

10-8-2014

Proton-sensing receptors- therapeutic targets in the management of asthma?

Raymond B. Penn, PhD
Thomas Jefferson University

Follow this and additional works at: <https://jdc.jefferson.edu/pulmcritcaregrandrounds>

 Part of the [Medicine and Health Sciences Commons](#)

[Let us know how access to this document benefits you](#)

Recommended Citation

Penn, PhD, Raymond B., "Proton-sensing receptors- therapeutic targets in the management of asthma?" (2014). *Division of Pulmonary and Critical Care Medicine Presentations and Grand Rounds*. Presentation 111.

<https://jdc.jefferson.edu/pulmcritcaregrandrounds/111>

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's [Center for Teaching and Learning \(CTL\)](#). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in Division of Pulmonary and Critical Care Medicine Presentations and Grand Rounds by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: JeffersonDigitalCommons@jefferson.edu.

Department of Medicine

Jefferson University Hospital



Raymond Penn, PhD

Division of Pulmonary and Critical Care Medicine

Jane and Leonard Korman Lung Center

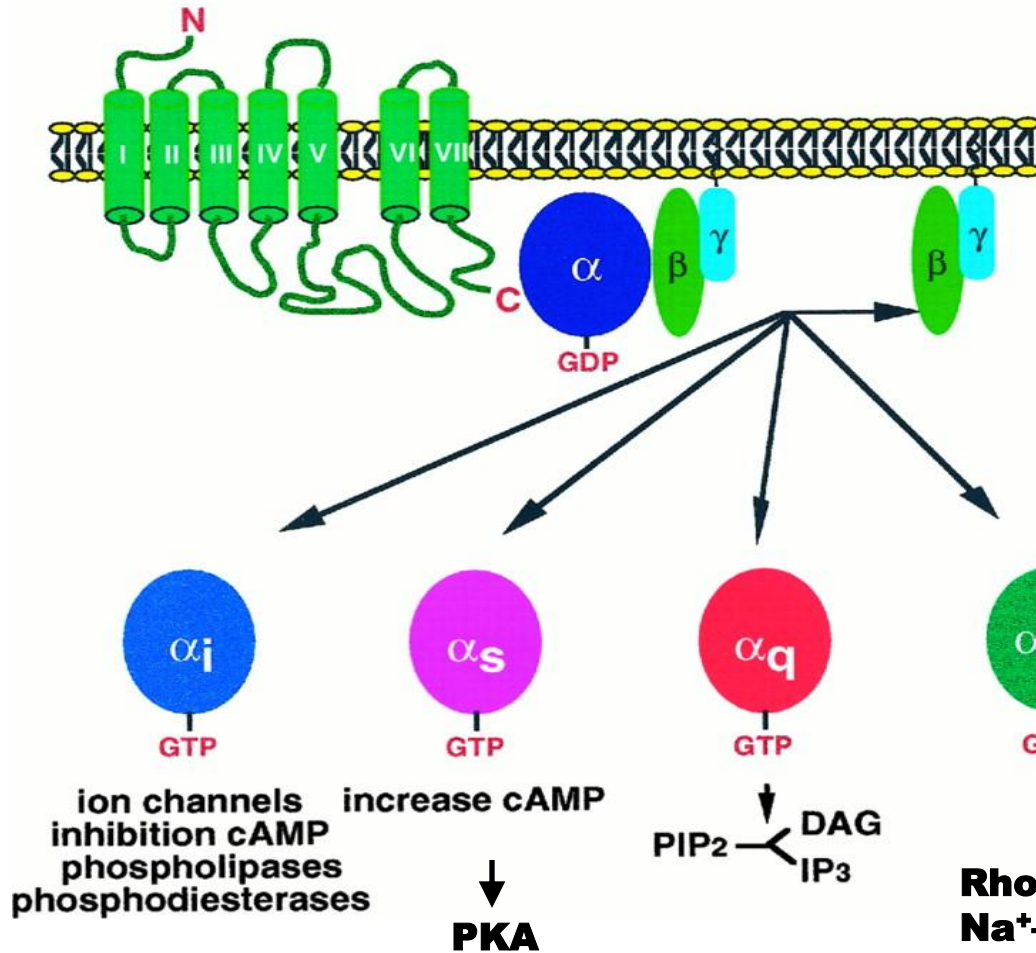
Department of Medicine, Thomas Jefferson University

**“Proton-sensing receptors-
therapeutic targets in the
management of asthma?”**

The Speaker Declares No Conflicts of Interest

G protein-coupled receptors

pretty much explain all biological phenomena
and are the only thing worth studying 🤖🧐



Biological functions

smell and taste
(~1000 types of receptors)
perception of light
neurotransmission
function of endocrine
and exocrine glands
chemotaxis
exocytosis
control of blood pressure
embryogenesis
development
cell growth and differentiation
HIV infection
oncogenesis

Rho GEF
Na⁺-H⁺ exchangers

GPCR agonists are physiological, pathological, and therapeutic regulators of ASM contractile state

1. Contractile state is a function of the dynamic balance of (pro-contractile) Gq-coupled vs (pro-relaxant) Gs-coupled GPCR activation.
2. Physiological: Parasympathetic innervation providing acetylcholine (ACh) activating Gq-coupled m3 muscarinic acetylcholine receptor (m3mAChR) is principal regulator of physiological tone.

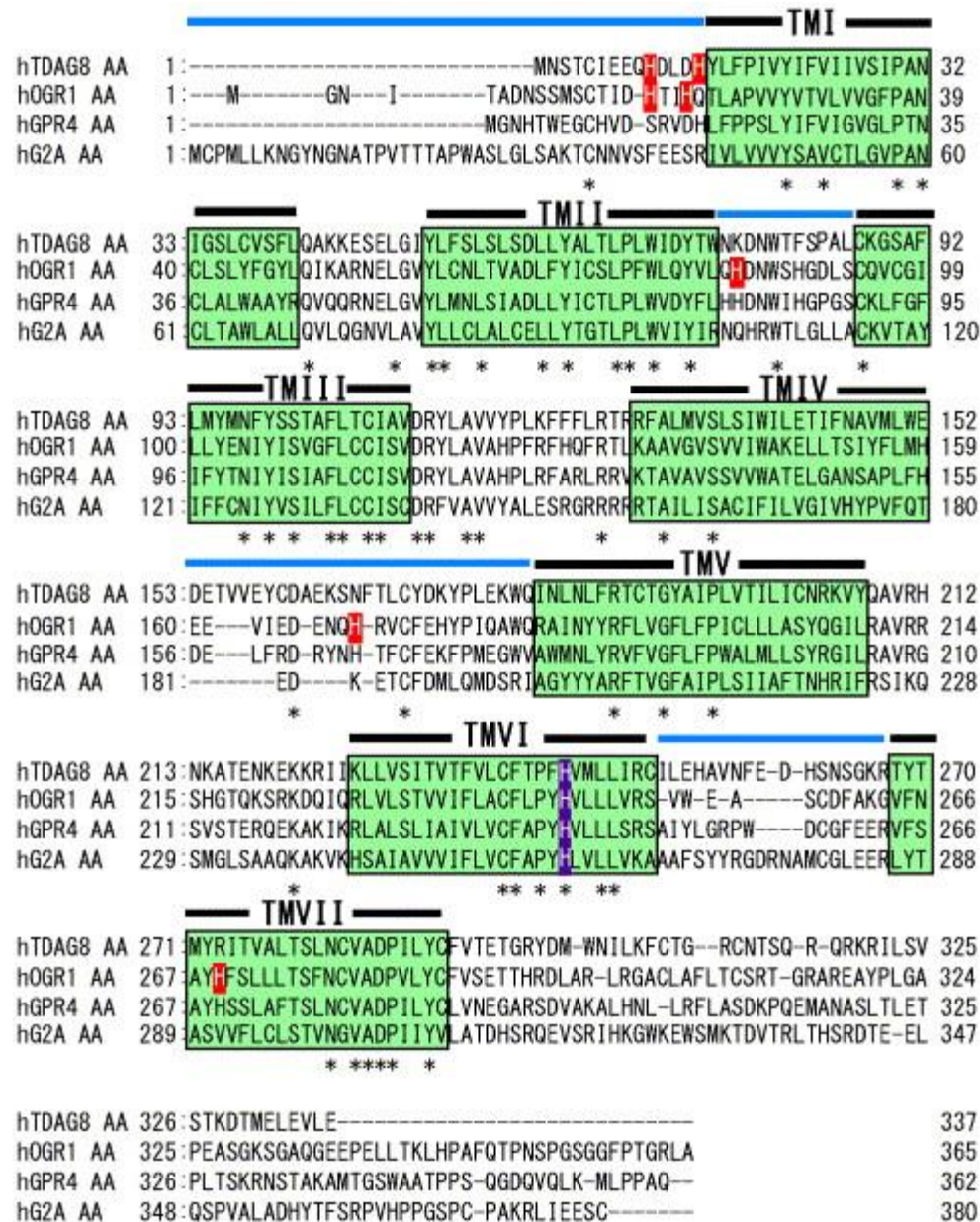
GPCR agonists are physiological, pathological, and therapeutic regulators of ASM contractile state

3. Pathological: Inflammation can cause increased parasympathetic ACh release (m3mAChR), and numerous inflammatory mediators (e.g. histamine, LTC4/LTD4, endothelin, serotonin) can activate Gq-coupled receptors on ASM.

4. Therapeutic: Many anti- asthma/COPD drug either: 1) block Gq-coupled receptors (monteleukast for CysLT1R, tiotropium for m3mAChR); or 2) activate bronchodilatory Gs-coupled receptors (beta-agonists).

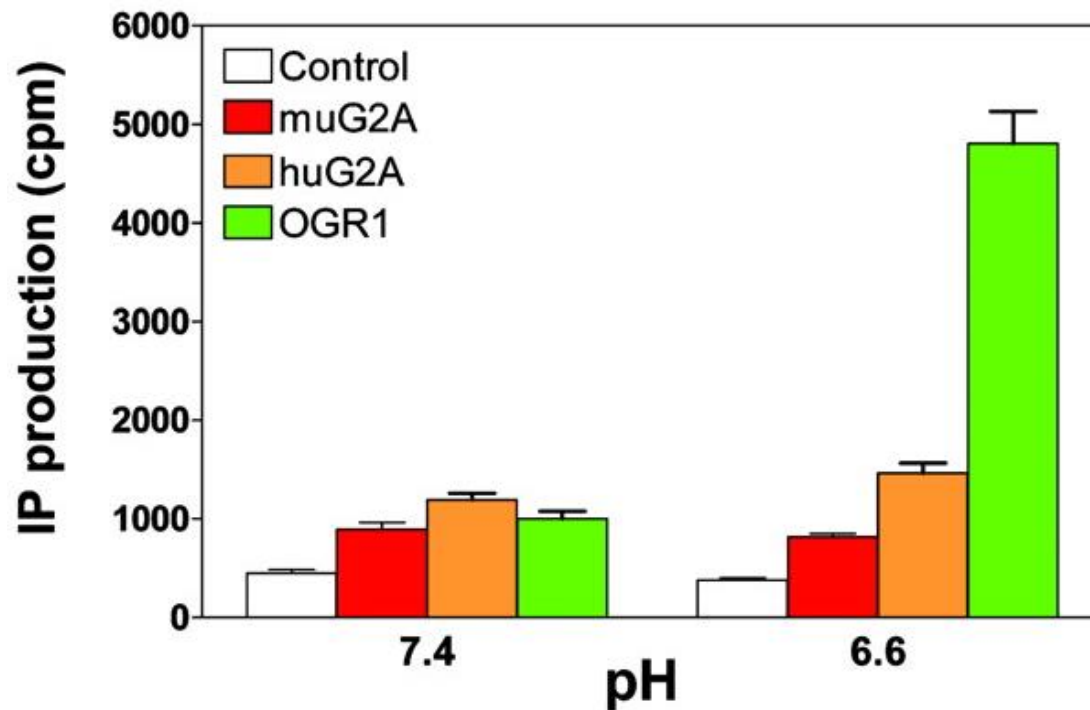
Proton-sensing GPCRs

- Subfamily of GPCRs linked by sequence similarity:
 - OGR1, G2A ? (Gq)
 - GPR4, TDAG8 (Gs)
- Can exhibit high level of constitutive activity
- Originally thought to be receptors activated by lysolipids
- Subsequently found to signal in response to lowering extracellular pH

