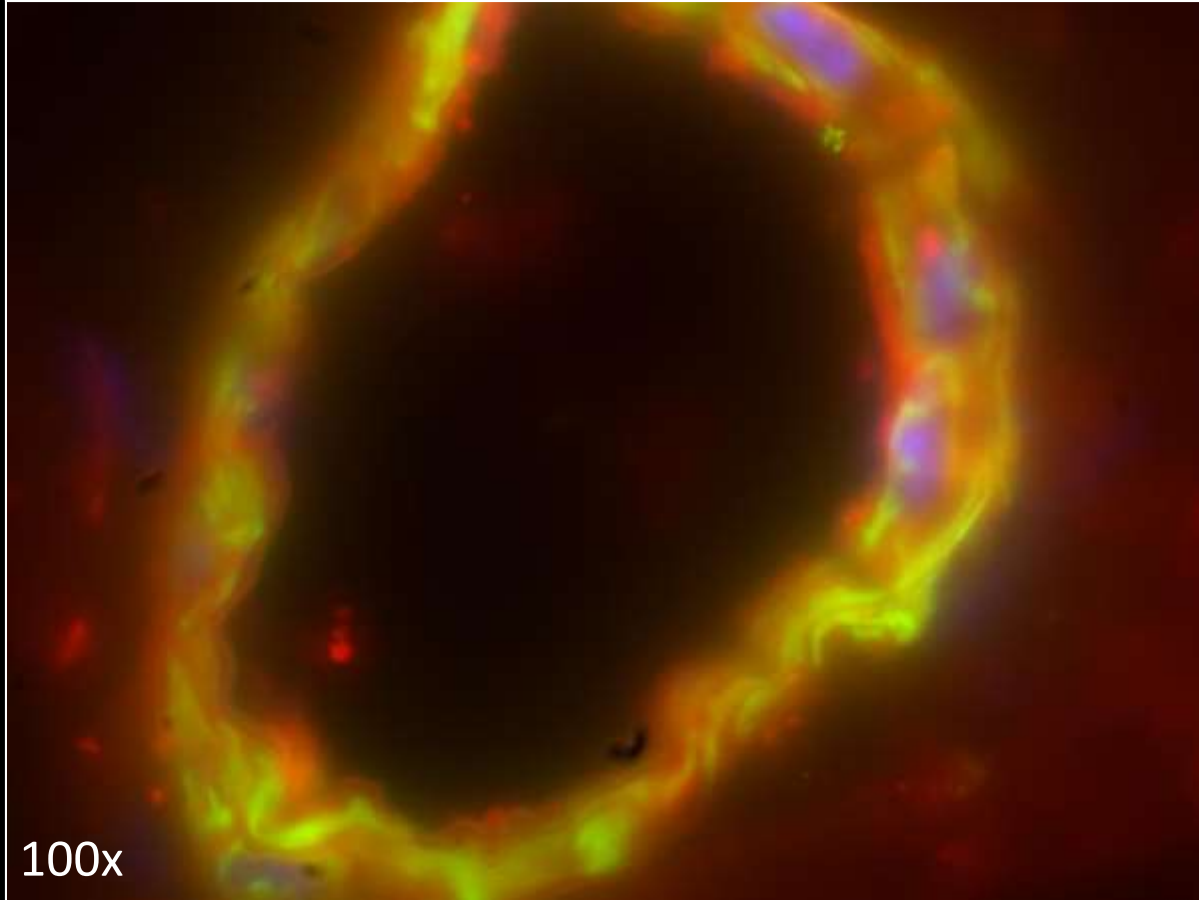
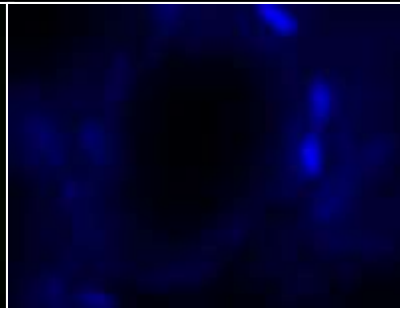
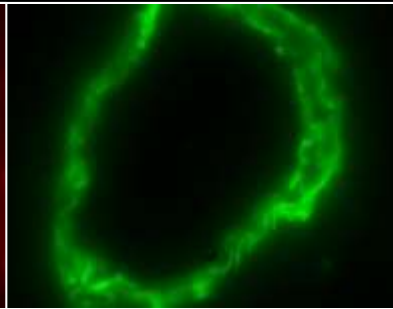
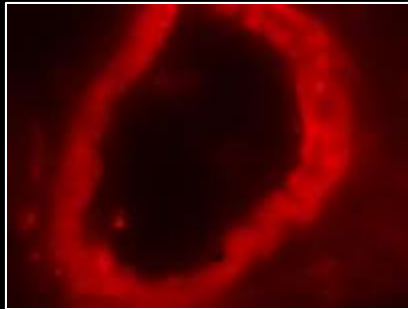
DAPI



