

# The pH-Sensing GPCR GPR68 Signals In cAMP/PKA/EPAC1-Independent Manner

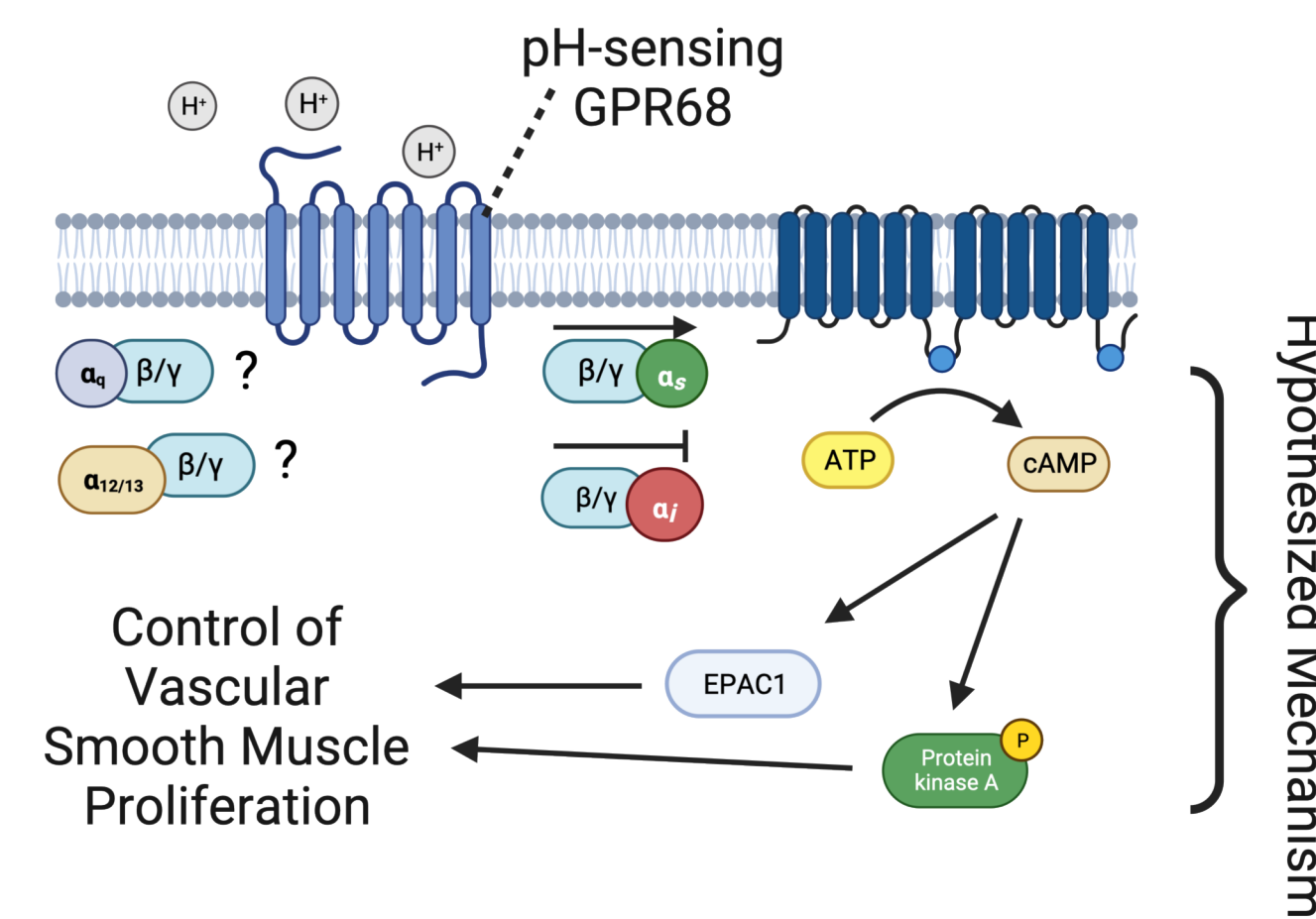
Cere Poovey, MOP<sup>1</sup>, Joshua S. Morgan, MS, PhD<sup>1</sup>, Madison Williams, MS<sup>1</sup>, and David Tulis, MS, PhD<sup>1</sup>