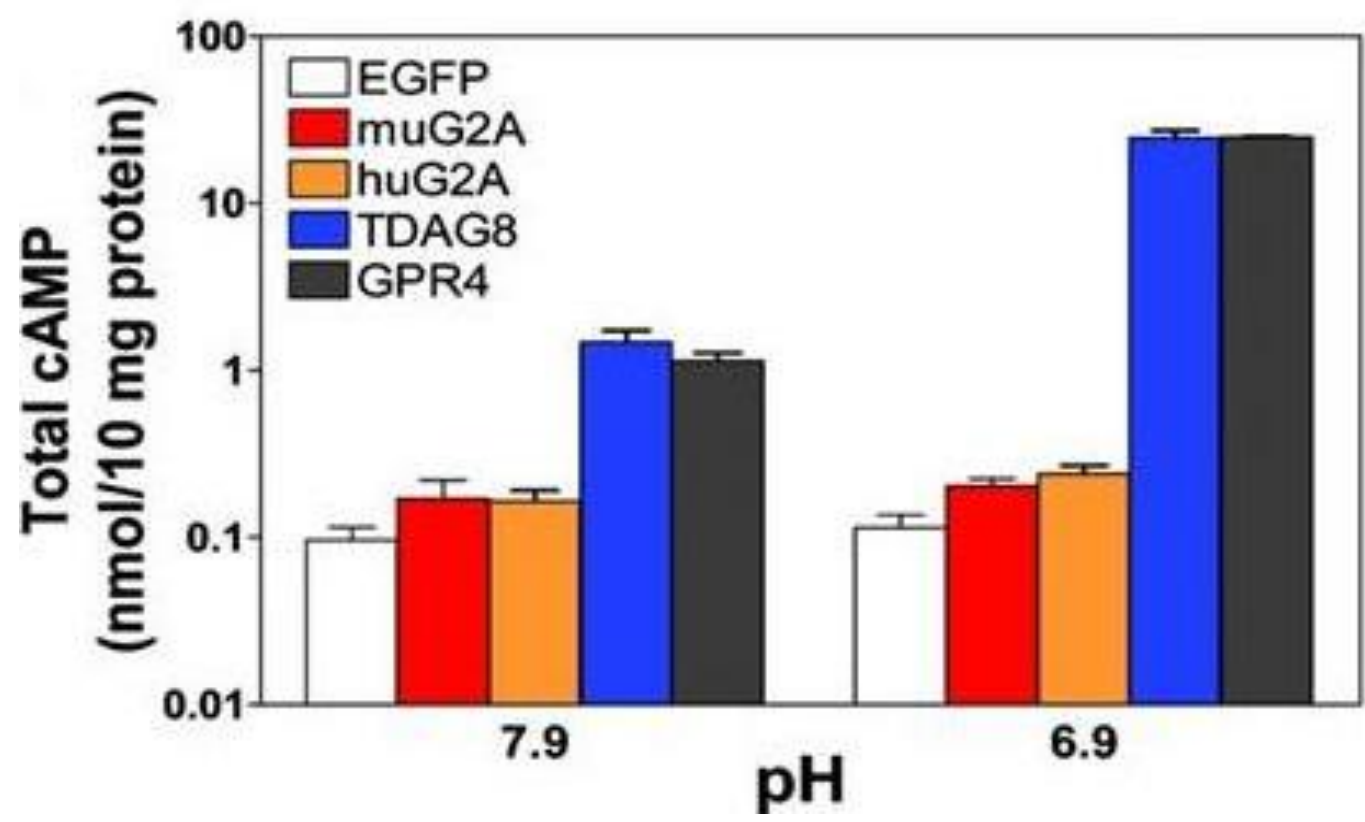


Sequence alignment identifies OGR1, GPR4, TDAG8, and G2A as family of GPCRs

Expressed OGR1 exhibits constitutive and pH-dependent IP production

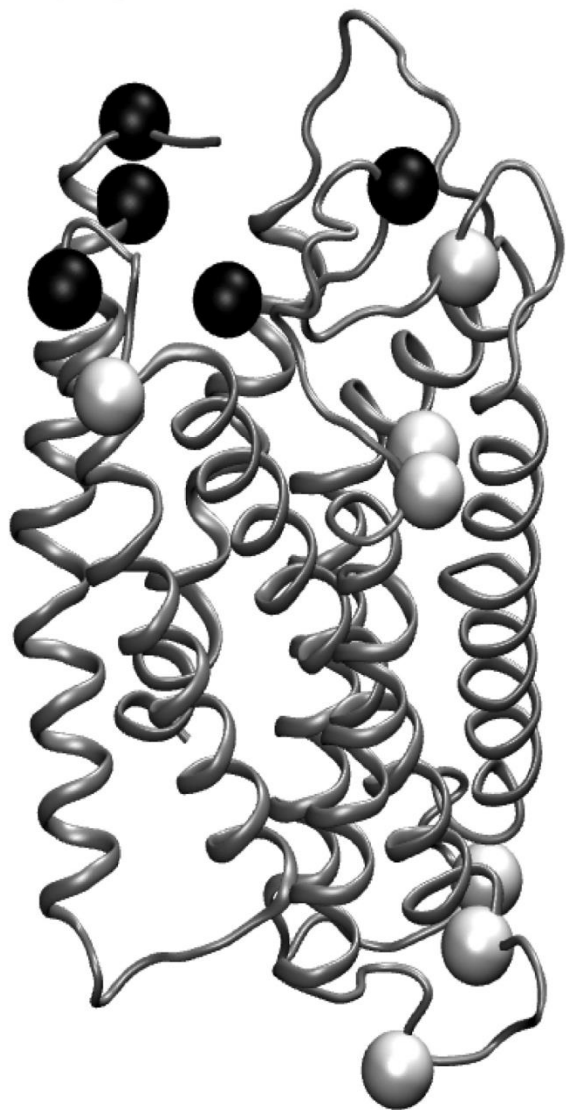


pH-dependent accumulation of intracellular cAMP by GPR4, TDAG8

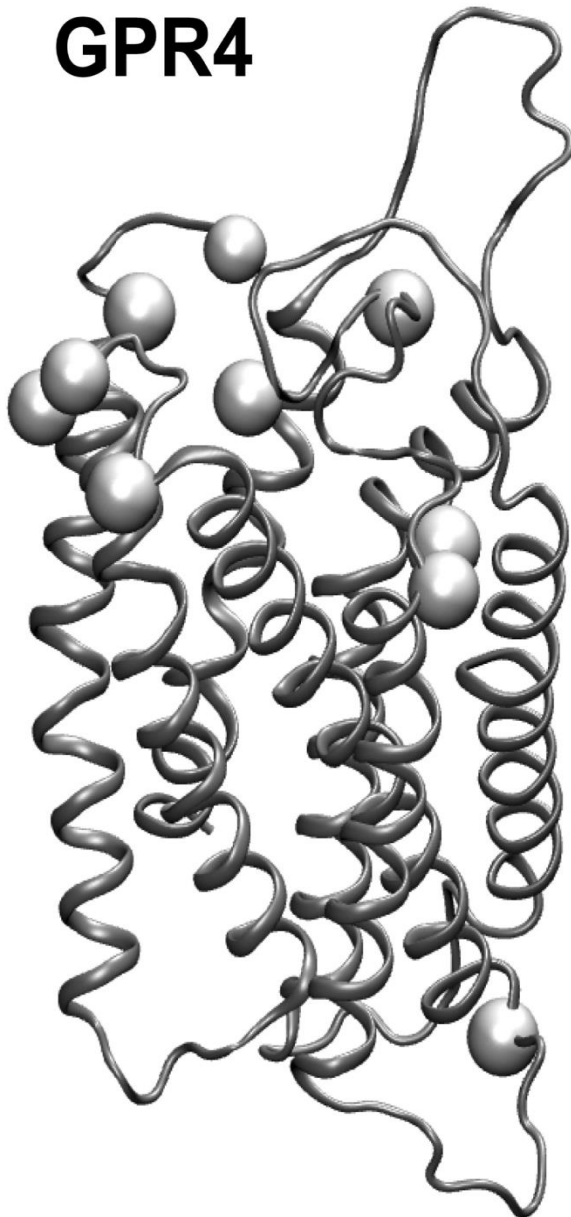


How do protons activate these GPCRs?

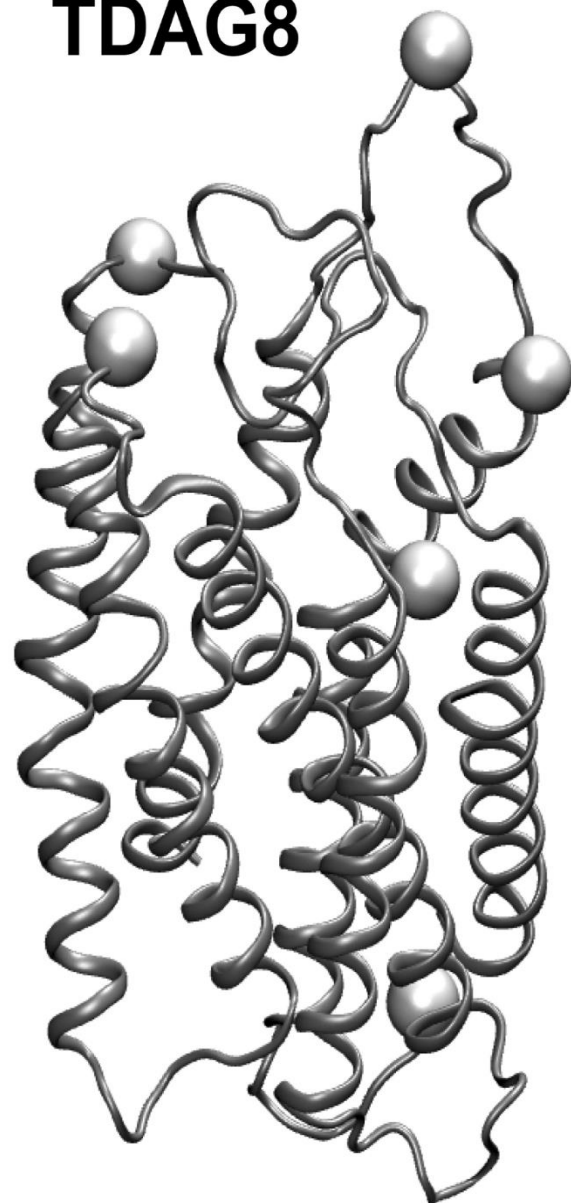
OGR1



GPR4

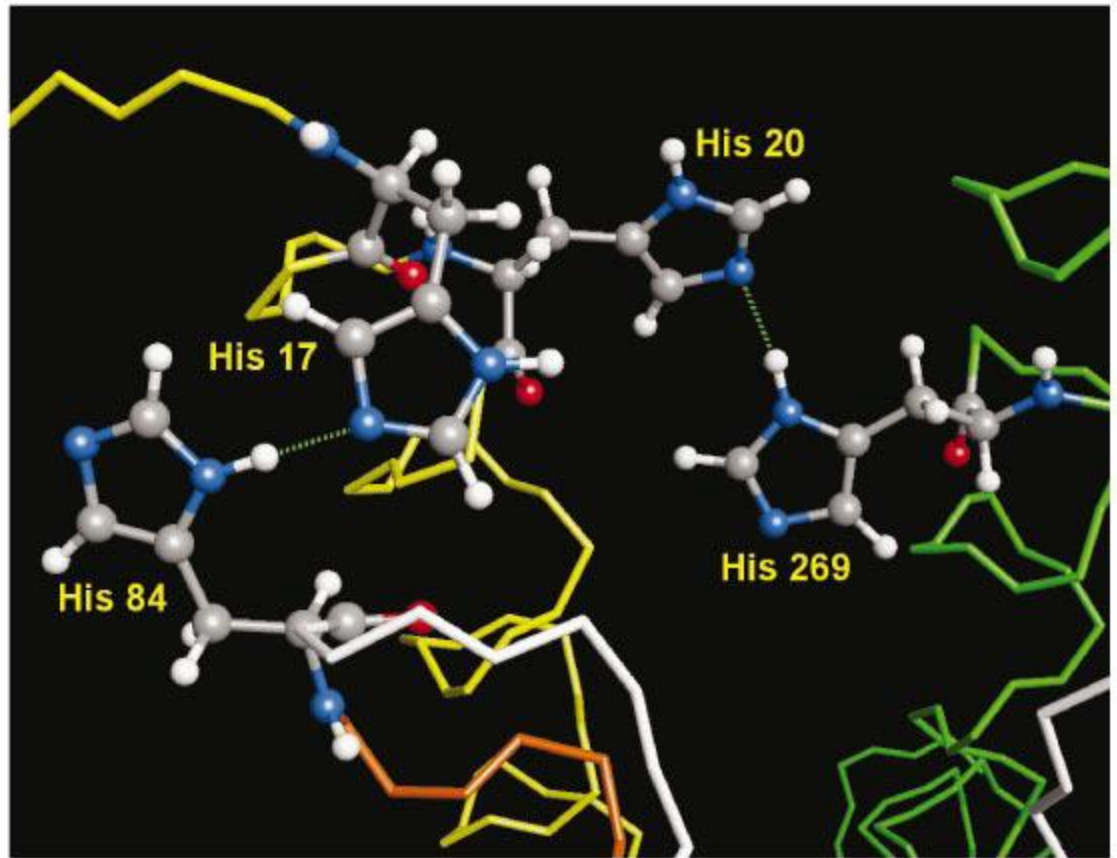
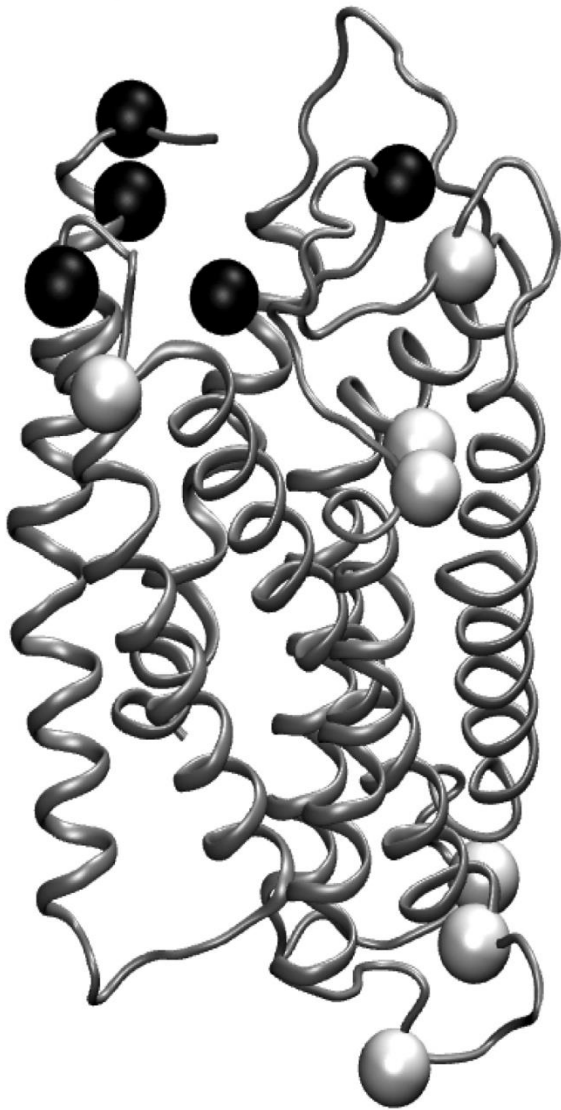