SPSY

Calponin-3

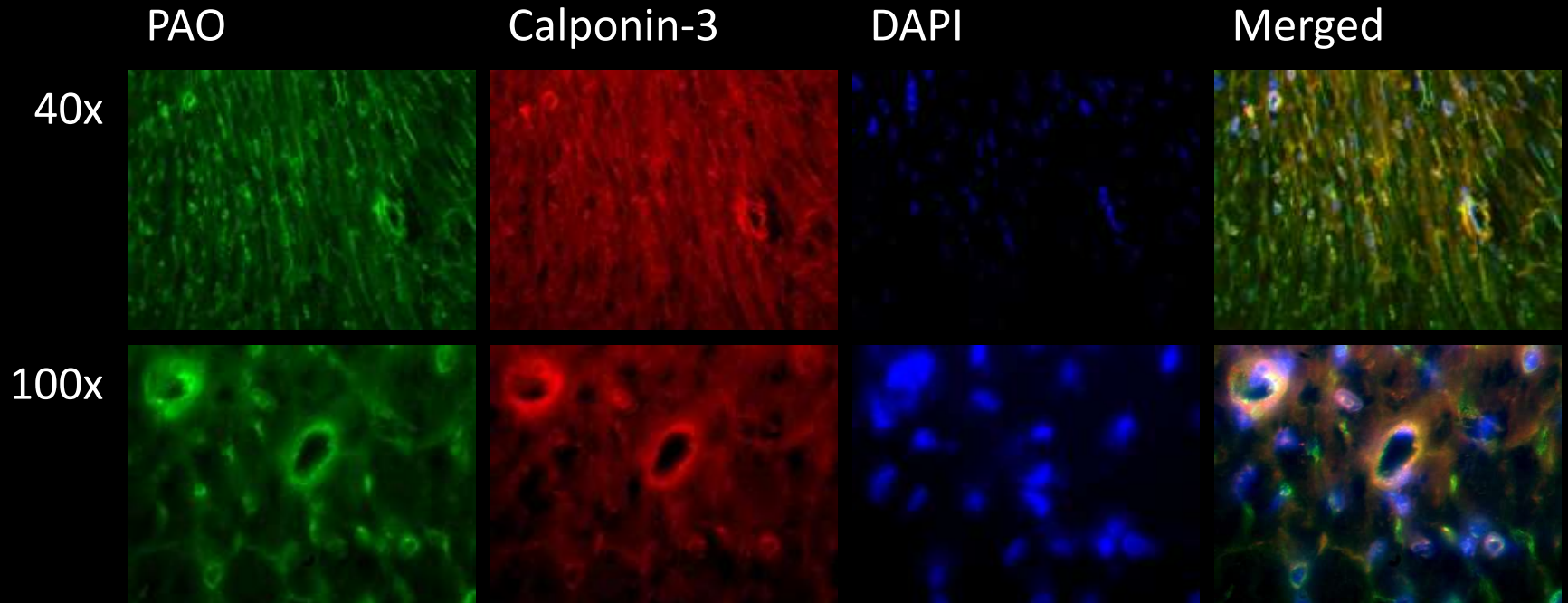
DAPI



100x



# Polyamine Oxidase (PAO)

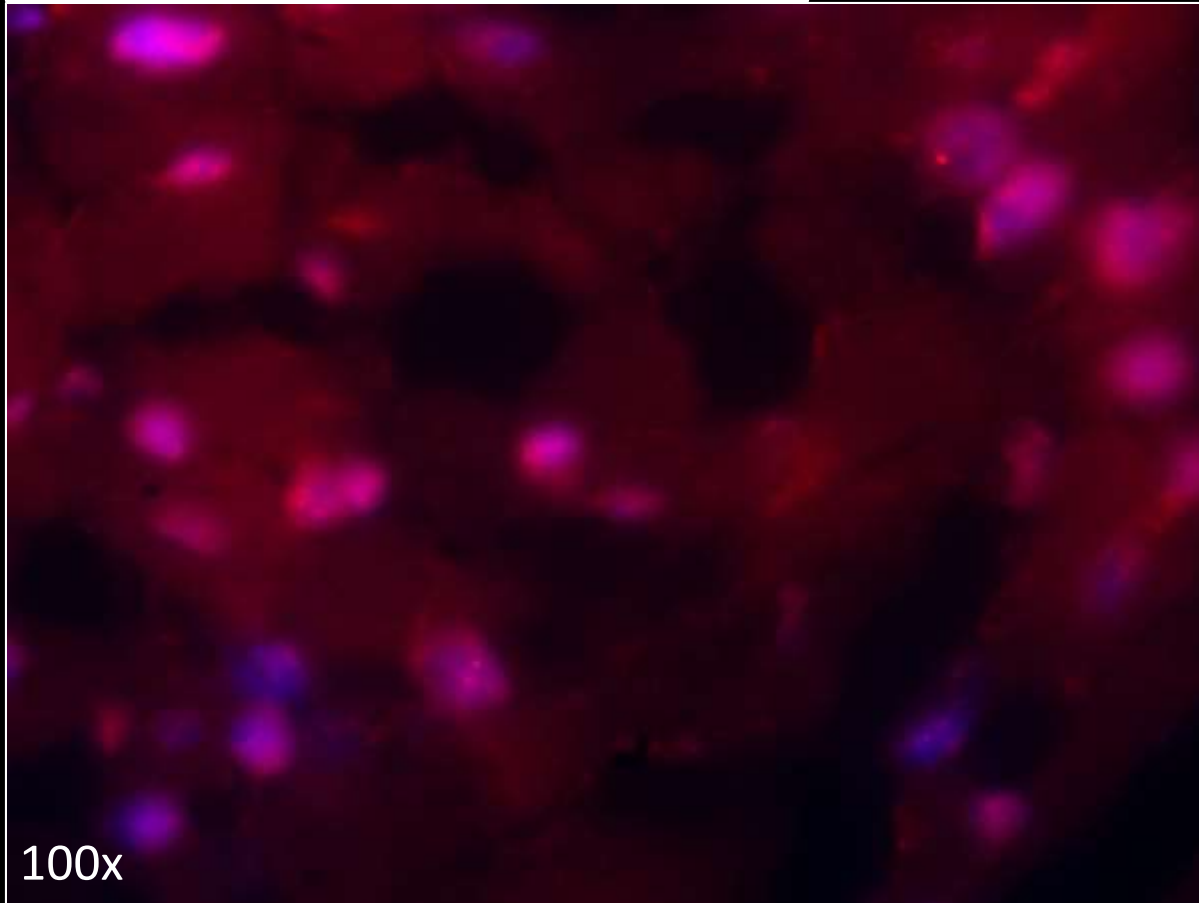
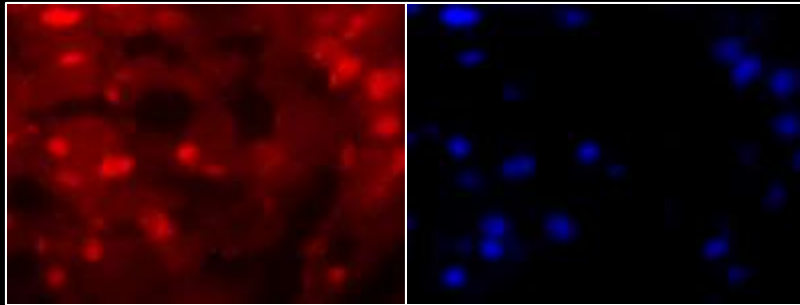


# Results/Discussion

- PAO:
  - seemed to colocalize with nuclei
  - also seemed to colocalize with the smooth muscle layer
  - did not seem to colocalize with intercalated discs

