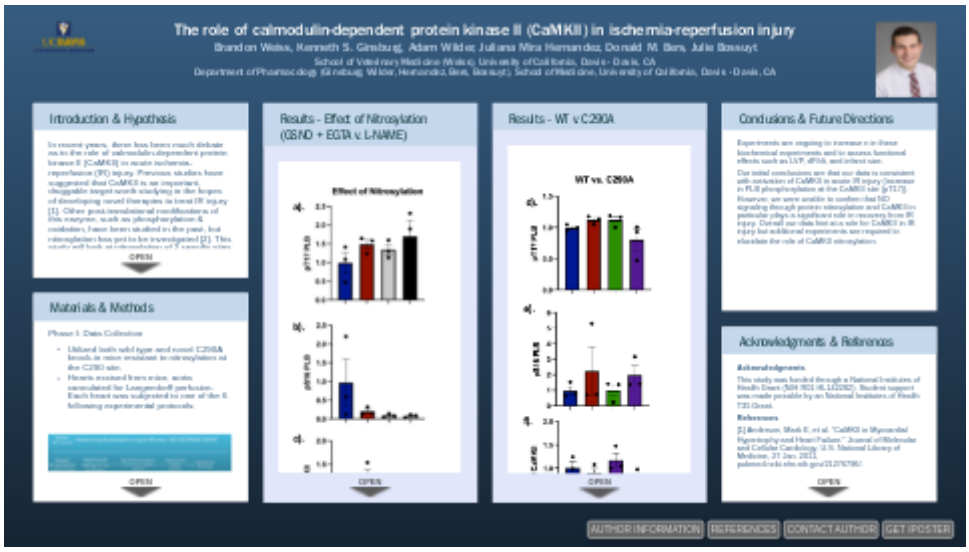
# The role of calmodulin-dependent protein kinase II (CaMKII) in ischemia-reperfusion injury

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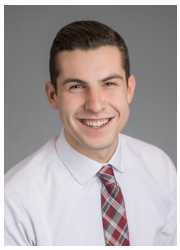