TDAG8

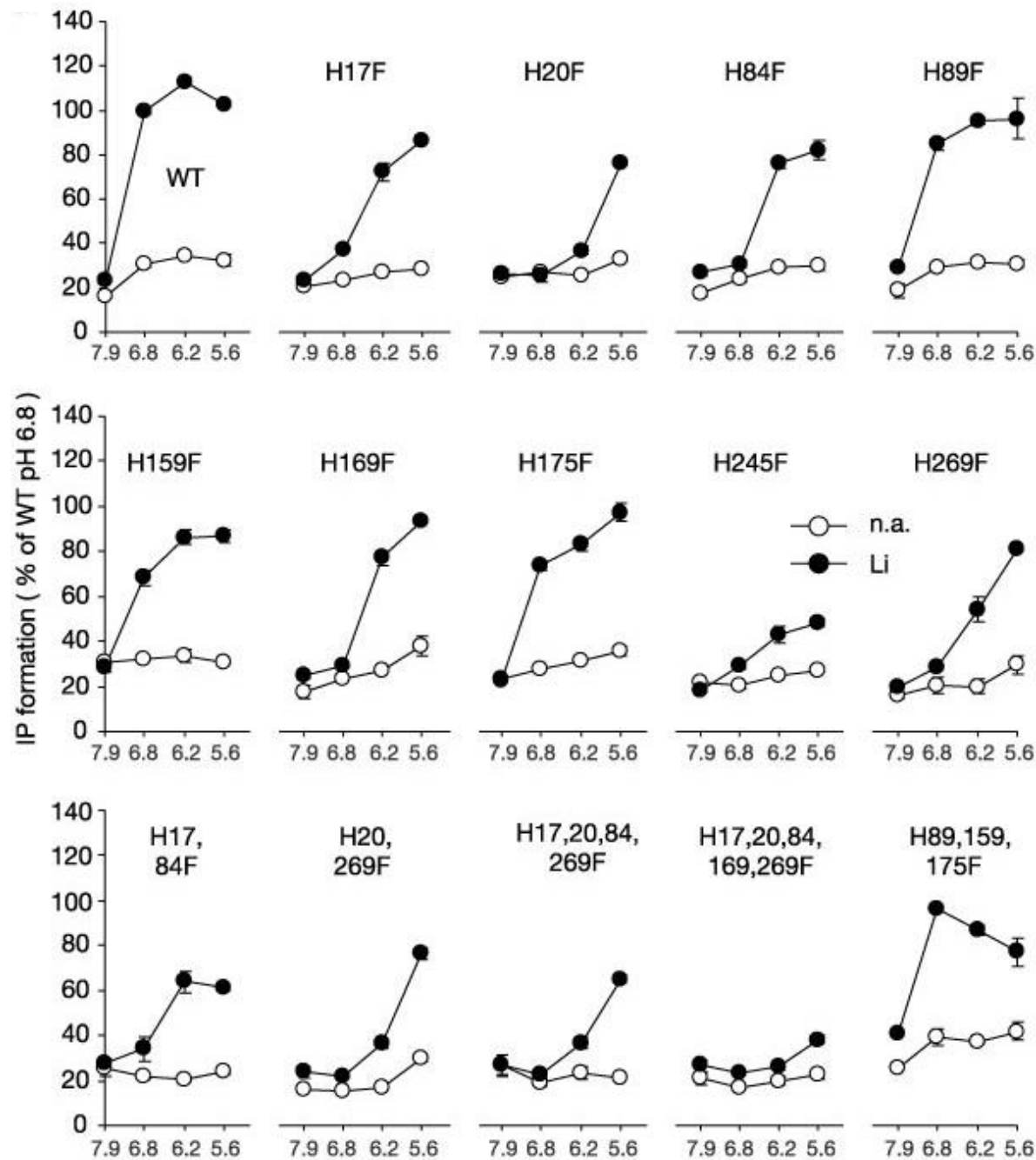


**Predicted structure, proton-sensing histidine residues in
OGR1, GPR4, and TDAG8**

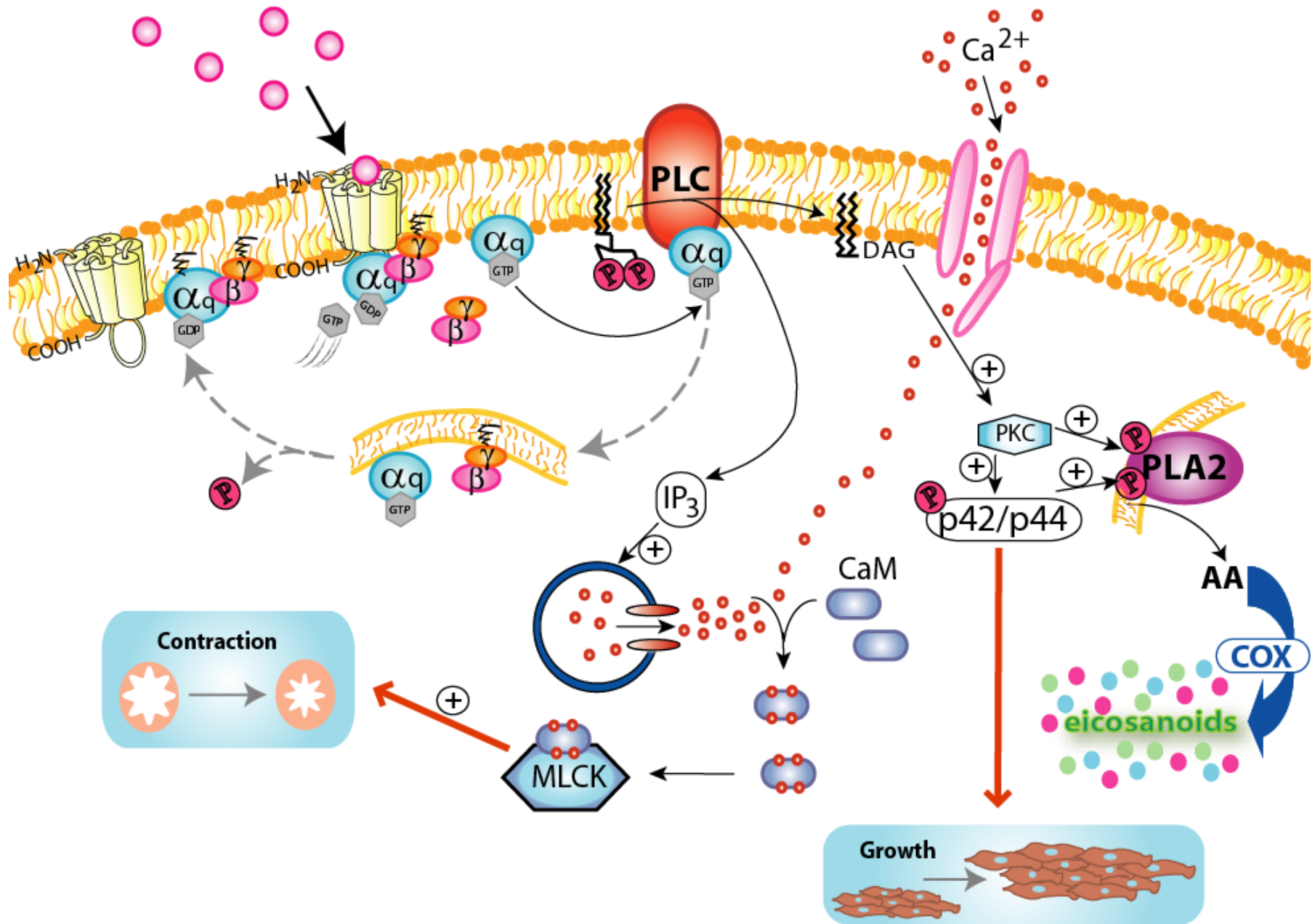
OGR1



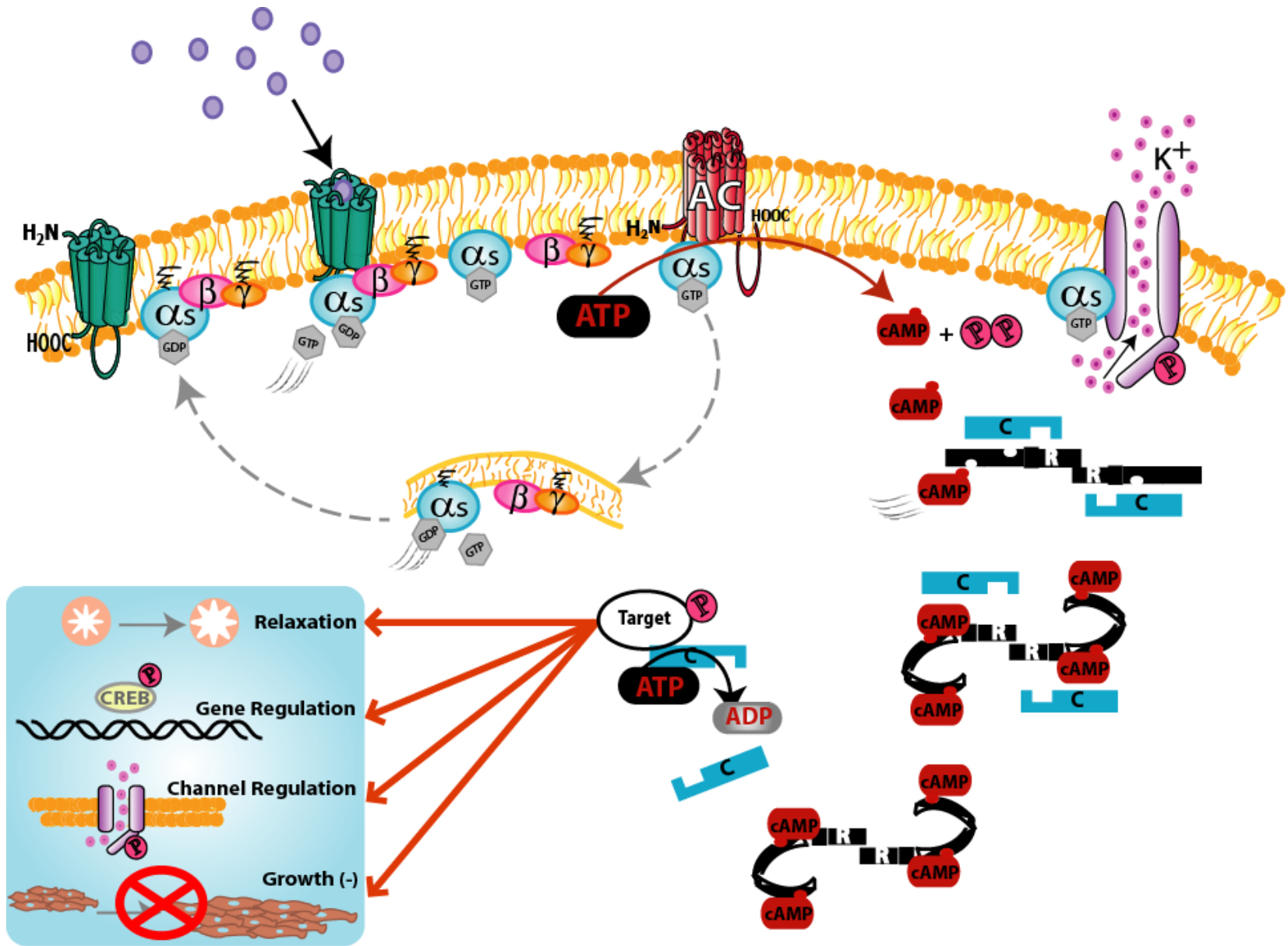
**Mutation of putative proton-sensing histidines inhibits
pH sensing by OGR1**



G_q -coupled receptor signaling in ASM

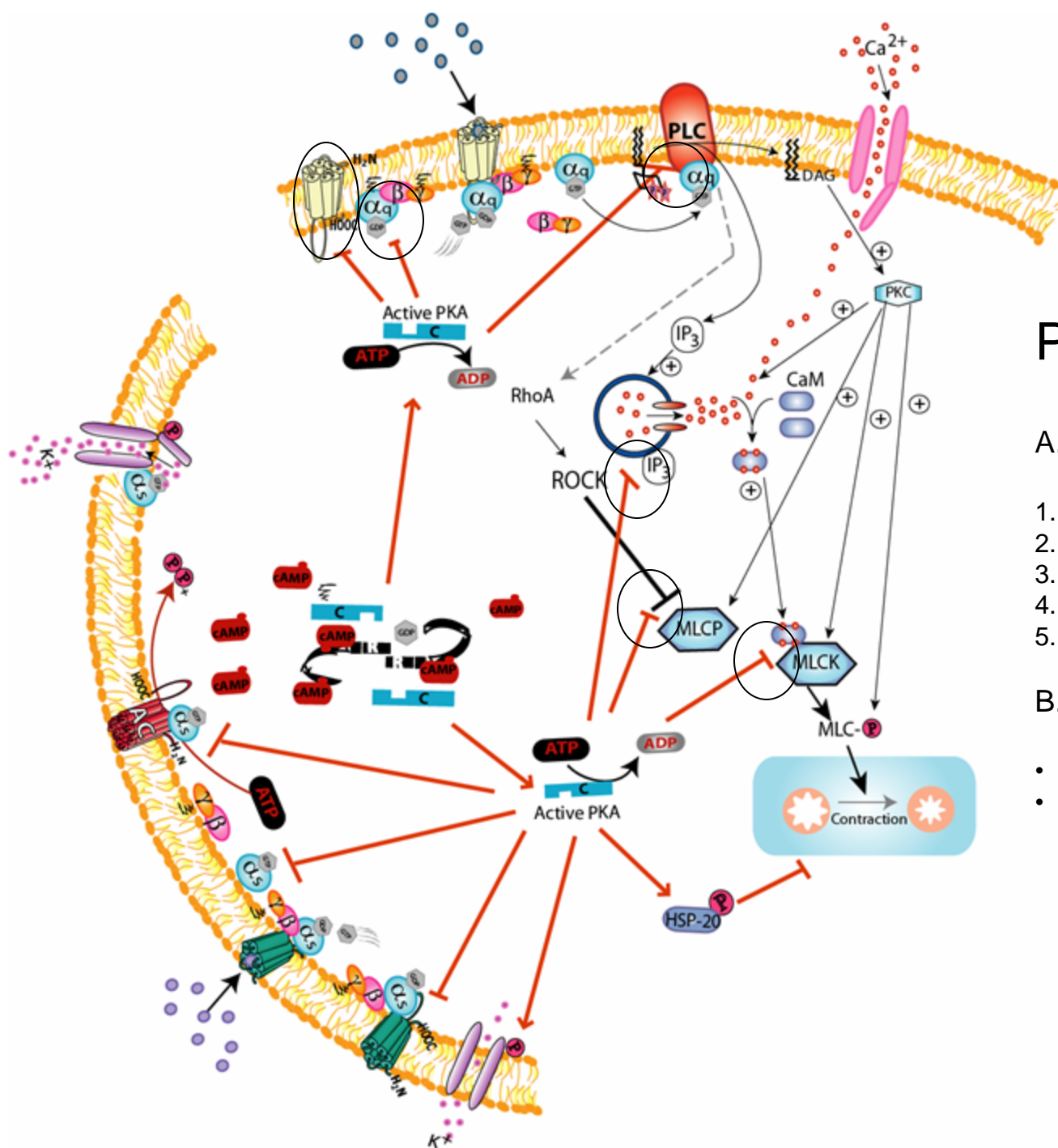


G_s-coupled receptor signaling



Gs-coupled receptors antagonize Gq-coupled receptor-mediated contraction

- Primarily via PKA activation
- Inhibits increase in intracellular Ca^{2+}
- Inhibits cellular sensitization to Ca^{2+}



PKA targets:

A. Controlling Ca²⁺ release entry:

1. Gq-GPCR
2. Gq
3. PLC
4. Phospholamban/IP3R
5. K⁺ and Ca²⁺ channels

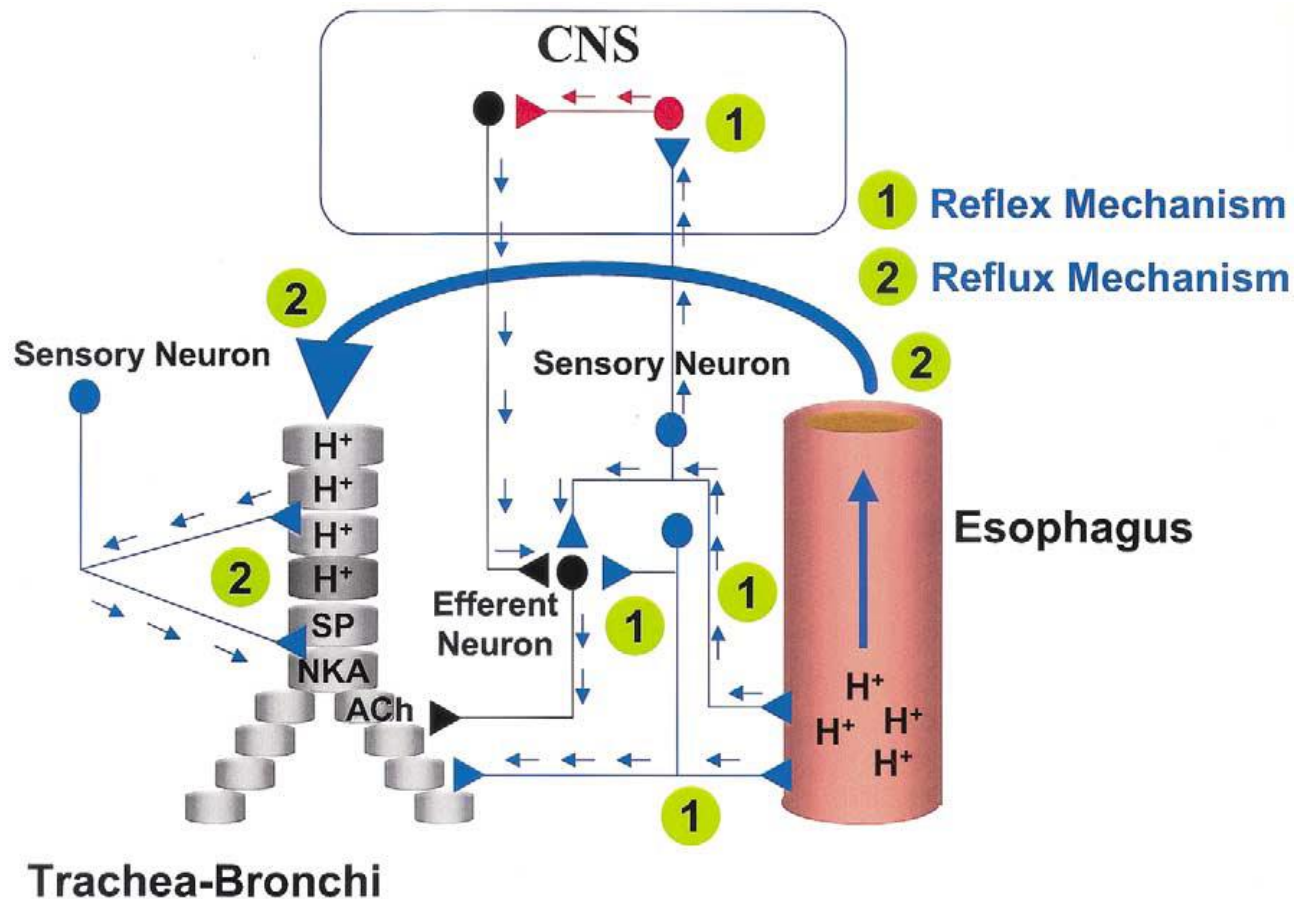
B. Calcium Sensitization:

- Calcium/calmodulin/MLCK
- Rho Kinase reg of MLCP

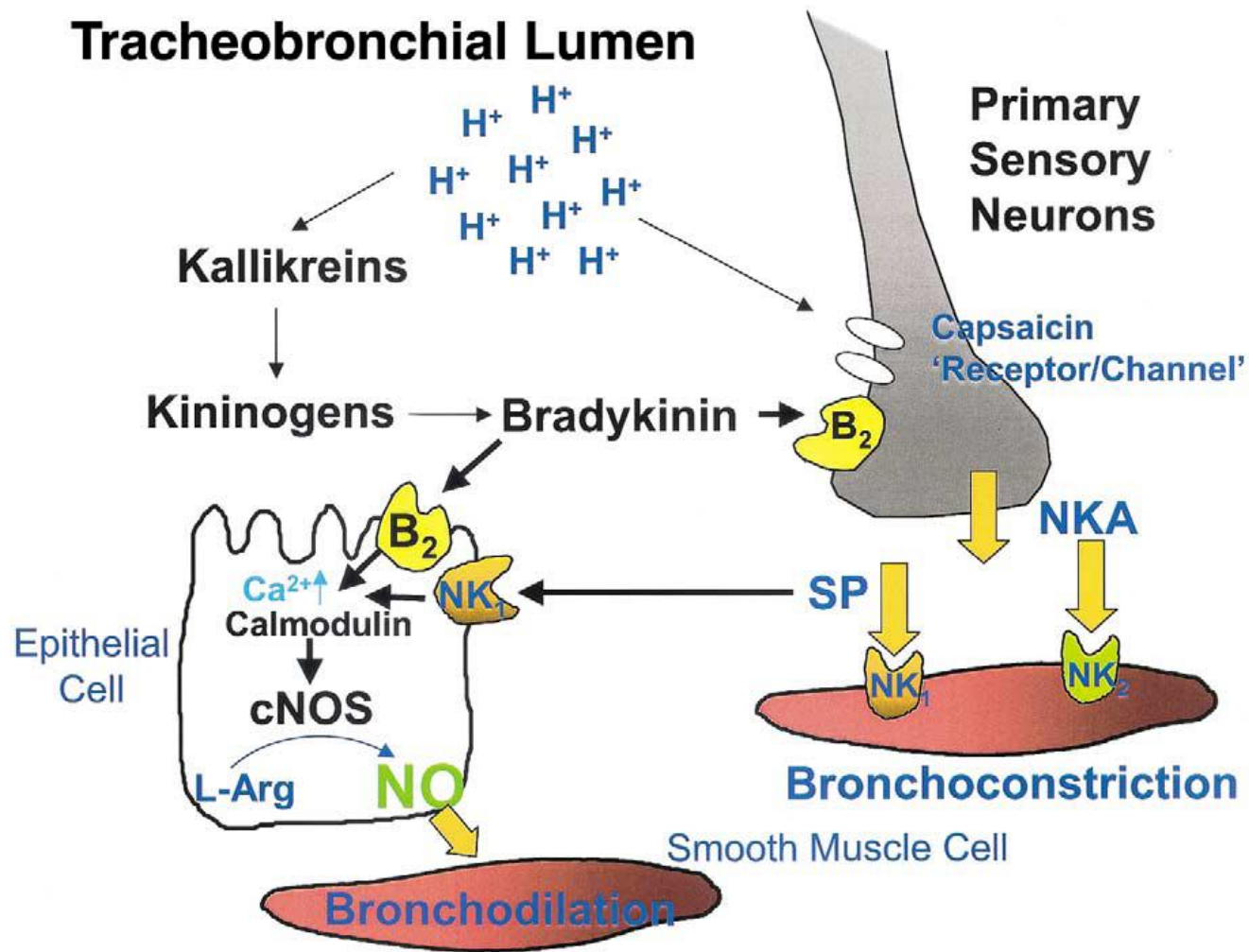
Objectives

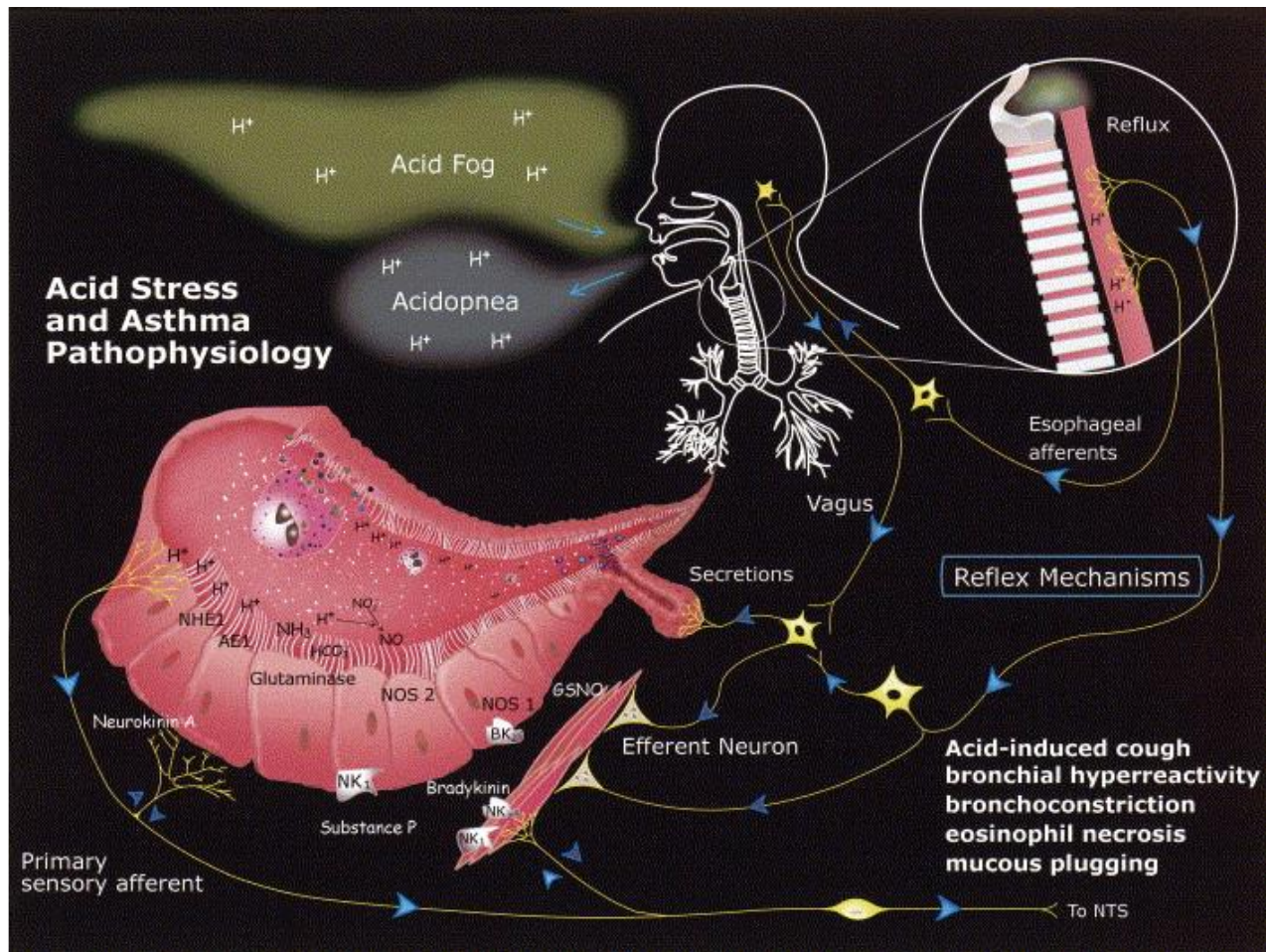
- Characterize the proton sensitive GPCRs in the ASM
- Determine the intracellular signaling mechanisms activated by proton sensitive receptors in the ASM
- Determine the functional consequences of changing pH in the microenvironment of the ASM

Current proposed mechanisms



Tracheobronchial Lumen

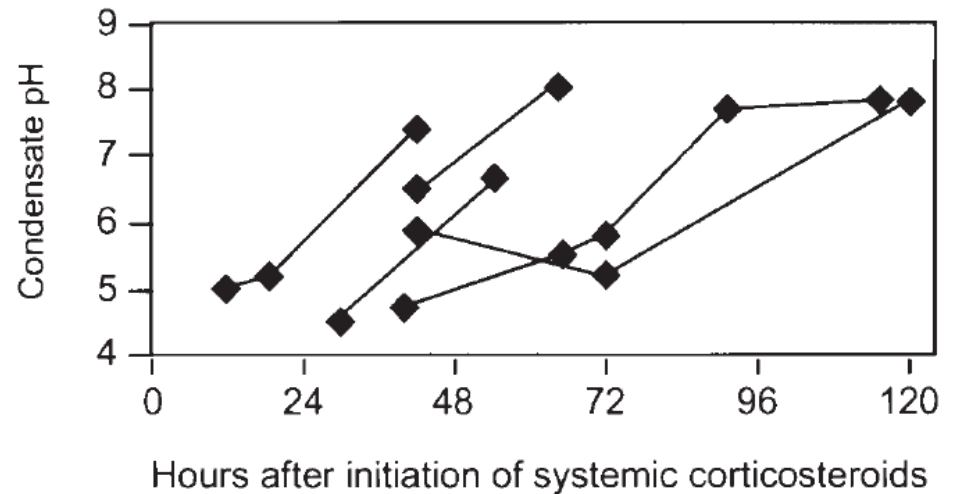
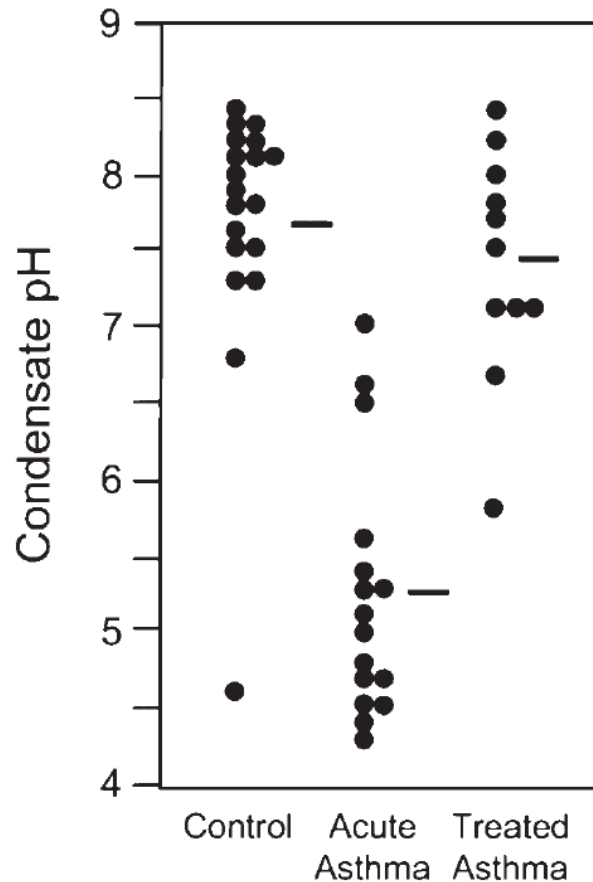




Airway pH tends to alkaline, but decreases with allergic inflammation

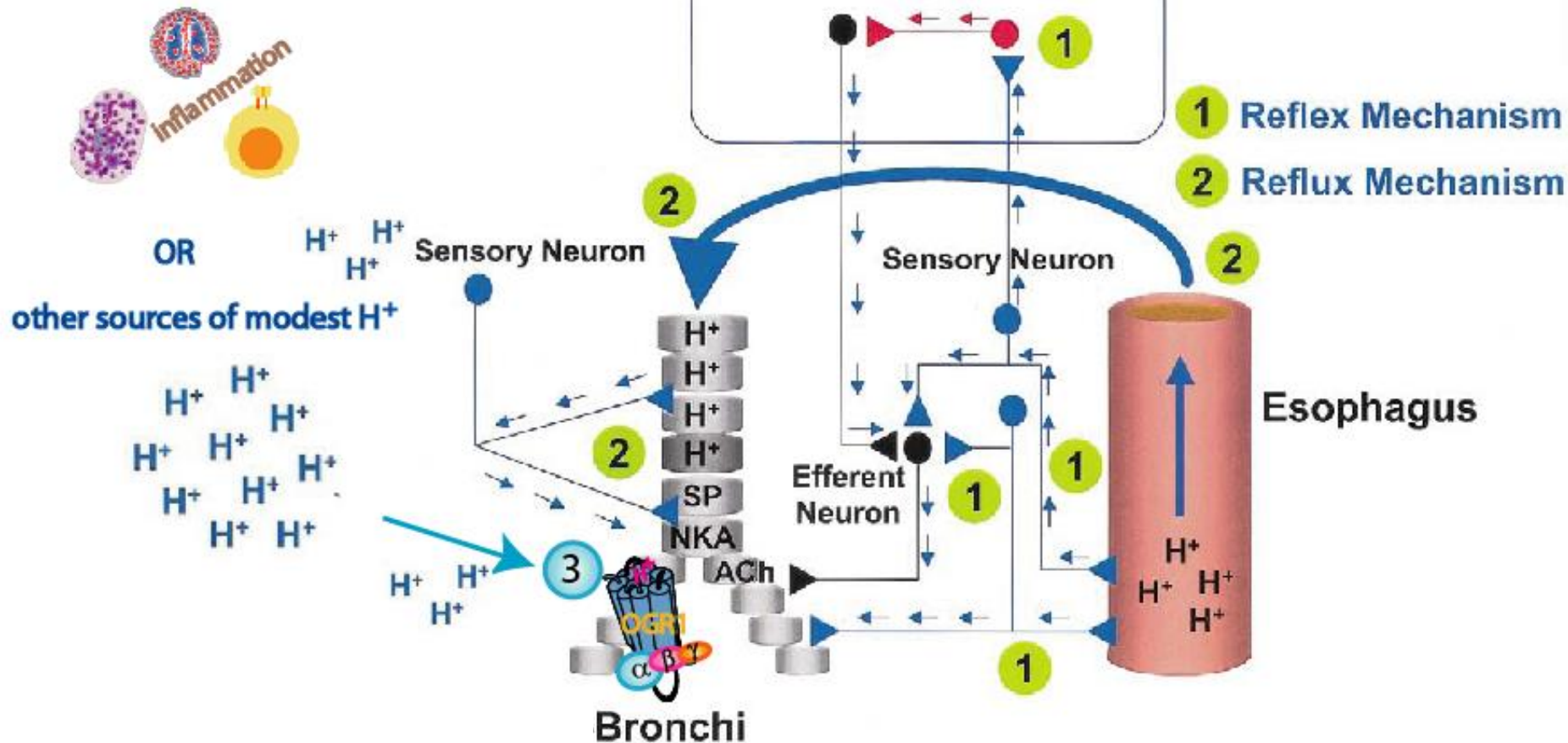
Respiratory fluid	Condition	Measured pH
EBC (deaerated to remove CO ₂)	Health	7.7±0.49
	Asthma exacerbation	5.2±0.21
	Stable mild asthma	7.6 (7.55-7.65)
	Stable moderate asthma	7.27 (CI, 7.15-7.39)
	Stable COPD	7.16 (CI, 7.09-7.23)
	COPD exacerbation	6.25
	Stable bronchiectasis	7.11 (CI, 7.04-7.19)
	Intubated: healthy	7.8±0.28
	Intubated: sepsis	5.92
	Intubated: ARDS	6
Tracheal fluid in vivo (transcricoid pH probe)	Health w/GER episodes	7.0 (6.5-7.4) To <4.0
Bronchial fluid in vivo (pH probe inserted through bronchoscope)	Health	7.1Â±0.1
Airway submucosal gland secretion (explanted tissue)	Health	6.97
Nasal fluid in vivo (pH probe)	Health	7.4-7.9
	Health	6.9-7.4
	Health	7.31 (7.2-7.5)
	Allergic rhinitis	7.8-8.5
	Chronic hypertrophic rhinitis	7.14 (6.6-7.6)

Corticosteroids reverse the reduced airway pH associated with acute asthma exacerbations

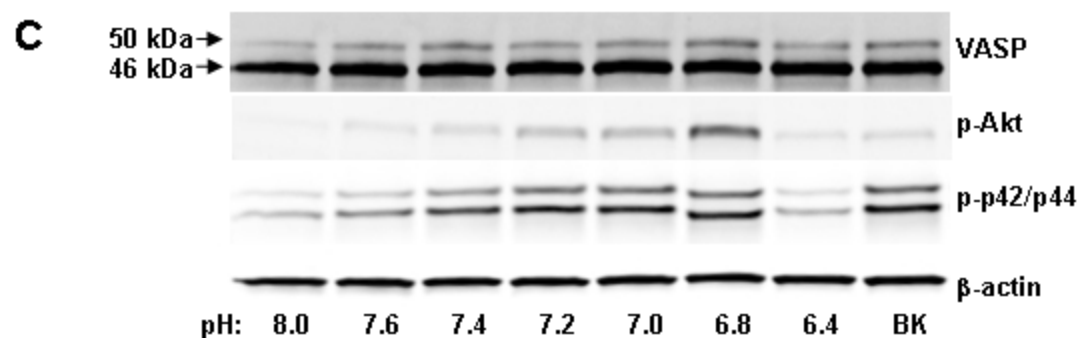
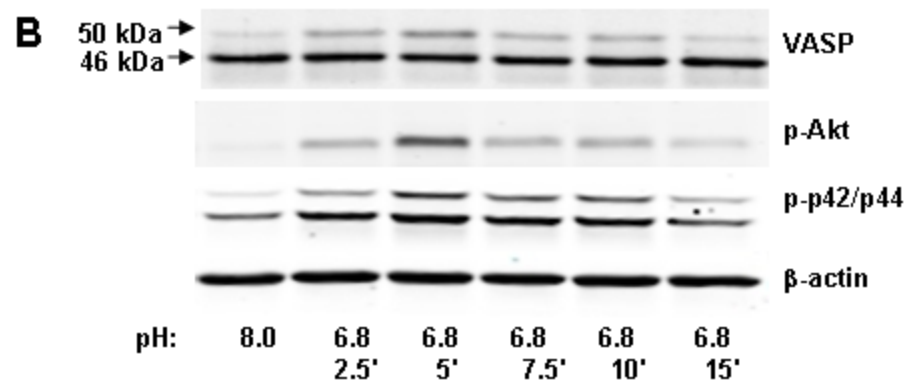
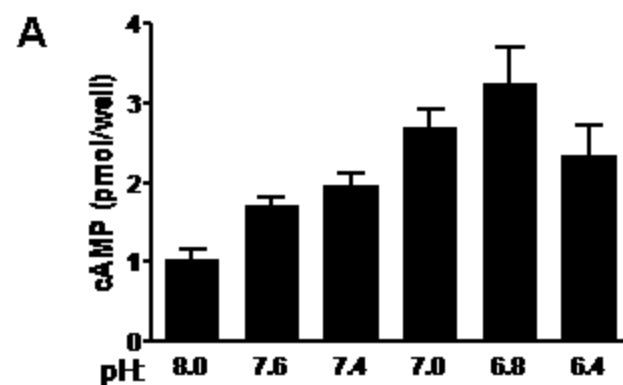