PAO

DAPI

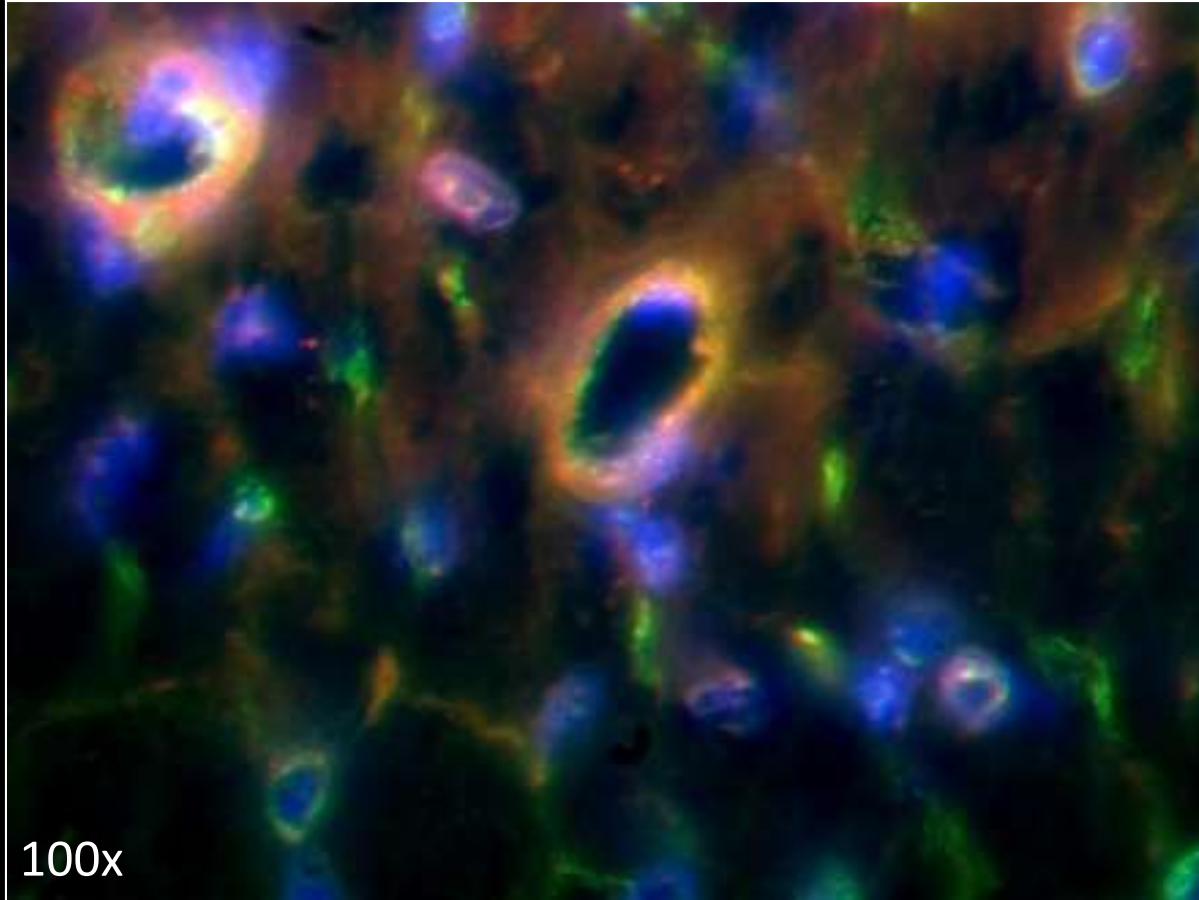
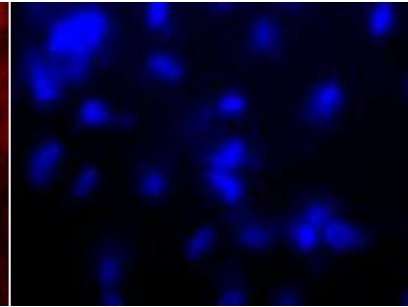
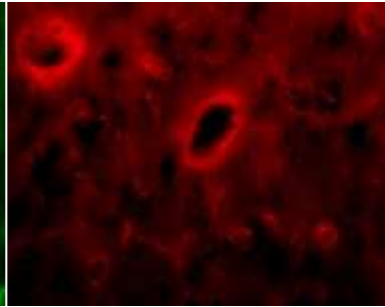
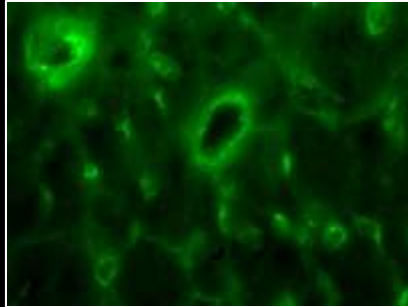