<sup>1</sup> Department of Physiology, Brody School of Medicine, East Carolina University

## BACKGROUND

- Cardiovascular disease is (CVD) a leading cause of death
- Ischemia is a major component of CVD
- Ischemia leads to anaerobic metabolism in affected tissues
  - Leads to a buildup of lactic acid
- GPR68 is a GPCR that is activated by low pH and is thought to play a role in vascular smooth muscle (VSM) growth

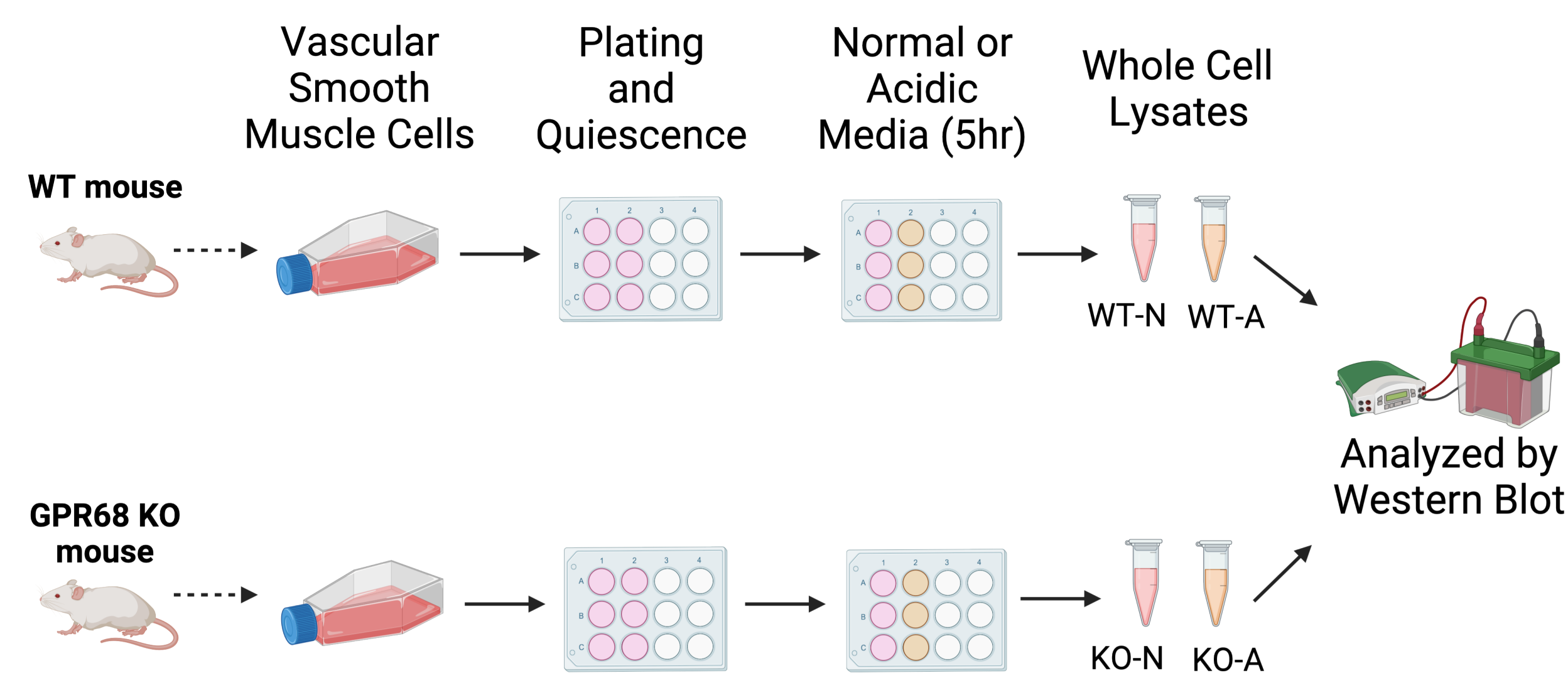
## HYPOTHESIS



## METHODS

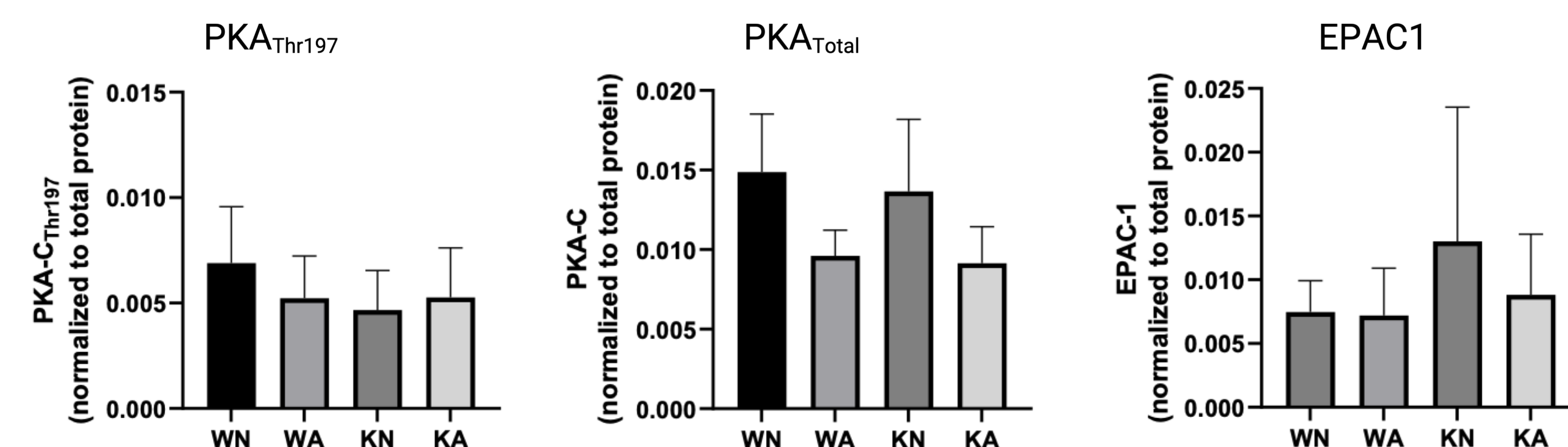
Littermate wild type (WT) C57BL/6J and littermate knockout (KO) GPR68 mouse primary VSM cell cultures were collected, plated, and exposed to experimental conditions for 5 hours:

- Normal media: pH ~7.5
- Acidic media: pH ~6.5



## RESULTS

Activation of GPR68 in acidic conditions showed no change in protein levels of PKA<sub>Thr197</sub>, PKA<sub>Total</sub>, EPAC1, or PKA<sub>Thr197</sub> to PKA<sub>Total</sub> ratio (see right side), when compared to normal biological pH after 5 hours of exposure. N=4