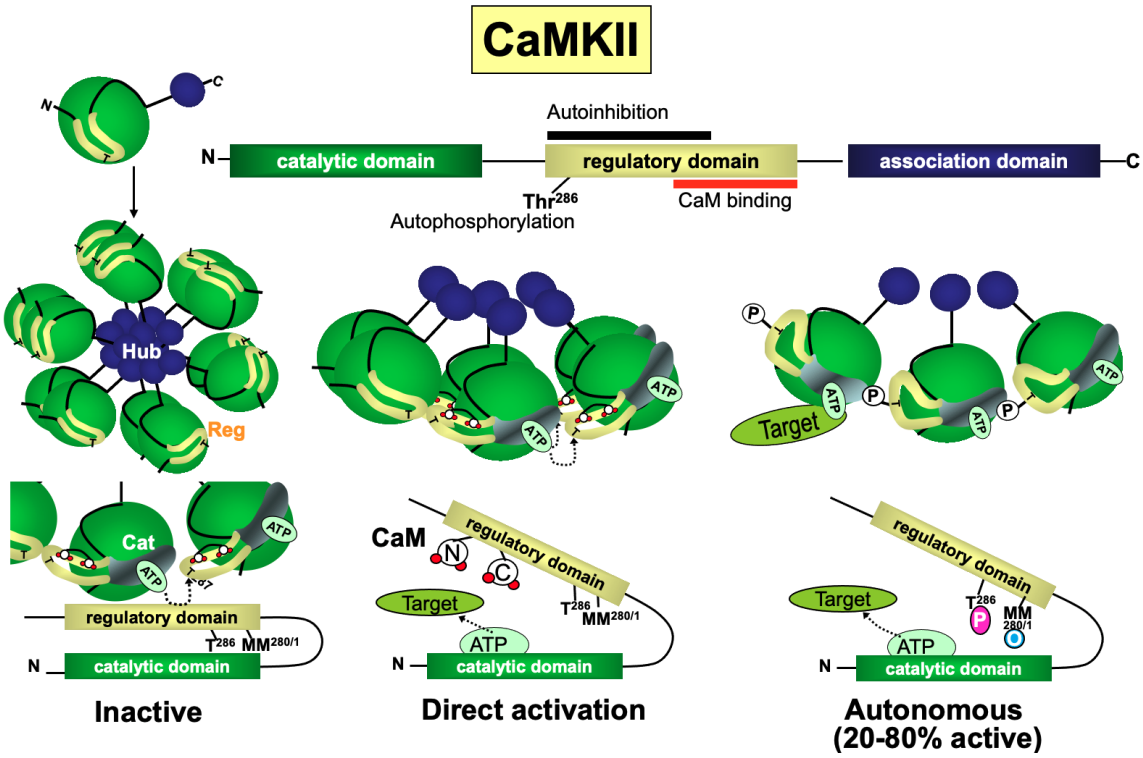
**PRESENTED AT:**

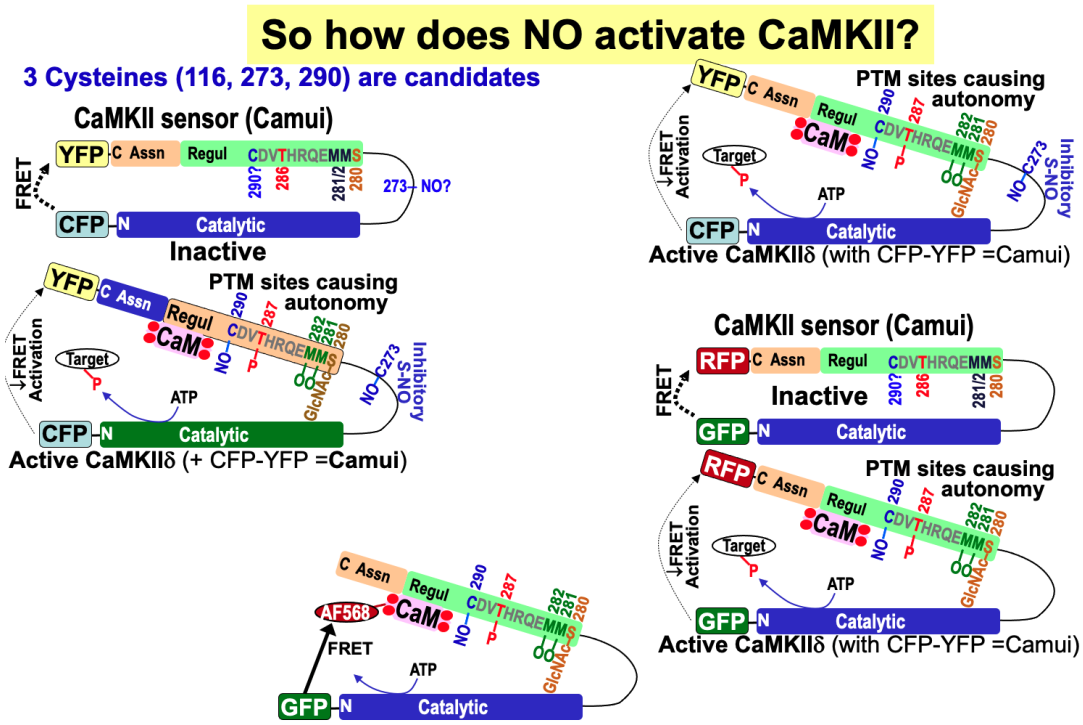
AAVMC American Association of Veterinary Medical Colleges

NVSS National Veterinary Scholars Symposium

# INTRODUCTION & HYPOTHESIS

In recent years, there has been much debate as to the role of calmodulin-dependent protein kinase II (CaMKII) in acute ischemia-reperfusion (IR) injury. Previous studies have suggested that CaMKII is an important, druggable target worth studying in the hopes of developing novel therapies to treat IR injury [1]. Other post-translational modifications of this enzyme, such as phosphorylation & oxidation, have been studied in the past, but nitrosylation has yet to be investigated [2]. This study will look at nitrosylation of 2 specific sites on CaMKII, C273 and C290. Previous projects have shown that nitrosylation of the C290 site causes autonomous activation of CaMKII, while nitrosylation at C273, inhibits enzyme activity [3].