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PAO

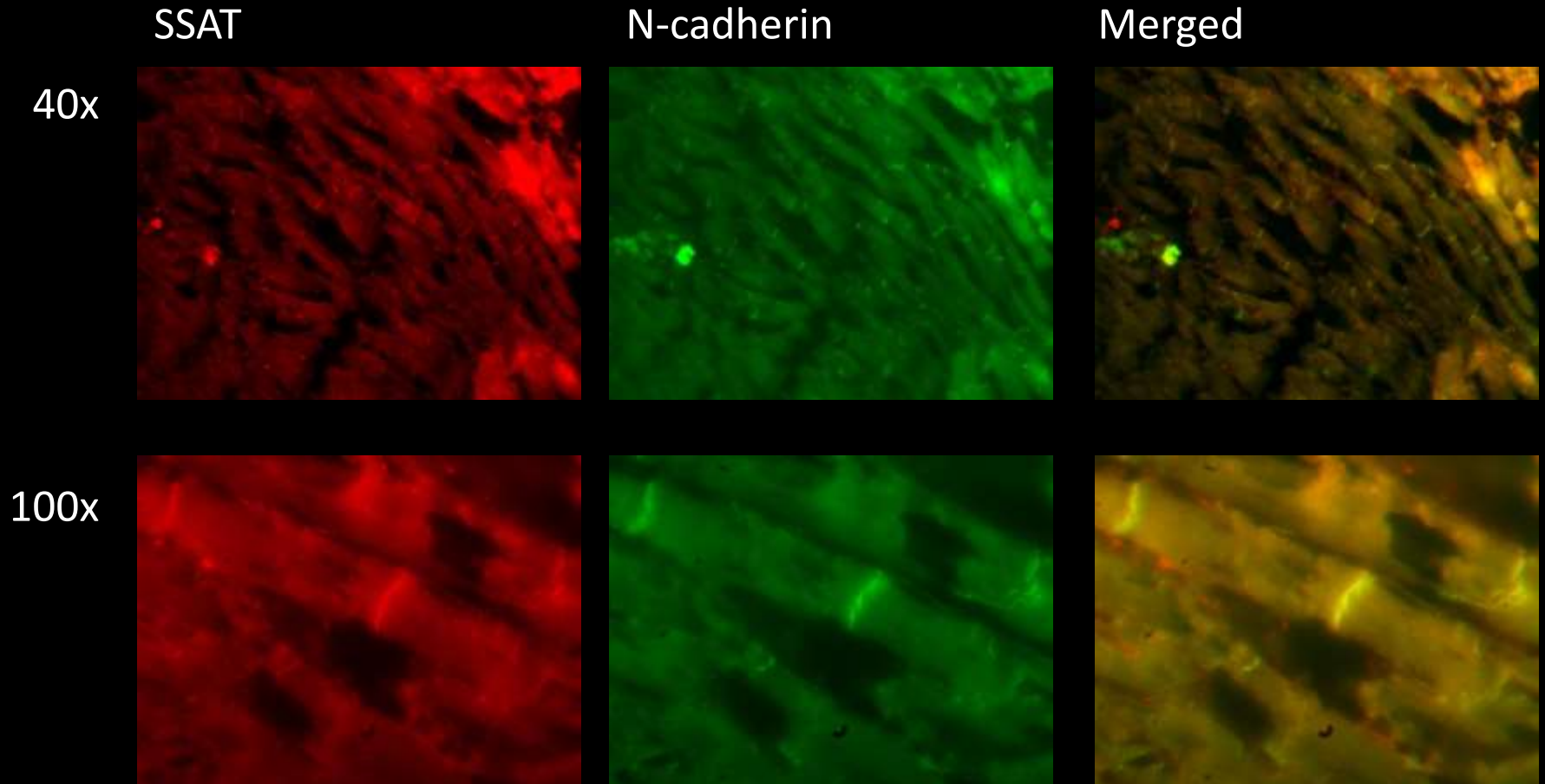
Calponin-3

DAPI



100x

# Spermidine/Spermine Acetyltransferase (SSAT)