Proton-sensing GPCRs may represent another mechanisms mediating effects of more subtle decreases in airway pH

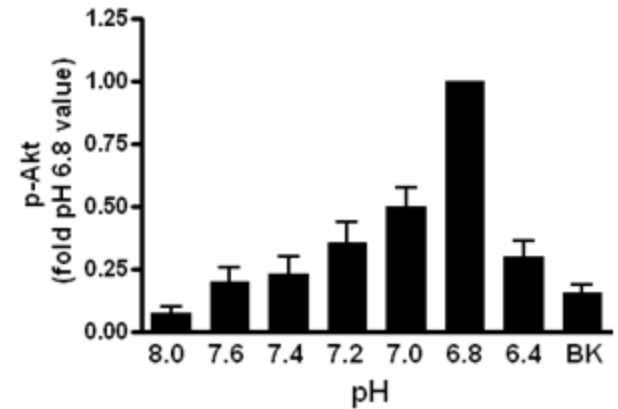
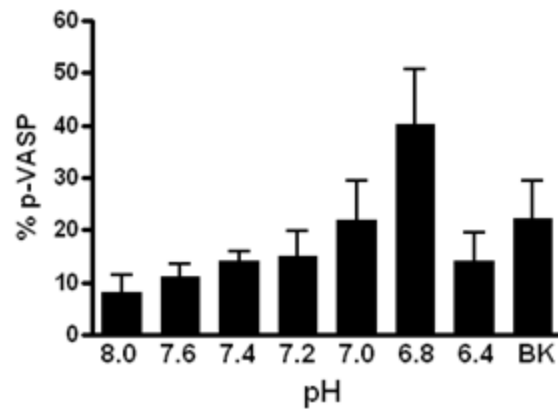
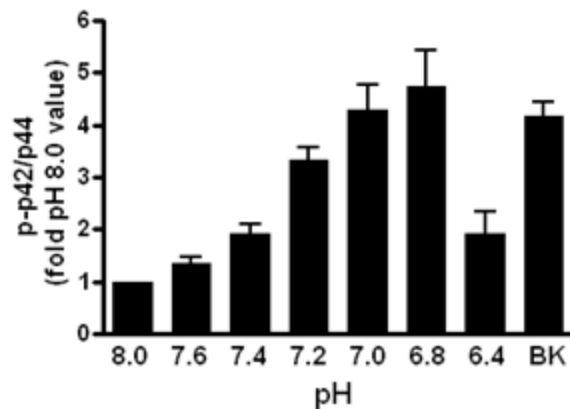
3 Alternative Mechanism



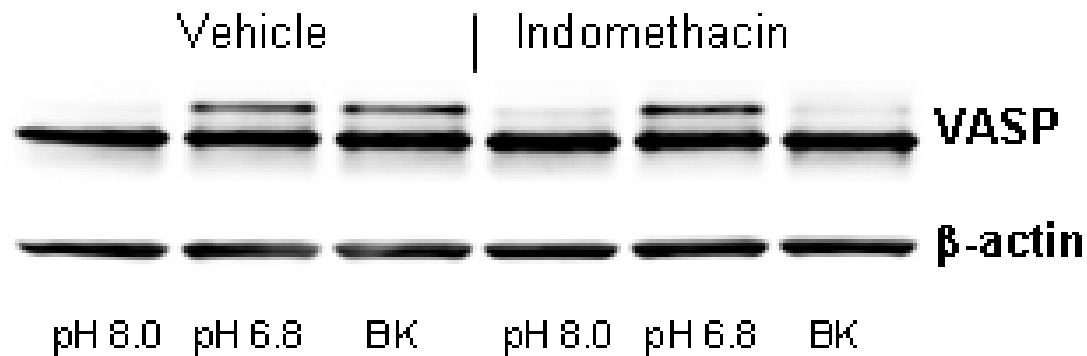
↓pHo activates cAMP/PKA, Akt, and p42/p44 in ASM



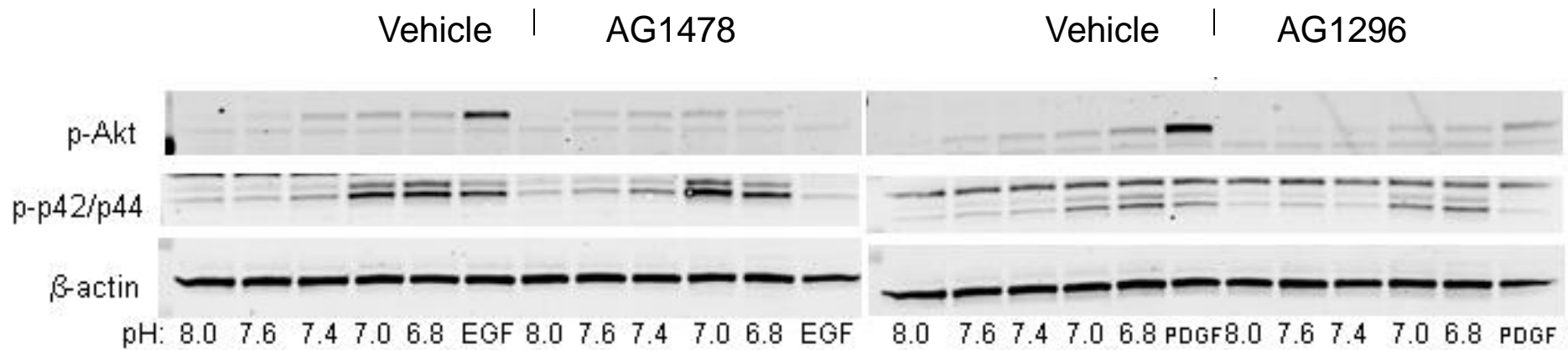
↓pHo activates cAMP/PKA, Akt, and p42/p44 in ASM



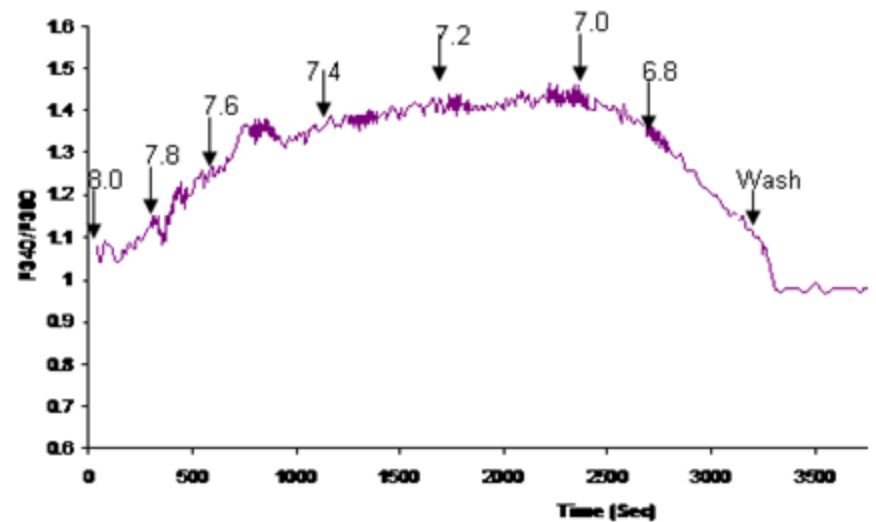
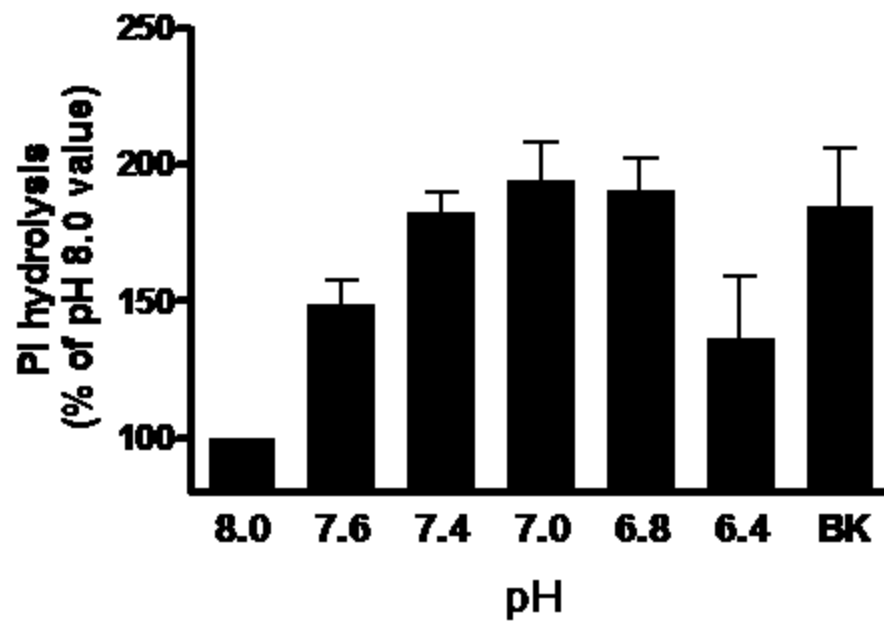
PKA activation by ↓pH_o not necessarily dependent on COX:
Pleiotropic signaling?