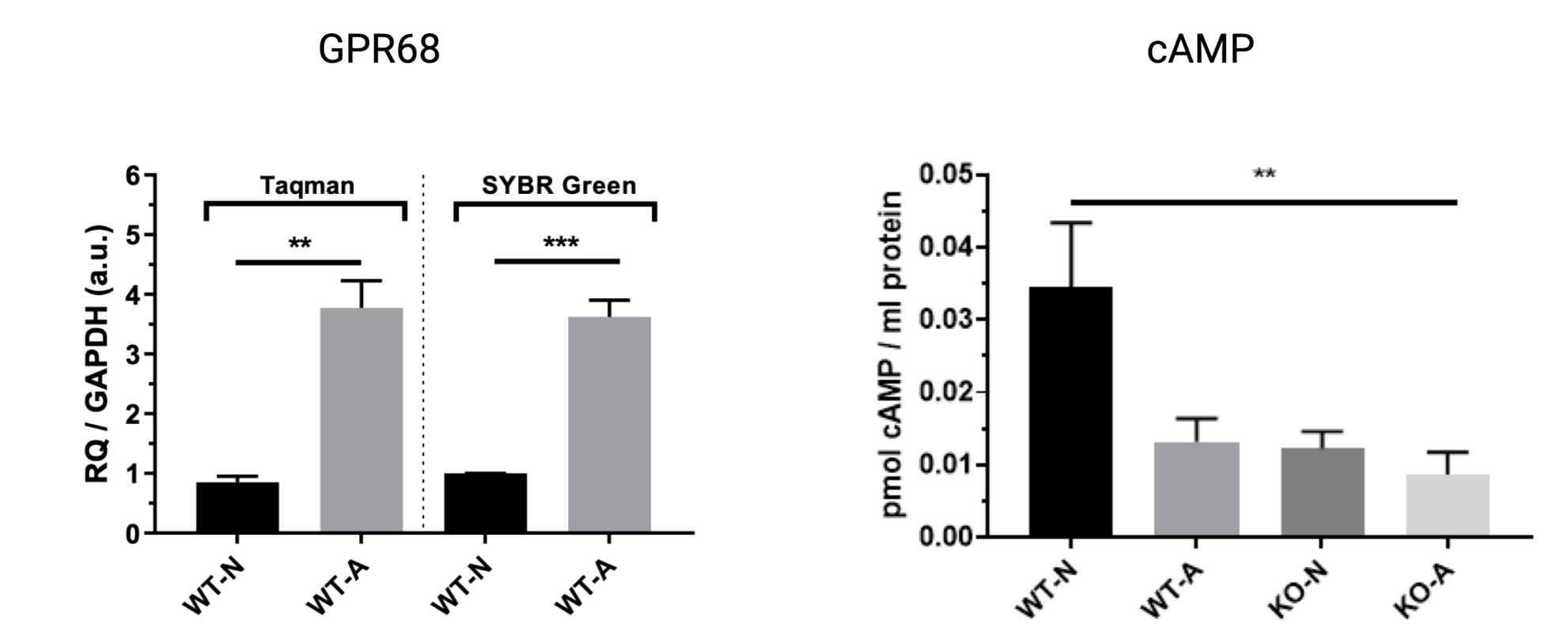


# GPR68 signals in cAMP/PKA/EPAC1-independent manner under acidic conditions in vascular smooth muscle cells.

## CONCLUSION

- GPR68 does not signal through cAMP/PKA/EPAC1 in response to acidic conditions in VSM cells
  - May still use this pathway under other conditions, such as hypoxia
- GPR68 may signal through G<sub>q</sub> and/or G<sub>12/13</sub> in acidic conditions