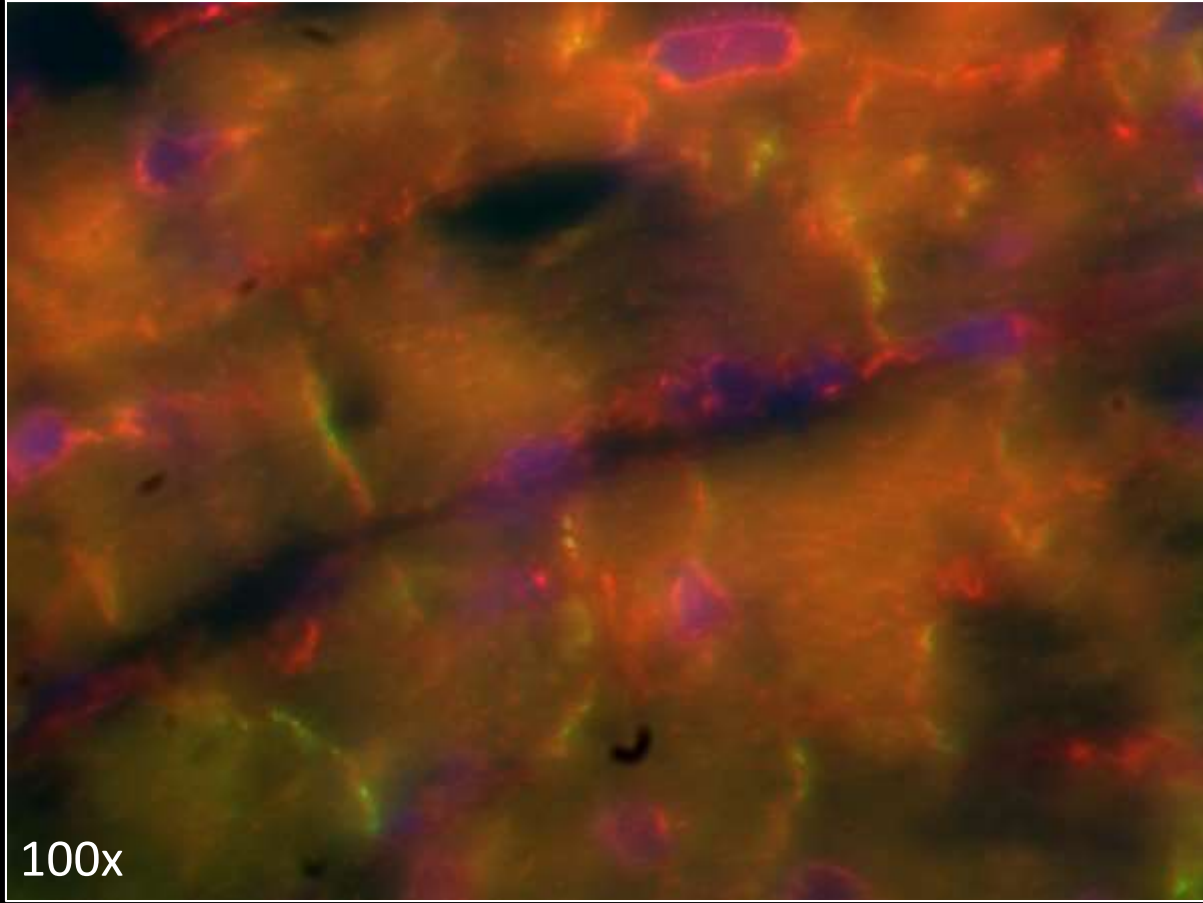
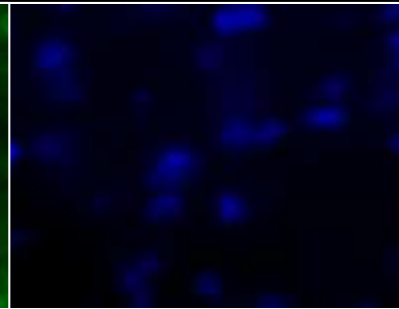
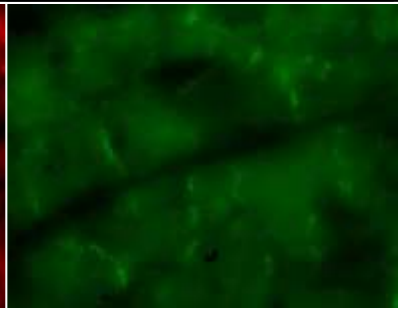
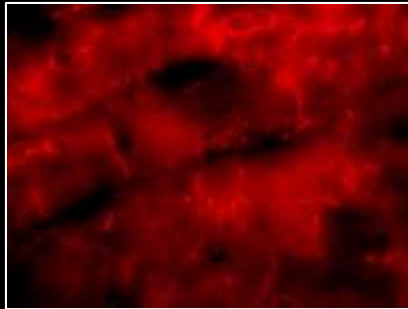
# Results/Discussion