**Hypothesis:**

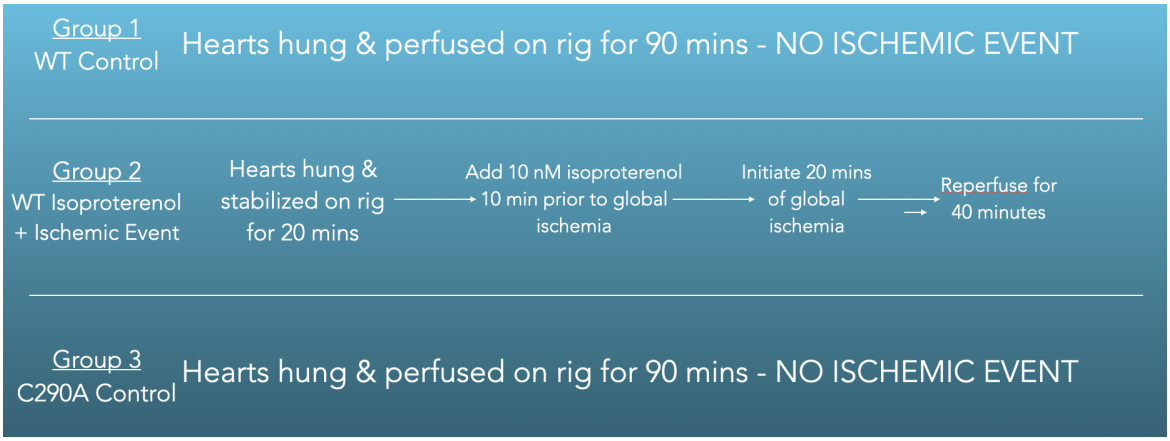
Nitrosylation at the C290 site is detrimental to recovery from acute IR injury, while nitrosylation at the C273 site improves recovery from IR injury.

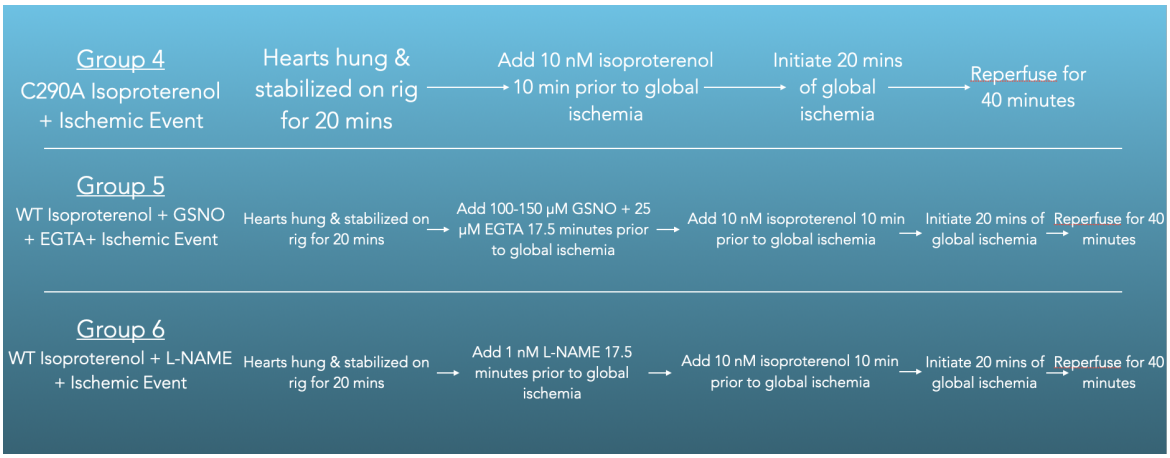
We will examine the role of CaMKII nitrosylation in IR injury by assessing the phosphorylation state of CaMKII and one of its prominent targets, phospholamban (PLB) in both wild-type and C290A knock-in mice that are resistant to nitrosylation at the C290 site.

# MATERIALS & METHODS

Phase I: Data Collection

- Utilized both wild type and novel C290A knock-in mice resistant to nitrosylation at the C290 site.
- Hearts excised from mice, aorta cannulated for Langendorff perfusion. Each heart was subjected to one of the 6 following experimental protocols:



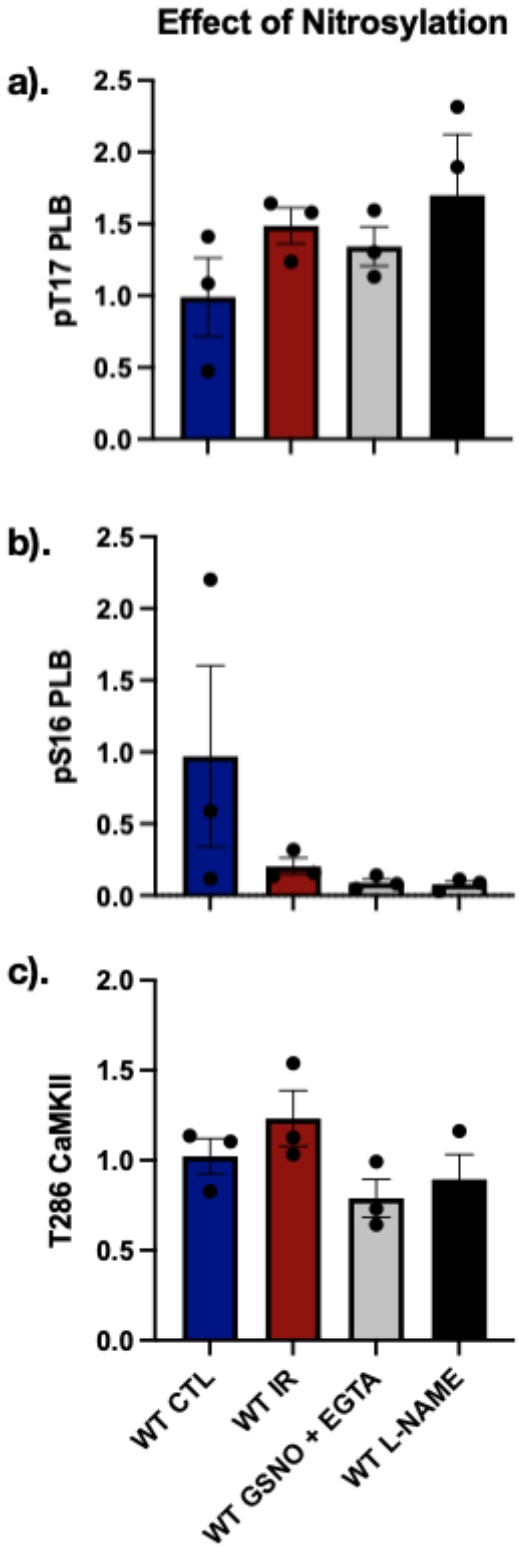




Phase II: Data Analysis

- Following perfusion protocol hearts were flash-frozen and stored at -80C
- Hearts were homogenized as previously described (ref https://pubmed.ncbi.nlm.nih.gov/33926209/)
- After protein quantification with BCA assay, samples were run on criterion TGX gels 4-20% before transfer to 0.2 µm nitrocellulose
- Blots were probed with one of the following antibody solutions
  - Anti-pT17 and anti-PLB (Badrilla)
  - Anti-pS16 and anti-PLB (Badrilla)
  - Anti-CaMKII T286 (Badrilla)
  - Anti-CaMKII delta (custom antibody)

- Followed by anti-rabbit IRDye800 and anti-mouse IR Dye680LT before scanning with the Sapphire Biomolecular Imager (Azure Biosystems).
- Blots were subsequently analyzed with Image J

# RESULTS - EFFECT OF NITROSYLATION (GSNO + EGTA V. L-NAME)




**a). pT17**

L-NAME (an NO scavenger) did not reduce phosphorylation of T17. GSNO pretreatment (triggering nitrosylation of CaMKII at C273) did not have a significant effect on PLB T17 phosphorylation.