Akt, p42/p44 activation by ↓pHo is not via RTK transactivation

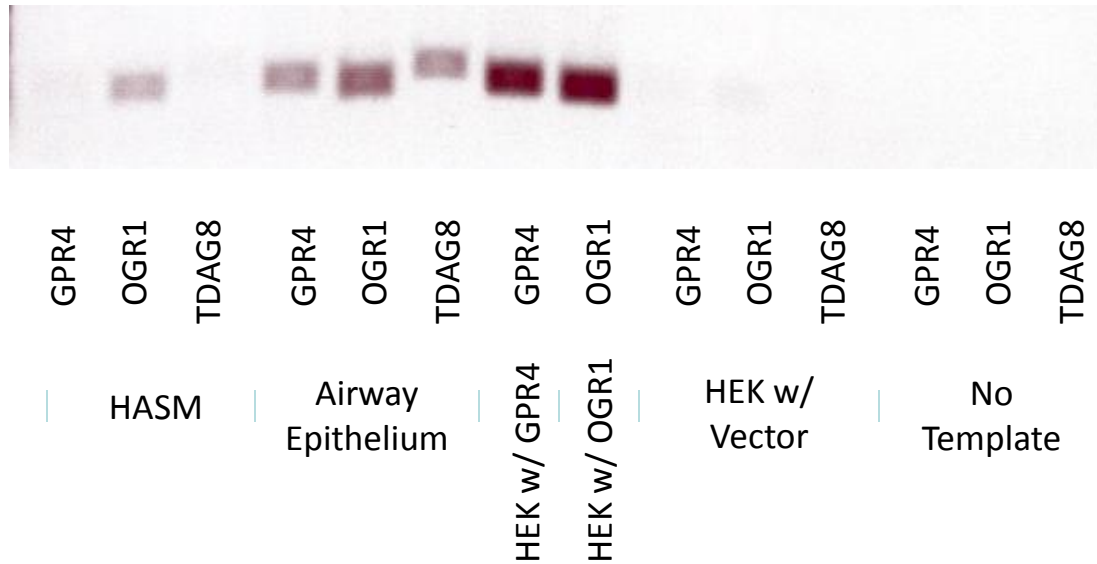


↓pHo stimulates PI hydrolysis, Ca^{2+} mobilization

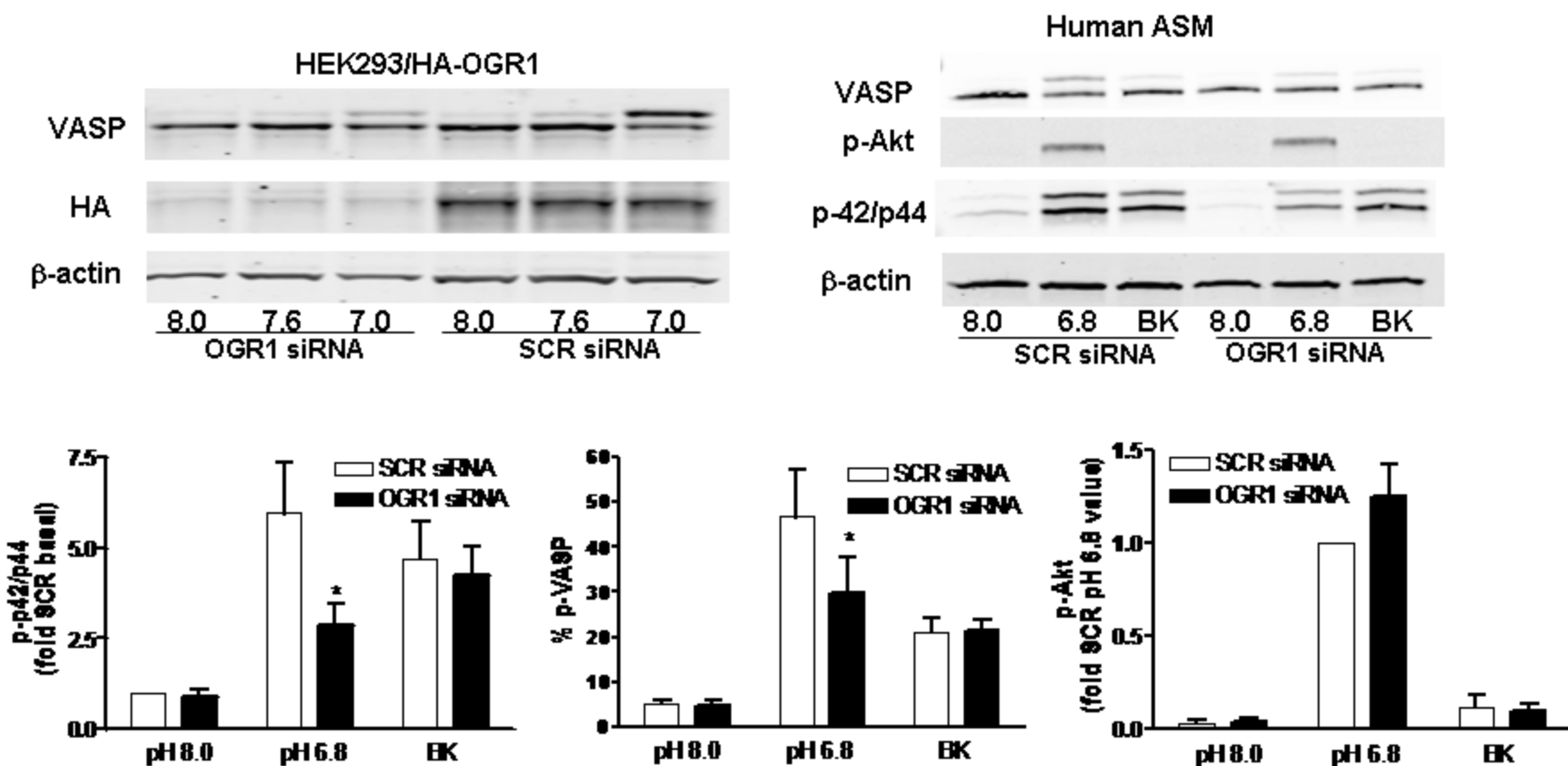


Expression of proton sensitive receptors in ASM

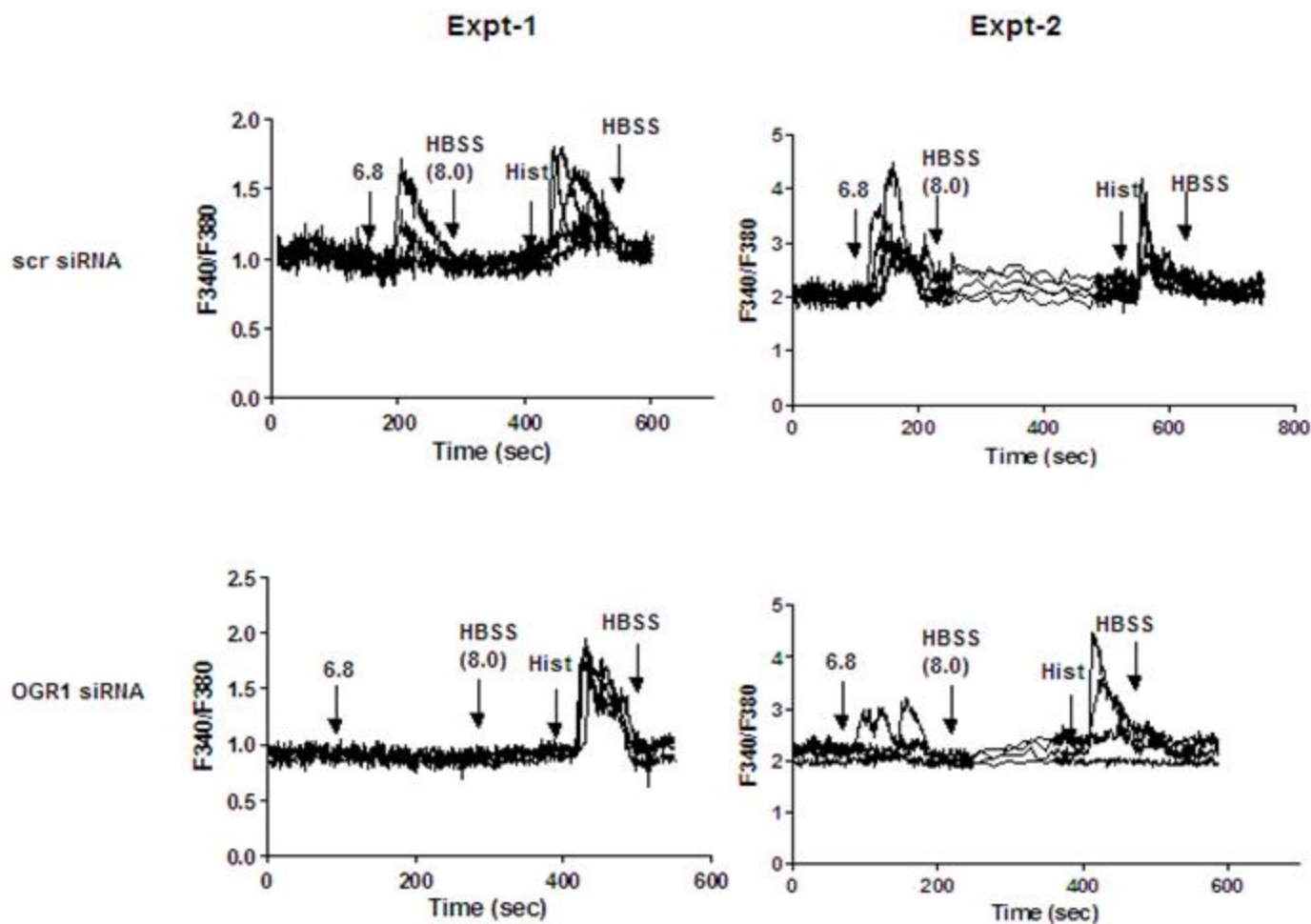
PCR of GPCRs in HASM and Airway Epithelium



OGR1 knockdown reduces PKA, p42/p44 activation by ↓pHo



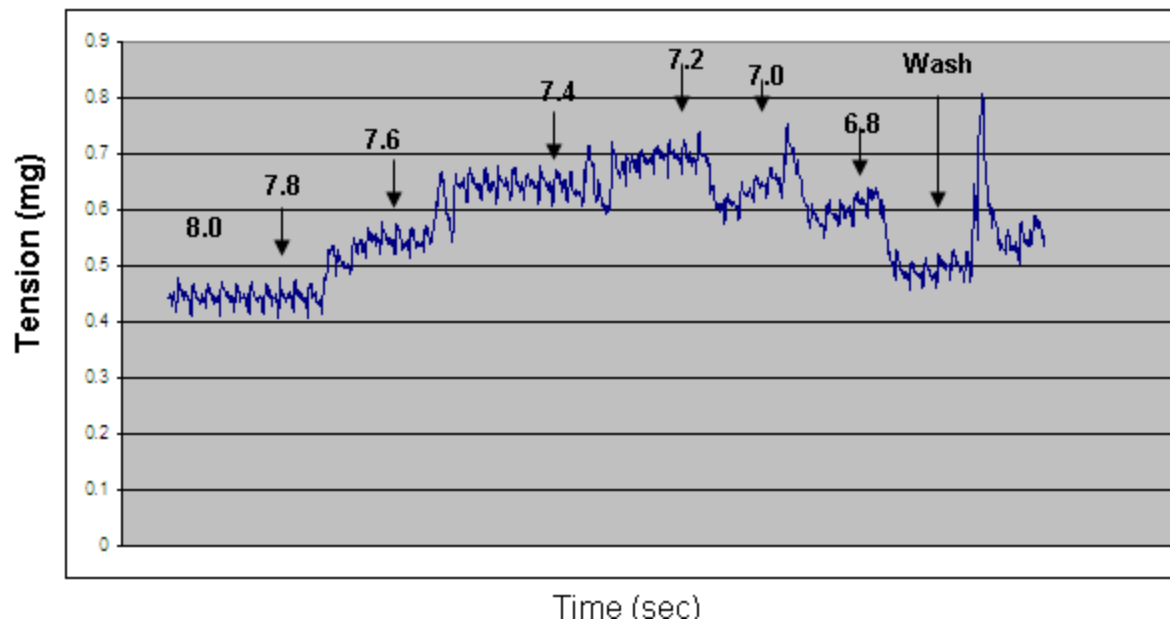
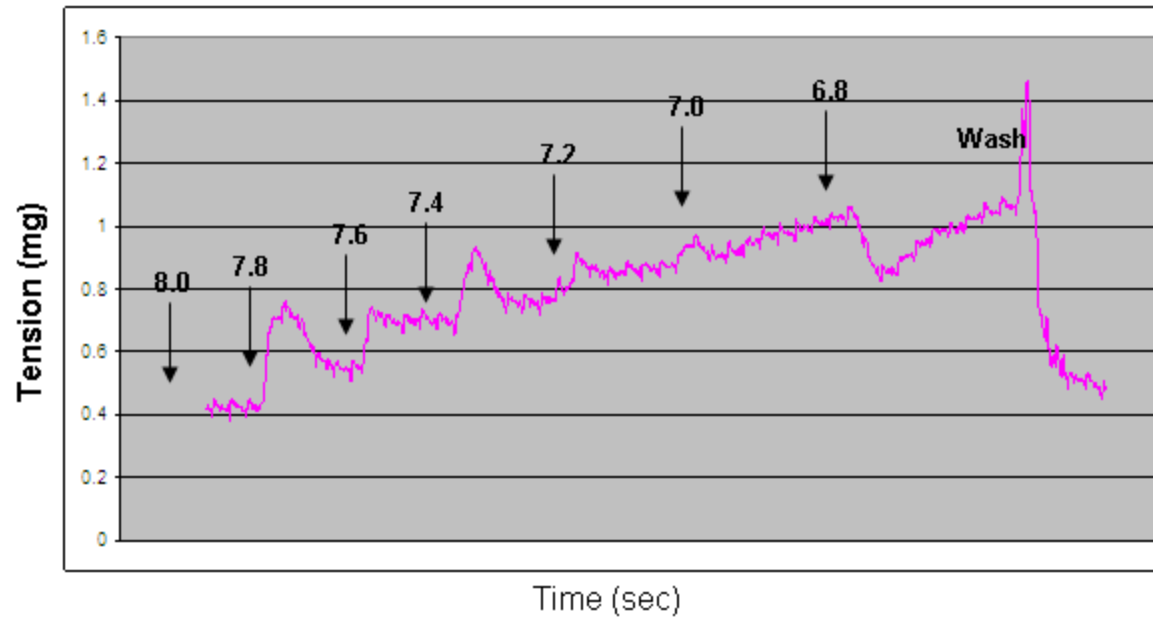
OGR1 knockdown reduces Ca^{2+} mobilization by $\downarrow\text{pH}_\text{o}$



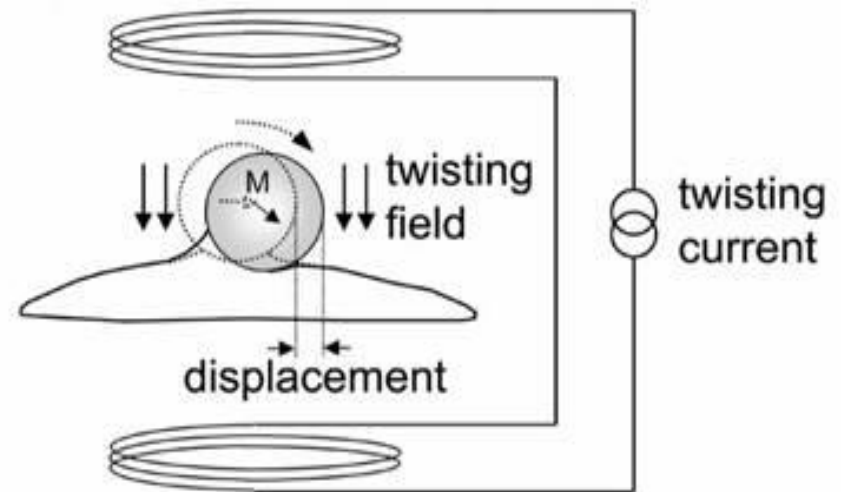
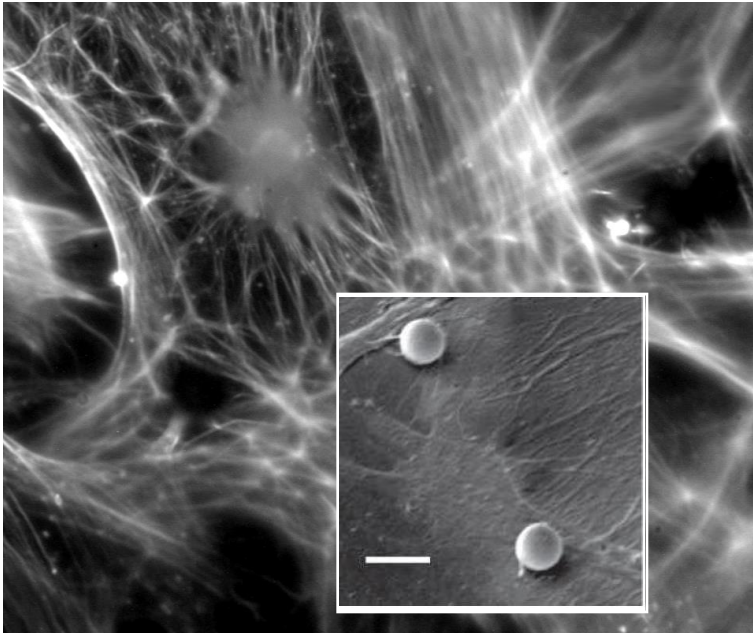
Signaling looks good.

What about function?

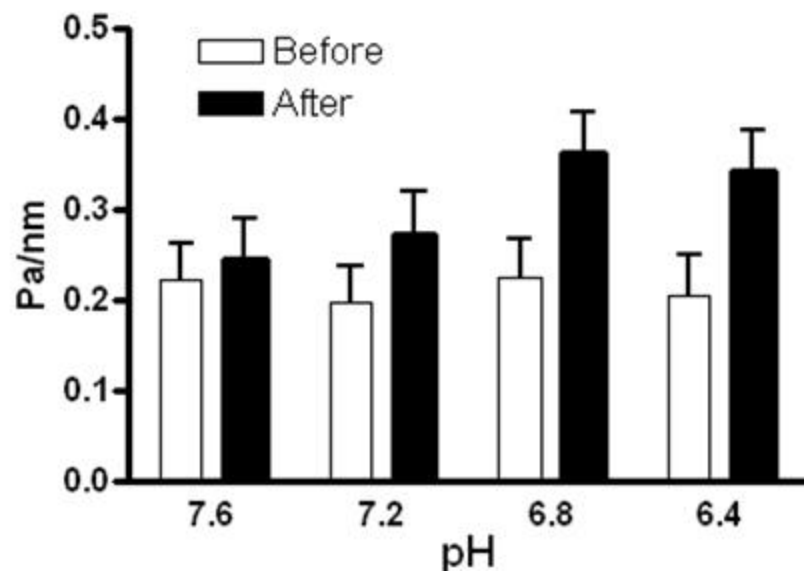
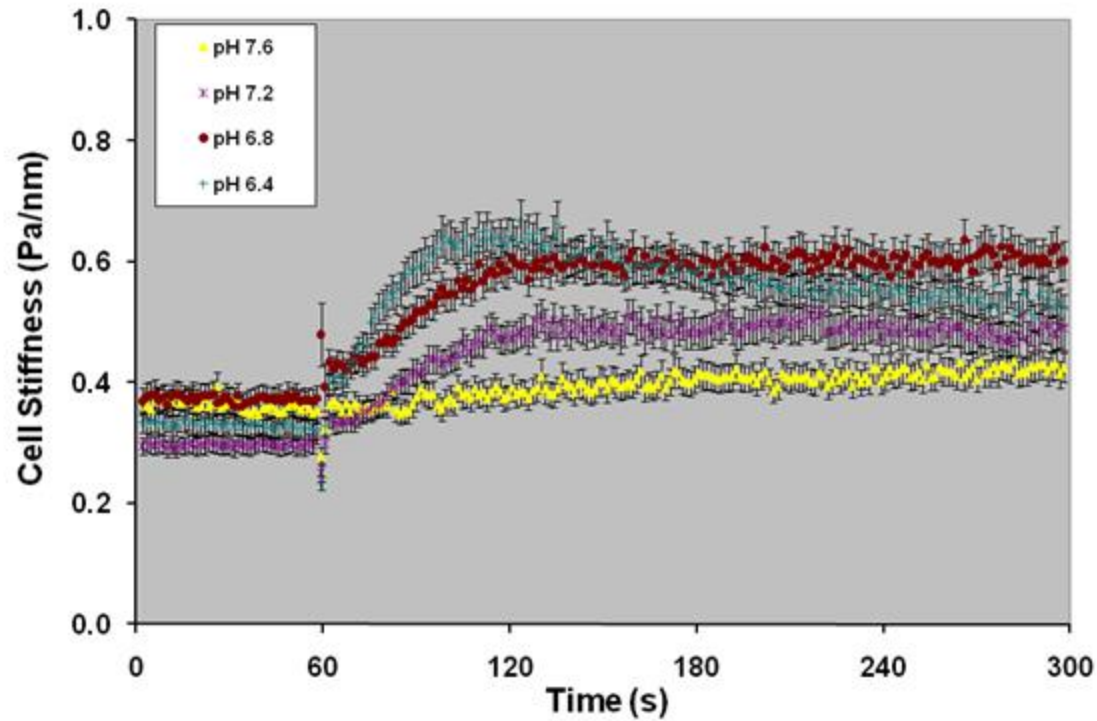
ASM tissue contracts in a pH dose-dependent manner