- SSAT:
  - colocalized with the intercalated discs
  - seemed to localize around the nuclei

SSAT

N-cadherin

DAPI



100x

# Future Goals

- Manuscript preparation
- Amino acid analysis
  - Determining the Arg and Cit concentrations in plasma from mice under various treatments
  - Do plasma Arg and Cit concentrations correlate with heart attack survival?



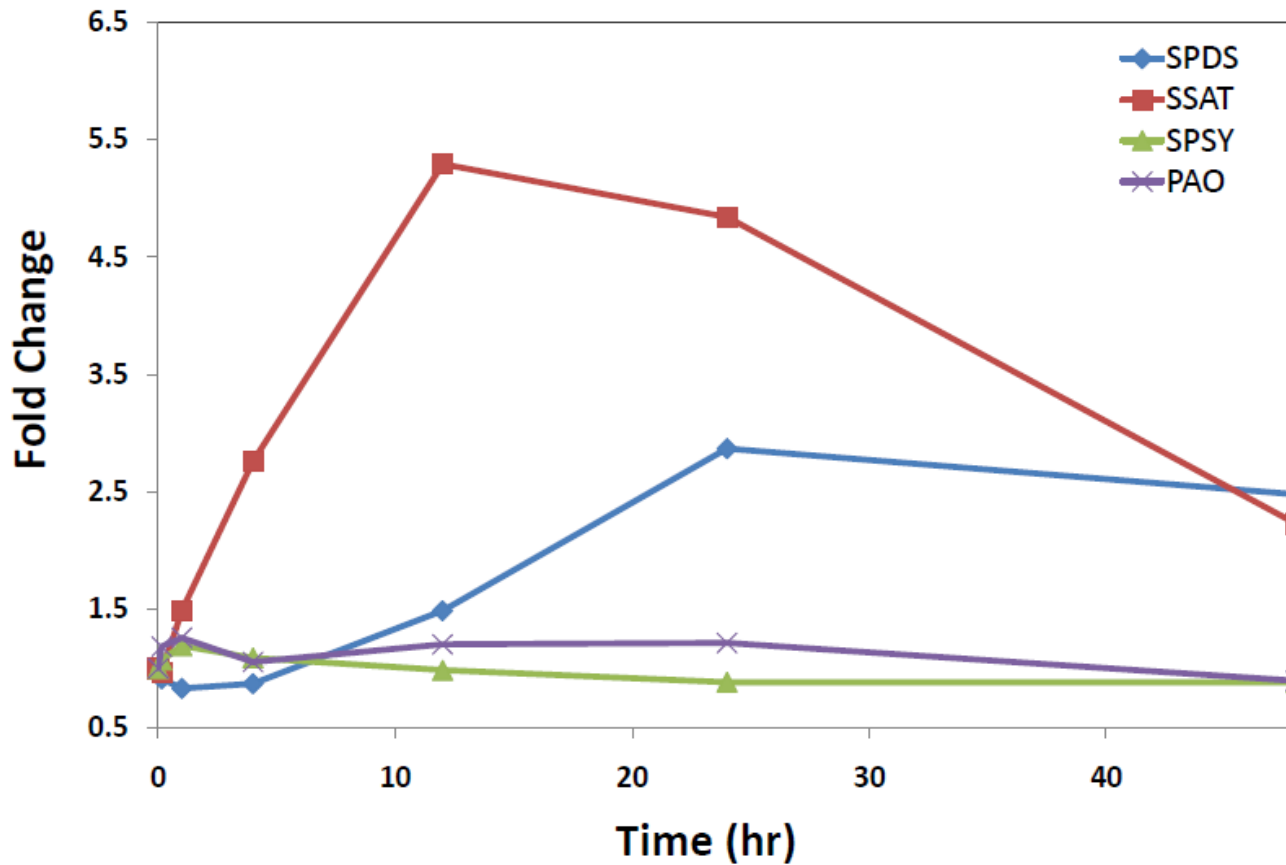
# Acknowledgements

- Dr. Mark Stayton
- Dr. Jacque Keele
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- Dr. Bill Flynn
- Dr. Gregory Krauss
  