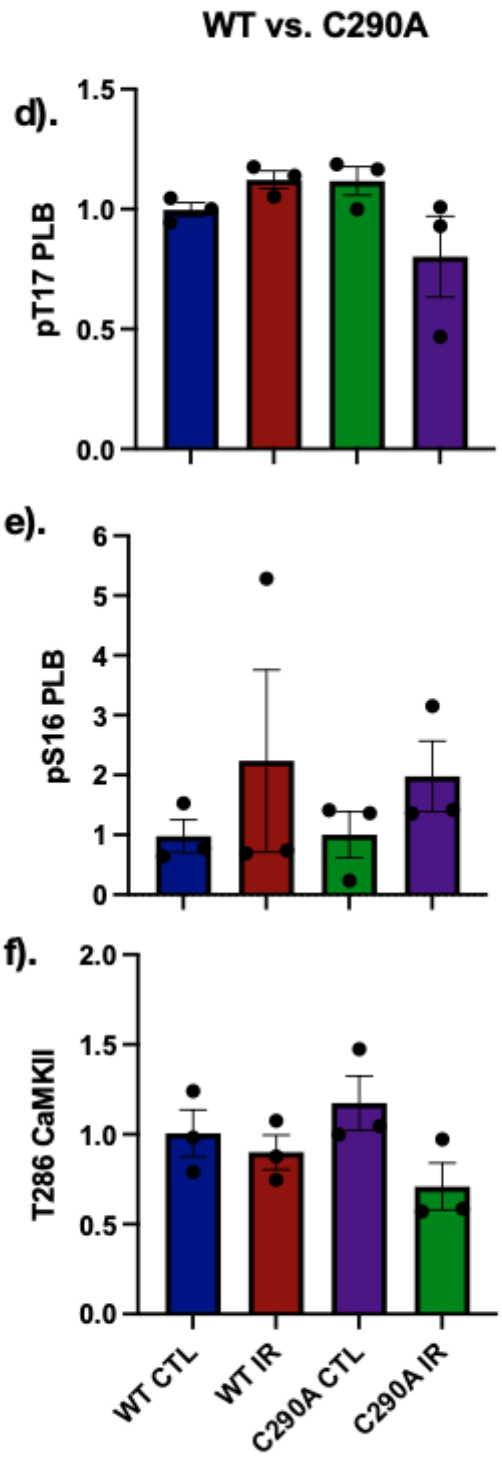
**b). pS16**

No significant changes in pS16 phosphorylation following IR, or LNAME or GSNO pretreatment were observed.

**c). T286**

pT286 antibody was not sensitive enough to display differences between CTL and IR, pretreatments or C290A and littermates.

# RESULTS - WT V. C290A




**d). pT17**

While phosphorylation of the T17 residue on phospholamban increased with IR injury in the WT mice, it appeared to decrease in the C290A mice. This is consistent with previous studies that found activation of CaMKII in IR injury and increased phosphorylation of its target PLB affecting SR Ca handling.

**e). pS16**

No significant differences between CTL and IR conditions in WT or C290A mice.

**f). T286**

pT286 antibody was not sensitive enough to display differences between CTL and IR, pretreatments or C290A and littermates.

# CONCLUSIONS & FUTURE DIRECTIONS

Experiments are ongoing to increase n in these biochemical experiments and to assess functional effects such as LVP, dP/dt, and infarct size.

Our initial conclusions are that our data is consistent with activation of CaMKII in acute IR injury (increase in PLB phosphorylation at the CaMKII site (pT17)). However, we were unable to confirm that NO signaling through protein nitrosylation and CaMKII in particular plays a significant role in recovery from IR injury. Overall our data hint at a role for CaMKII in IR injury but additional experiments are required to elucidate the role of CaMKII nitrosylation.

# ACKNOWLEDGMENTS & REFERENCES

**Acknowledgments**

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**References**

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[2] Salas MA, Valverde CA, Sanchez G, Said M, R odriguez JS, Portiansky EL, Kaetzel MA, Dedman JR, DonosoP, Kranias EG, Mattiazzi A. The signalling pathway of camkii-mediated apoptosis and necrosis in the ischemia/reperfusion injury. J Mol Cell Cardiol . 2010; 48:1298-1306

[3] Erickson, J. R., Nichols, C. B., Uchinoumi, H., Stein, M. L., Bossuyt, J., & Bers, D. M. (2015). S-Nitrosylation Induces Both Autonomous Activation and Inhibition of Calcium/Calmodulin-dependent Protein Kinase II δ. The Journal of biological chemistry, 290(42), 25646–25656. https://doi.org/10.1074/jbc.M115.650234

# AUTHOR INFORMATION

Brandon Weiss is a third year DVM candidate at the UC Davis School of Veterinary Medicine. He was born in Los Angeles, CA and attended UC Berkeley for his undergraduate education. There, he completed a Bachelor of Arts degree in Ecology, Evolution, & Organismal Biology and developed a fervent passion for California wildlife, especially birds. Brandon is deciding between many of his passions in veterinary medicine and will most likely either specialize in an area of small animal medicine or practice as an emergency doctor.

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