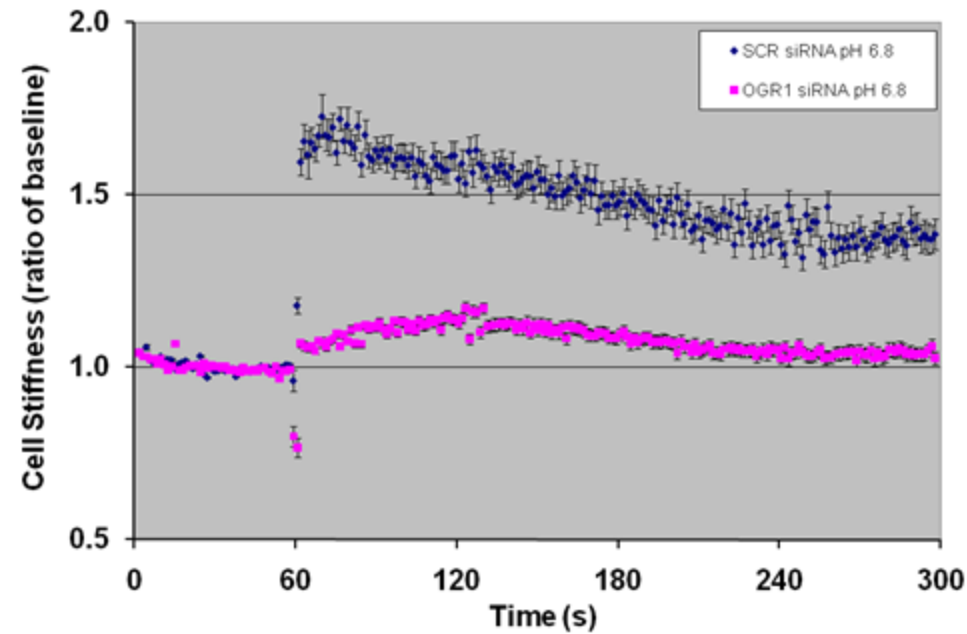
ASM cell contraction: Magnetic Twisting Cytometry (MTC)



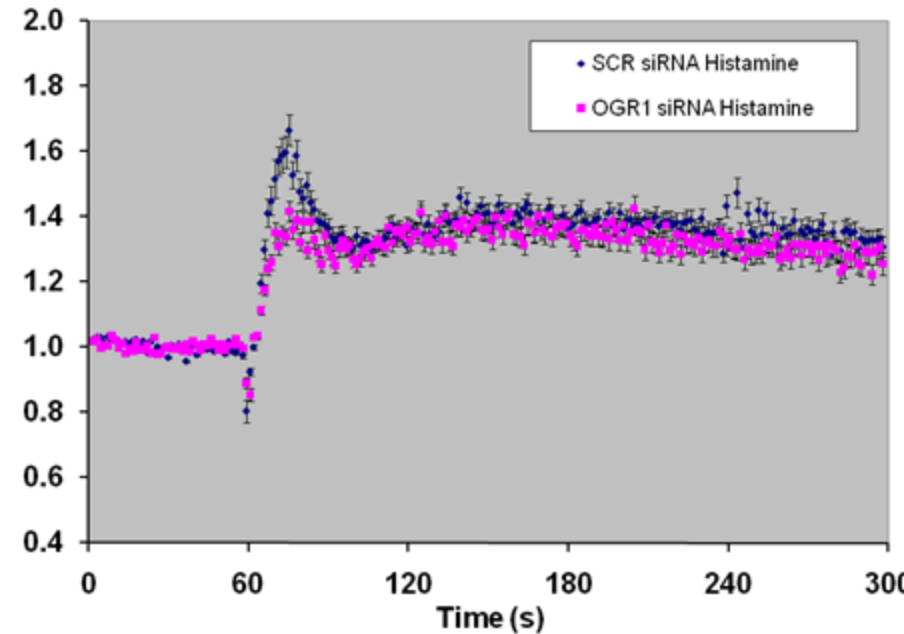
Cells contract, too



...and OGR1 knockdown inhibits this contraction

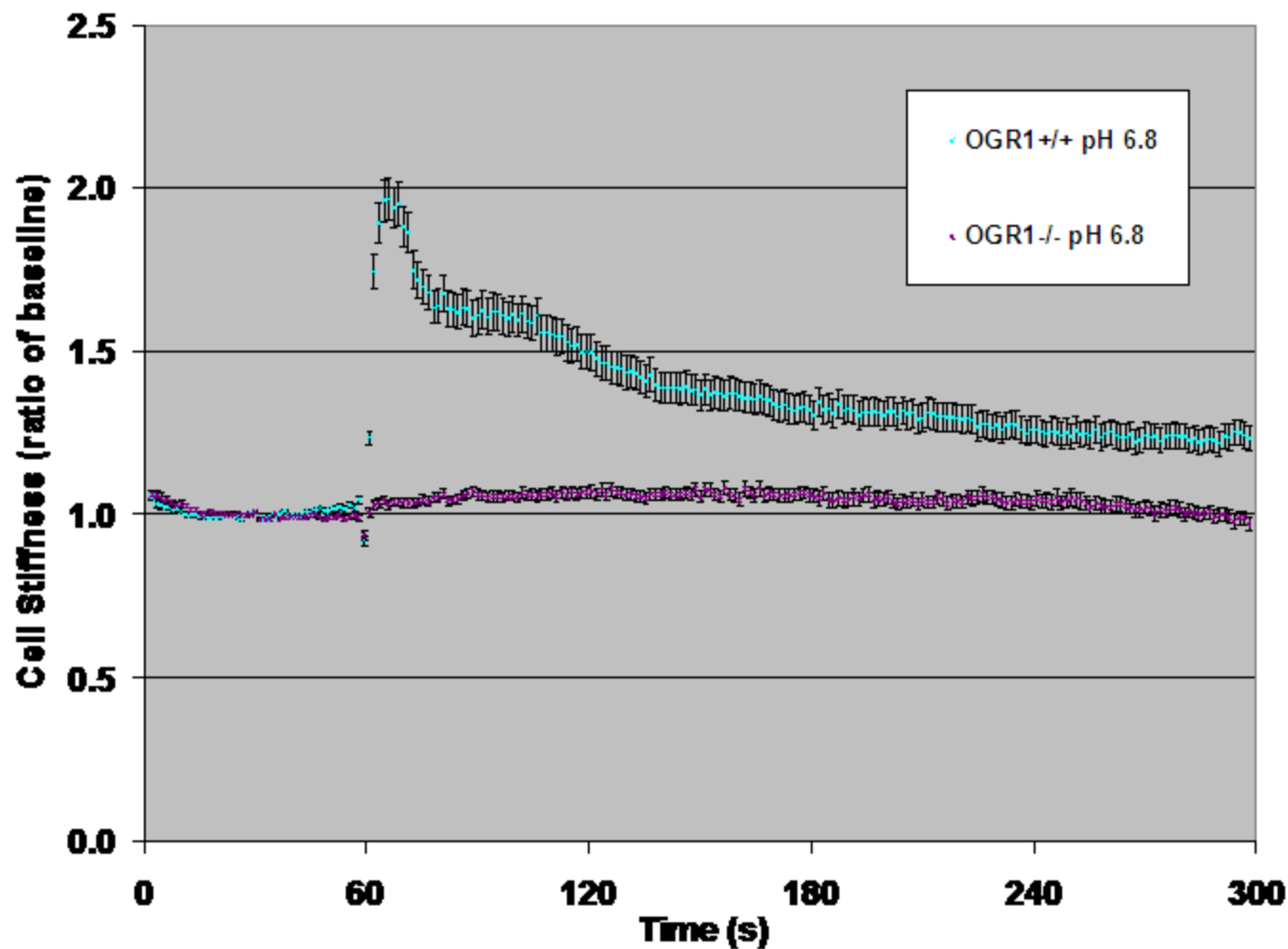


pH 6.8



Histamine

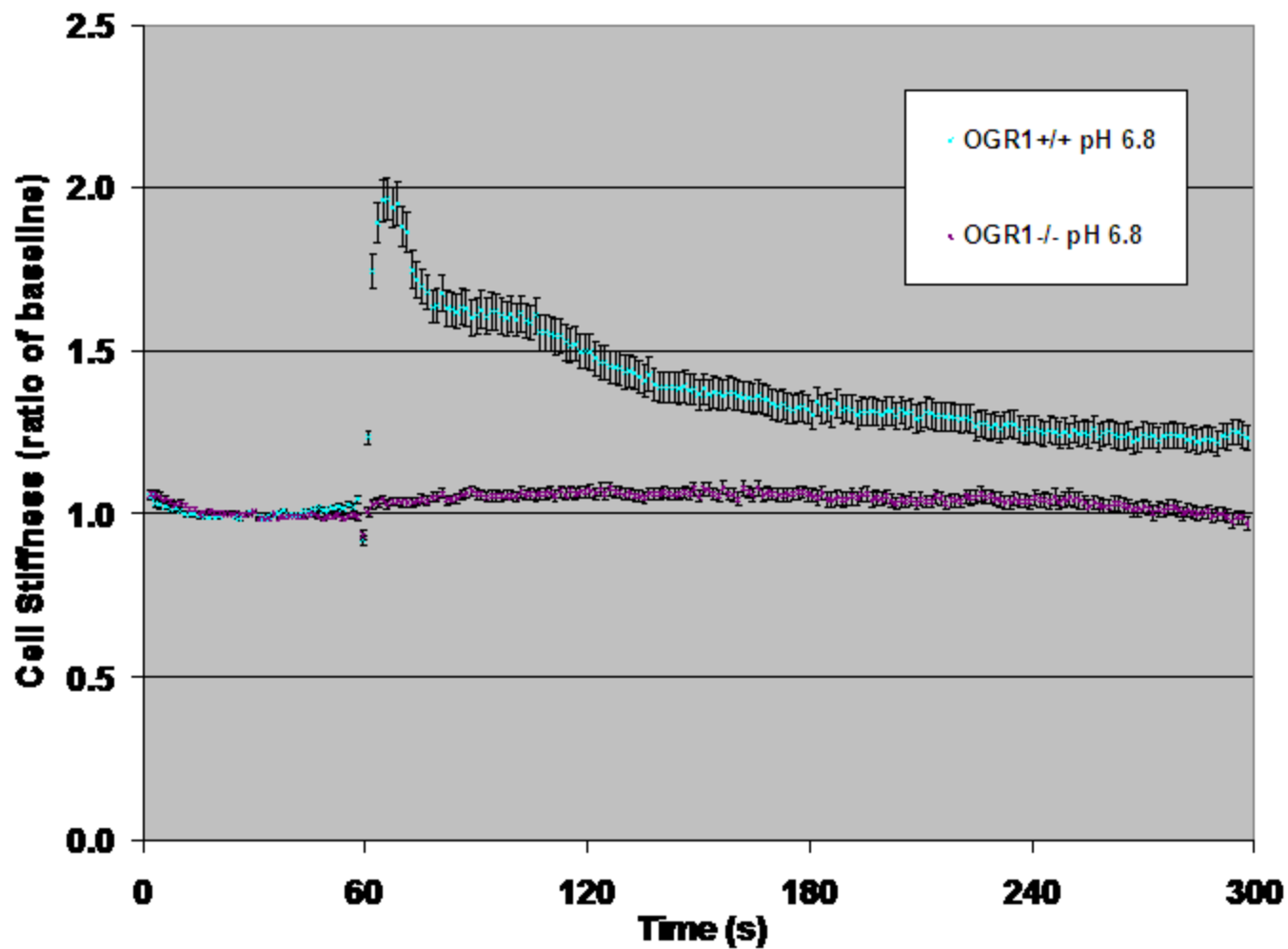
...and in the obligatory mouse experiment



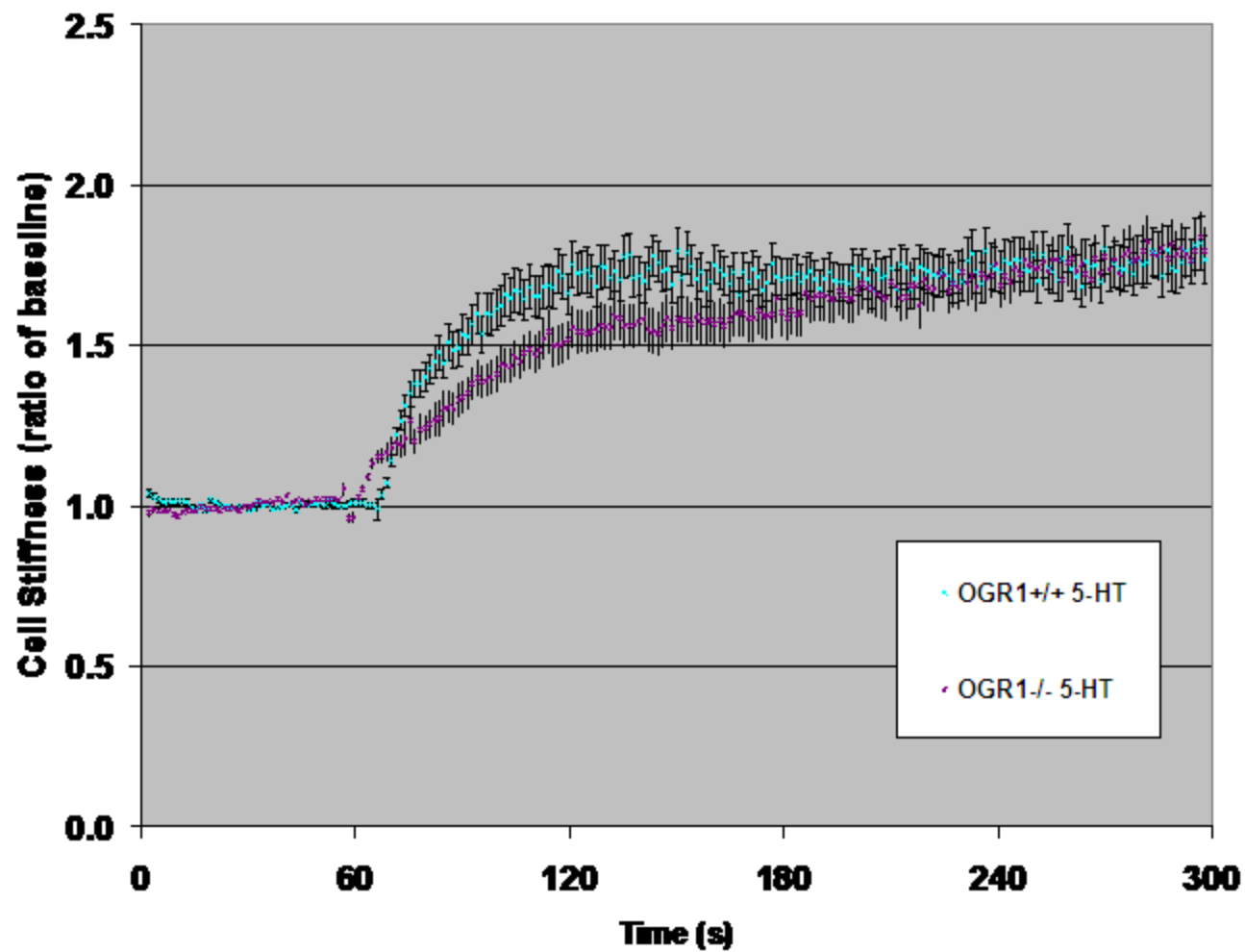
pH 8.0→6.8



...and in the obligatory mouse experiment



pH 8.0→6.8



5-HT

Summary of results

- Stimulation of ASM cells with increasing concentration of protons leads to activation of p42/p44 MAPK and calcium elevation suggesting Gq-mediated responses
- Acid stimulation of ASM cells also results in the activation of PKA that is not necessarily COX dependent
- Protease activity cannot account for activation of RTK pathways
- Acid contracts ASM tissue and ASM cells