- INBRE for funding





# Microarray data



# Polyamine Functions and Structures

A. Gugliucci / *Clinica Chimica Acta* 344 (2004) 23–35

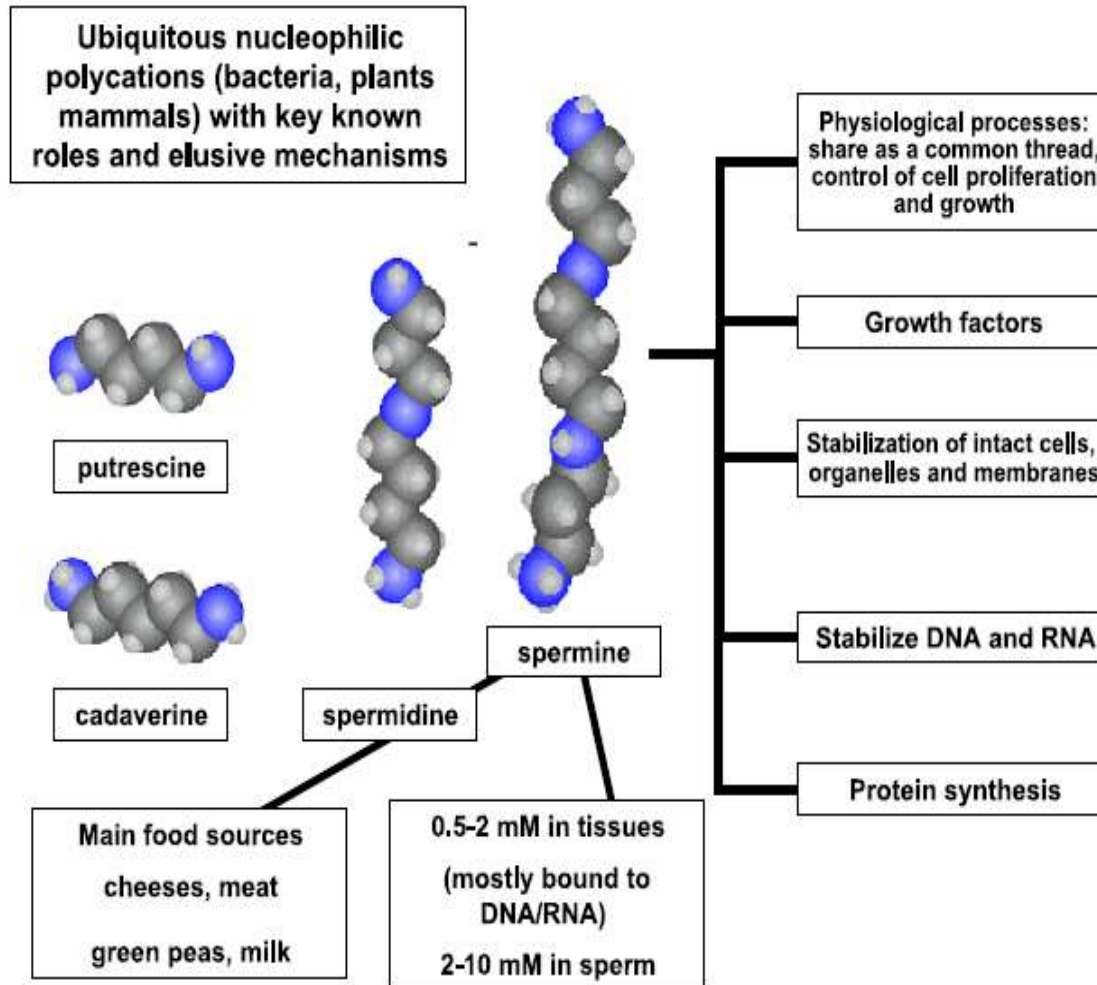
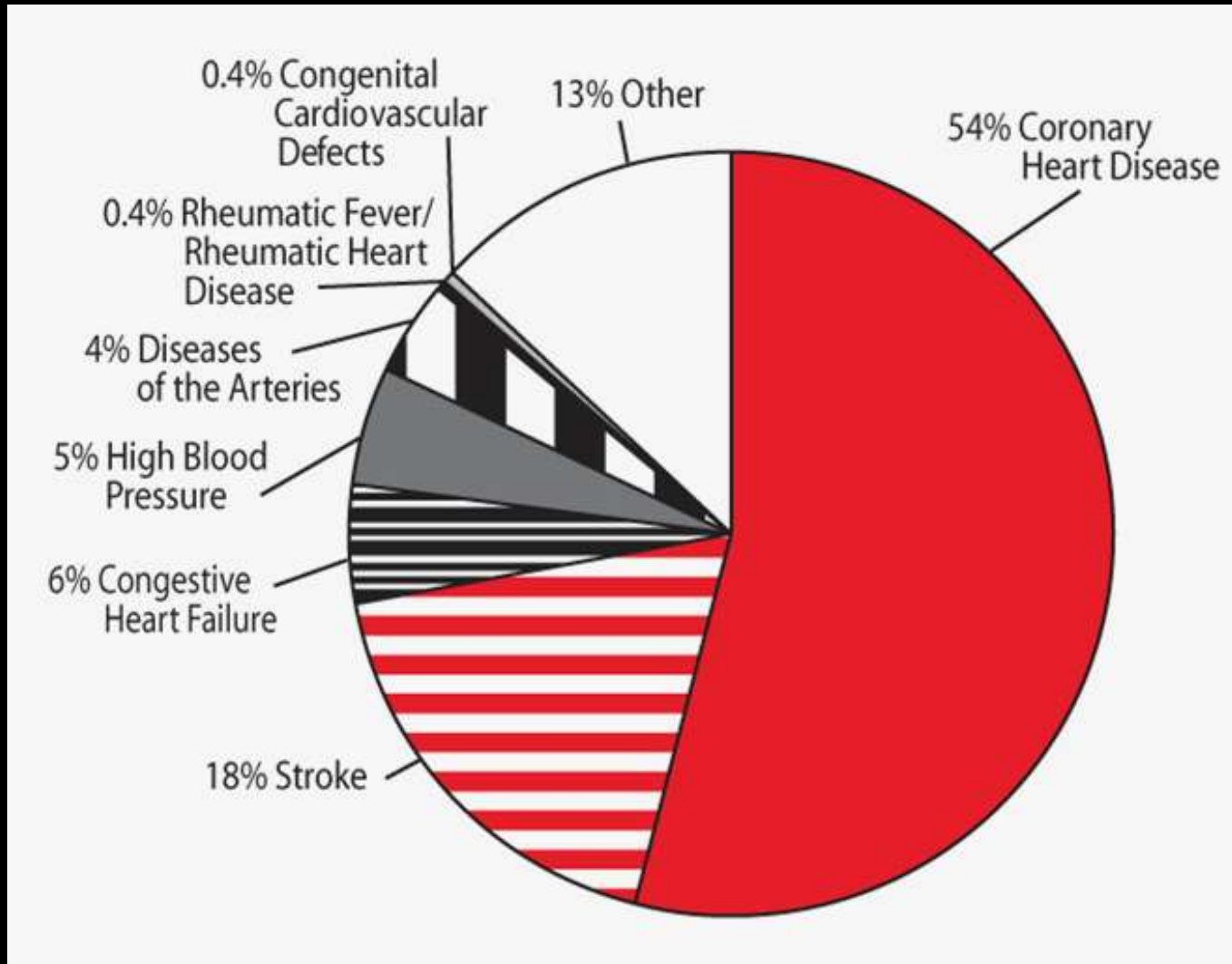


Fig. 1. Overview of the structure of the di- and polyamines as well as their main known physiological roles.

# Percentage Breakdown of Deaths from Cardiovascular Diseases (United States: 2001)



Source: CDC/NCHS.