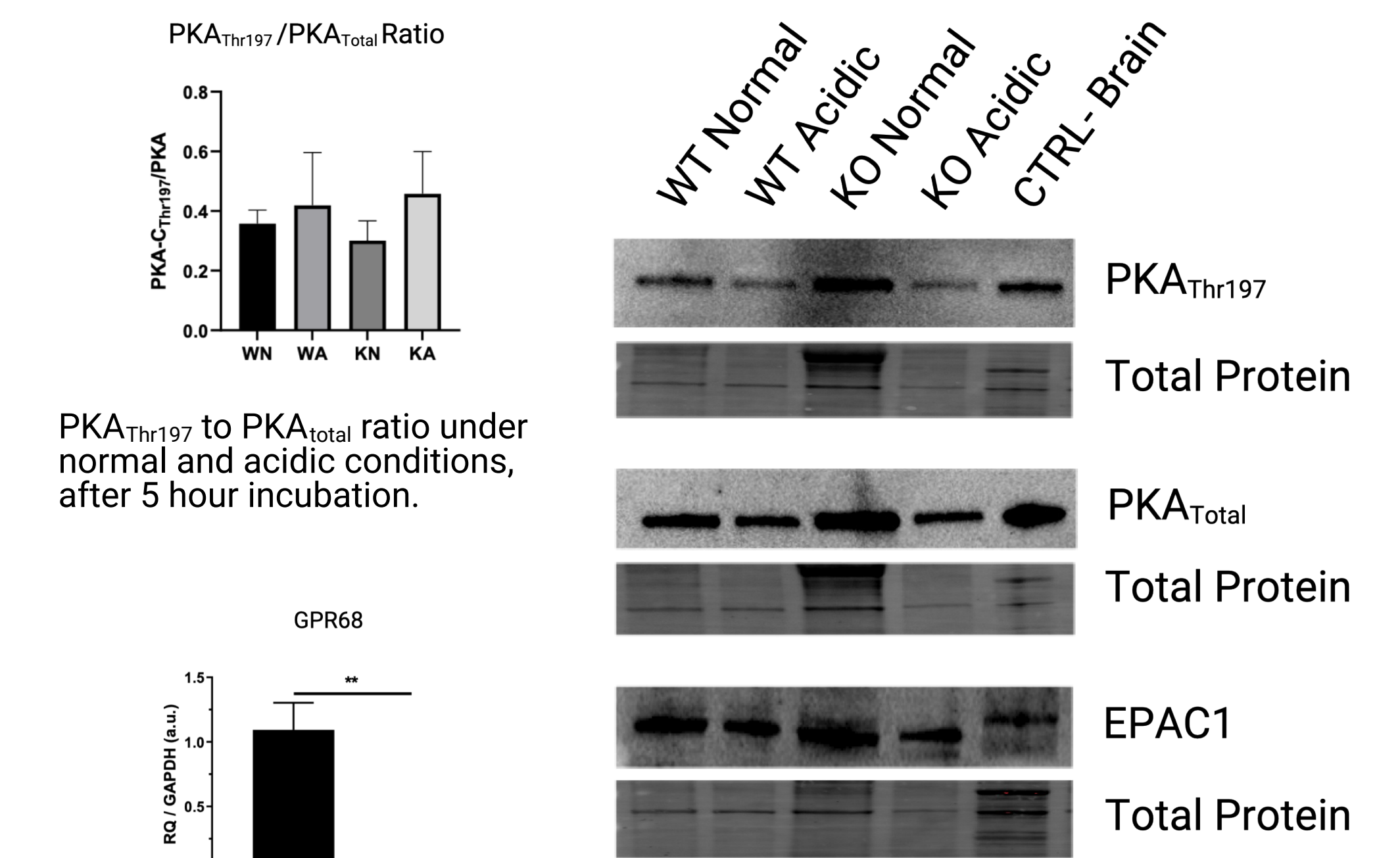
## EXTRA FIGURES / GRAPHS



LEFT: Real time PCR data showing increased transcription of GPR68 under acidic conditions in VSM cells.