Summary of results

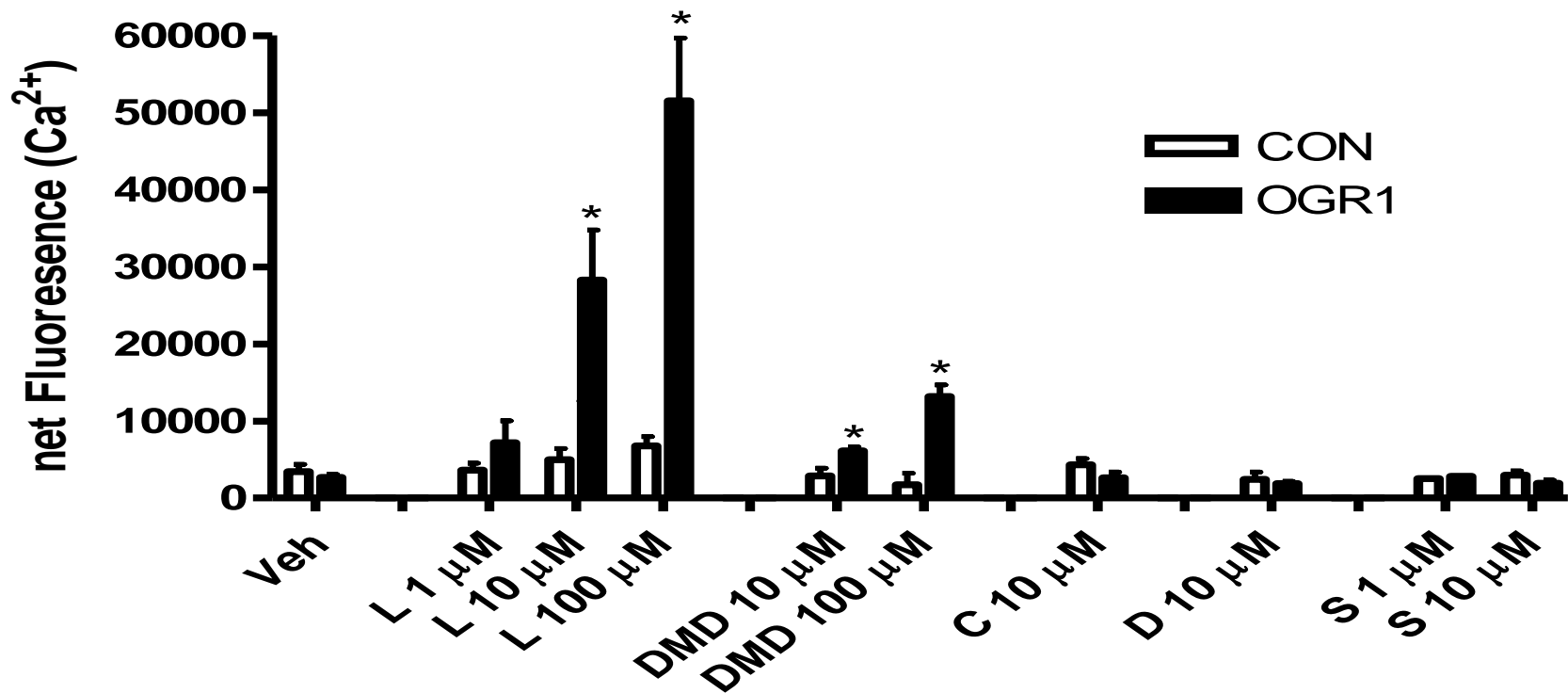
- OGR1 is the predominant proton sensitive receptor in the ASM cells
- OGR1 knockdown in human ASM inhibits acid-induced PKA and Ca^{2+} mobilization
- OGR1 knockdown in human ASM inhibits acid-induced contraction
- OGR1 knockout in murine ASM inhibits acid-induced contraction

Mixed bag of effects (context-dependent)- What to do?

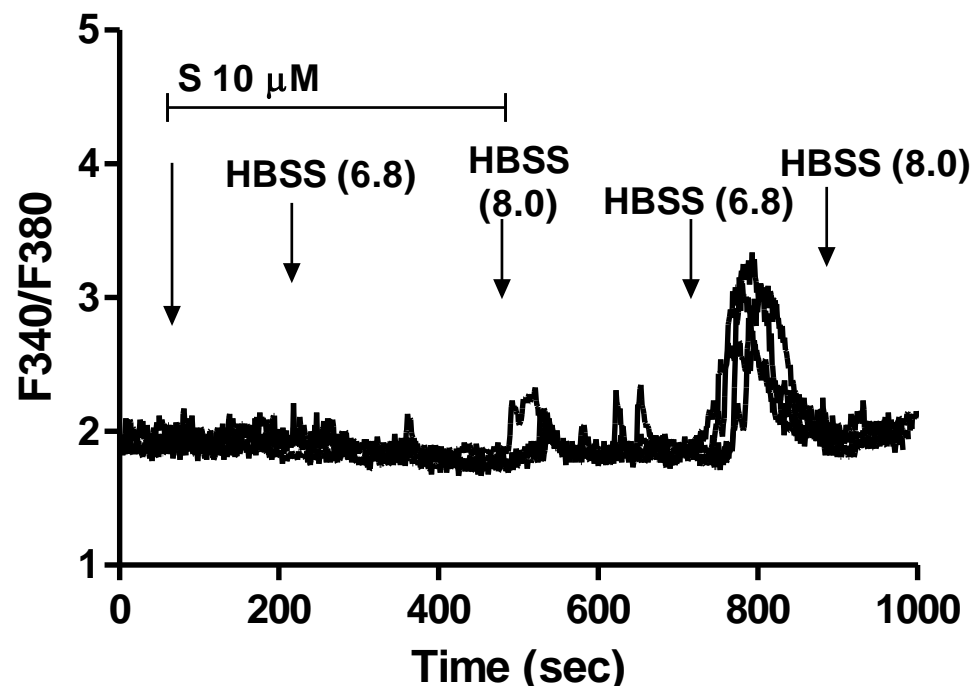
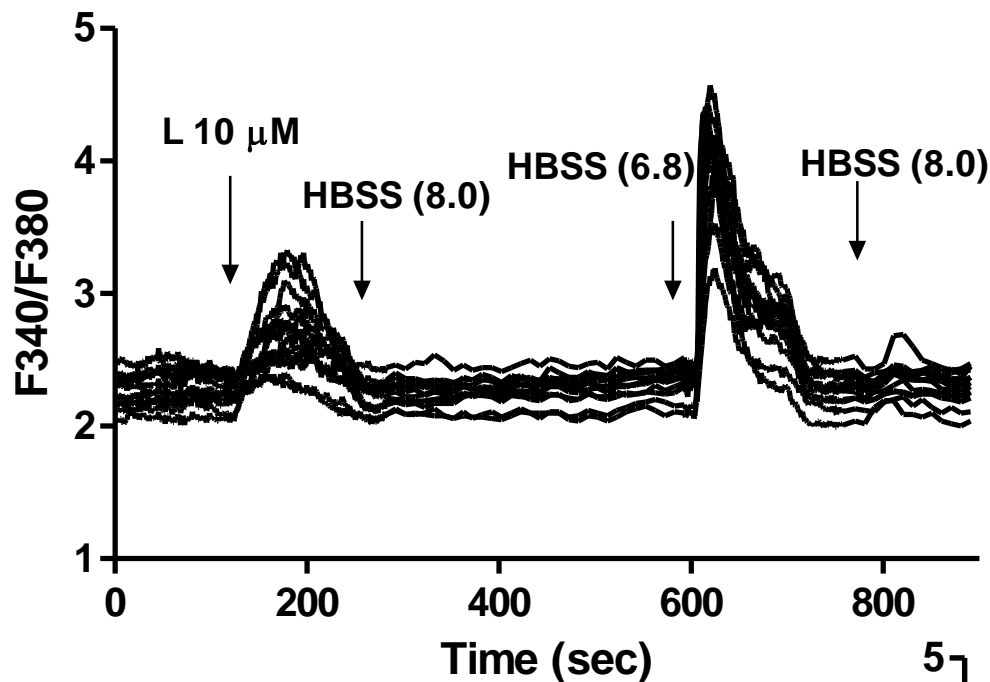
- Control pHo or pHi
- Block, activate, or bias GPCR signaling:
 - a. Downstream signaling- usual suspect pathway inhibitors, COX inhibitors, antagonists of induced GPCR ligands, tyrphostins.
 - b. At the receptor level for proton-sensing GPCRs; this quite hard given no ligands!

We have characterized a class of OGR1 ligands
(allosteric modulators actually)
and data to date that suggest we can bias
OGR1 signaling

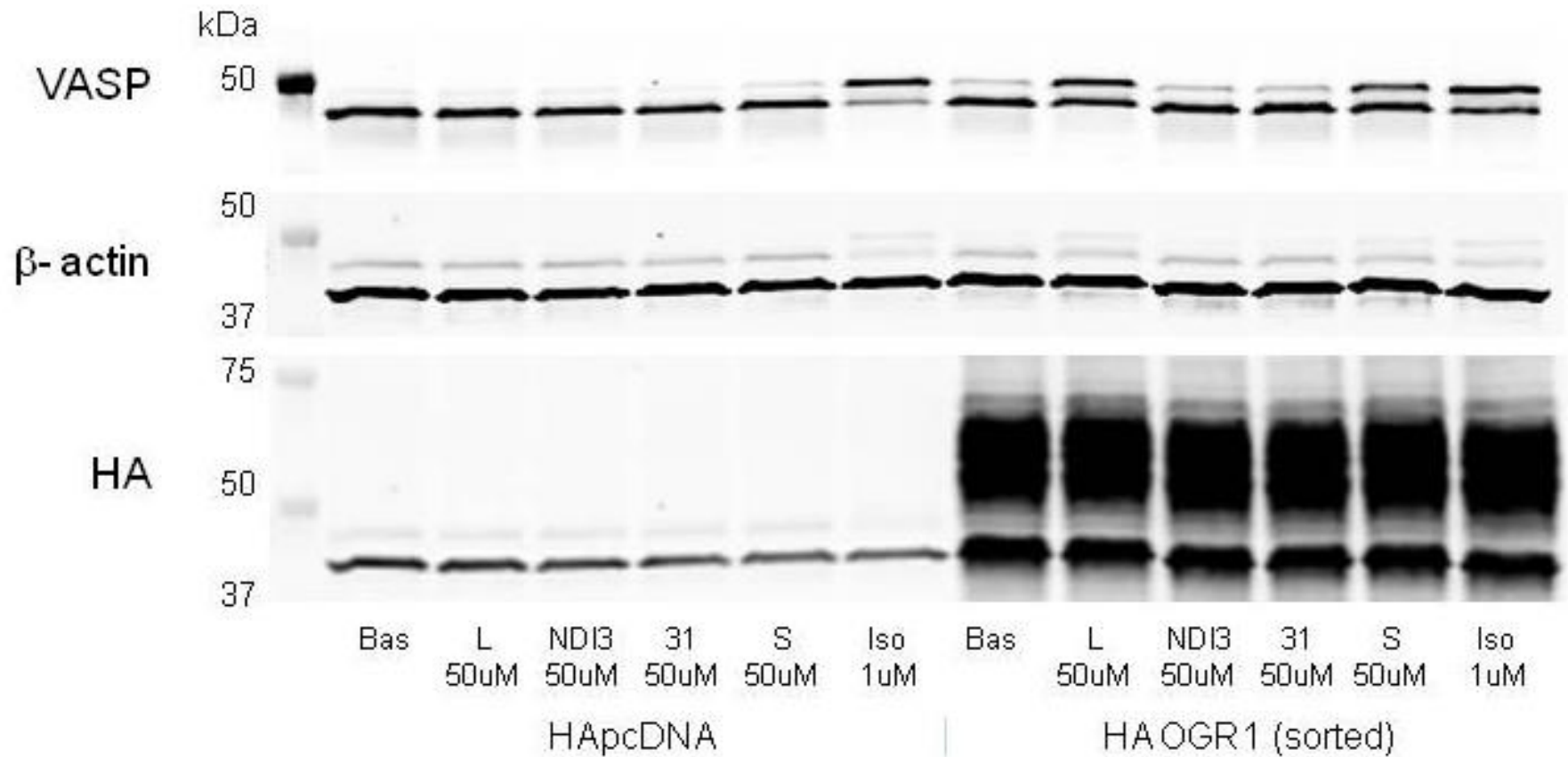
**A subclass of (can't tell you which because confidential!)
regulate OGR1-mediated Ca^{2+} mobilization.**



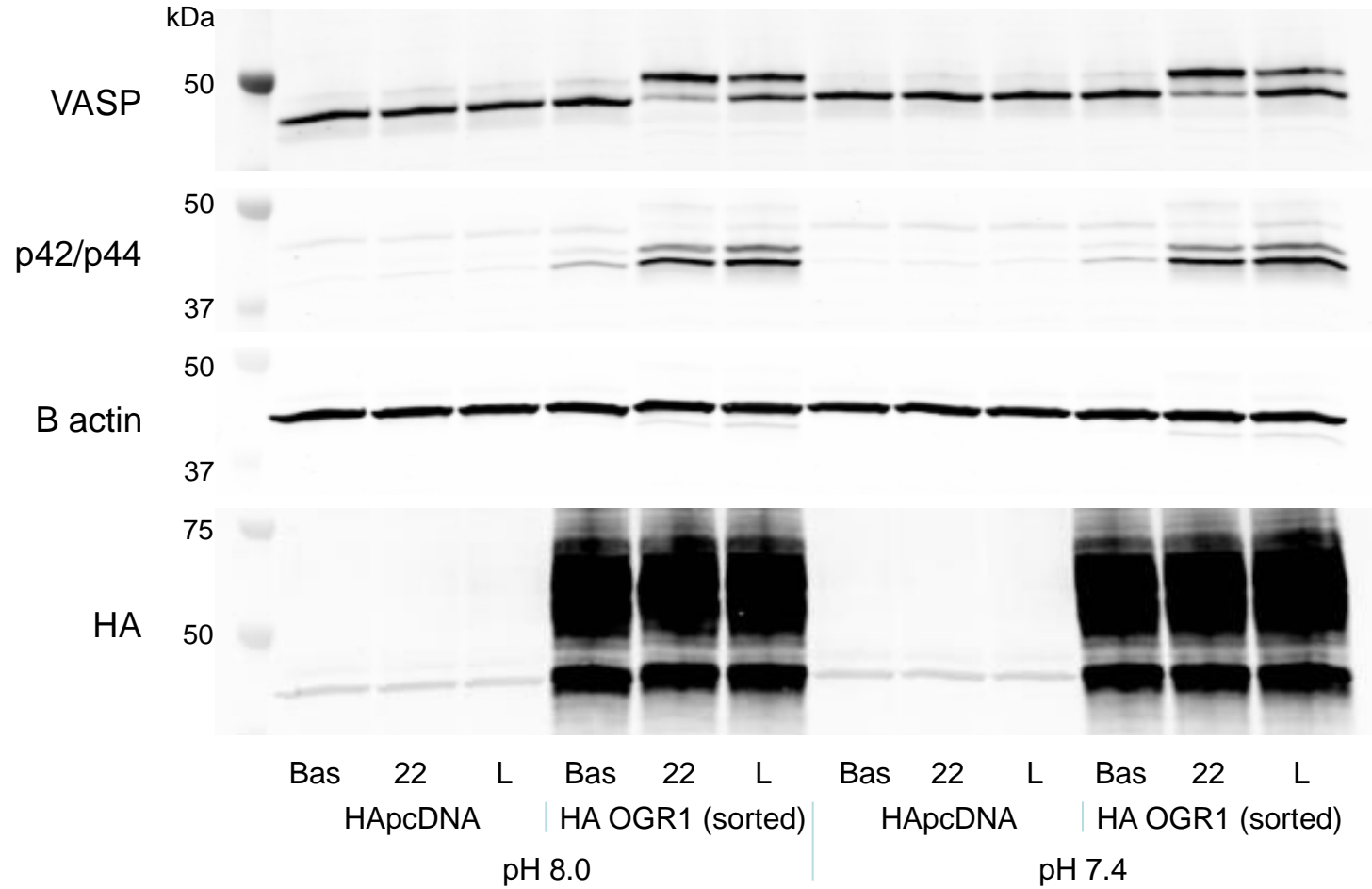
Some ligands appear balanced (L), some biased (S)



S stimulates Gs/ PKA but not Gq/Ca²⁺



HA OGR1 HEK stimulations



50uM L and 22
 Stimulated for 10 min in HBSS
 3/26/13

Up next: 🤖

Characterizing newly discovered small molecule
OGR1 ligands and their capacity
for biasing Gs vs Gq signaling
(Gs bias means bronchodilation!)

- a. Human ASM cells and tissue
- b. Guinea pig model of airway regulation by acid
- c. OGR1 $-/-$ mouse



Contributors

- Himansh Saxena
- Brian Tiegs
- Huandong Yan
- Richard Battafarano
- Whitney Burrows
- Sarah Horvat
- Deepak Deshpande (UMB)
- Steven An (JHU)
- Yan Xu (IU)
- Bryan Roth (UNC)