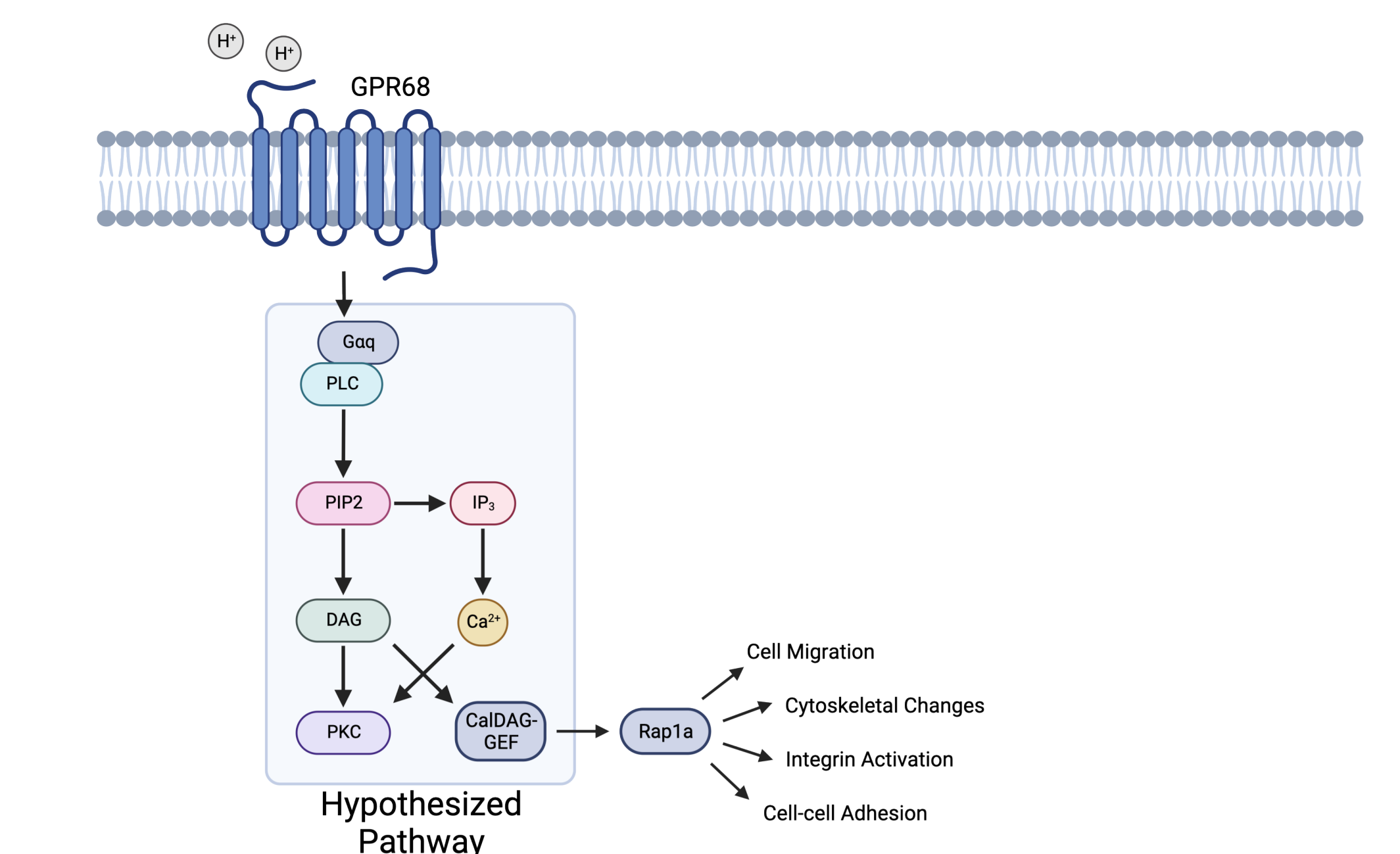
RIGHT: Preliminary data showing decreases in cAMP in acidic conditions for WT cells, but not in KO cells.

Data provided by: Joshua S. Morgan



PKA<sub>Thr197</sub> to PKA<sub>Total</sub> ratio under normal and acidic conditions, after 5 hour incubation.

Representative Western Blot of each target, 20ug of protein loaded in each lane and 3.5ul of mouse brain control. Antibodies and control from Cell Signaling Technologies. PKATotal, PKA<sub>Thr197</sub> were diluted 1:1000. EPAC1 diluted 1:500.



Future studies will explore the G<sub>q</sub> signaling cascade as the potential pathway for GPR68 signalling.

## ACKNOWLEDGEMENTS

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