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

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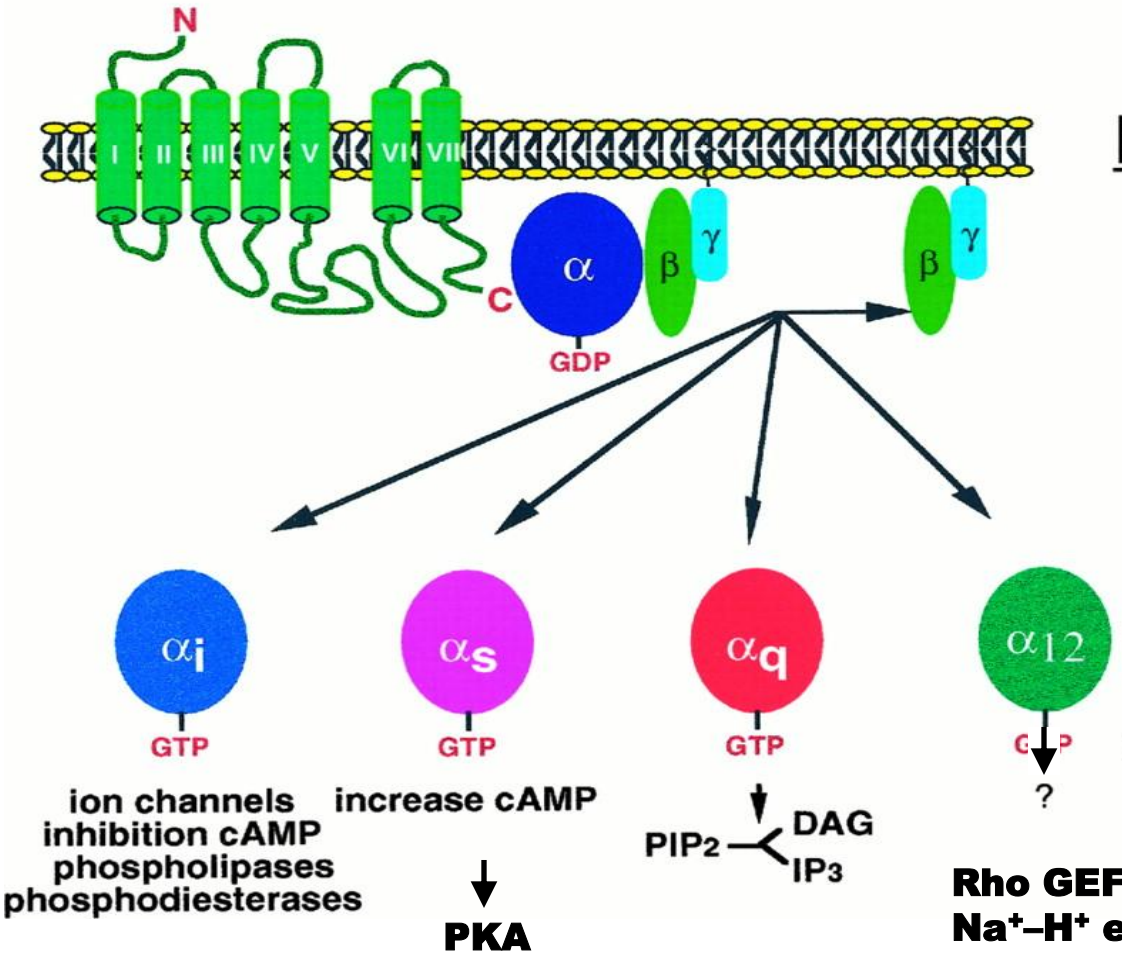
“Proton-sensing receptors-therapeutic targets in the management of asthma?”

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

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 acetycholine (ACh) activating Gq-coupled m3 muscarinic acetylcholine receptor (m3mAChR) is principal regulator of physiological tone.

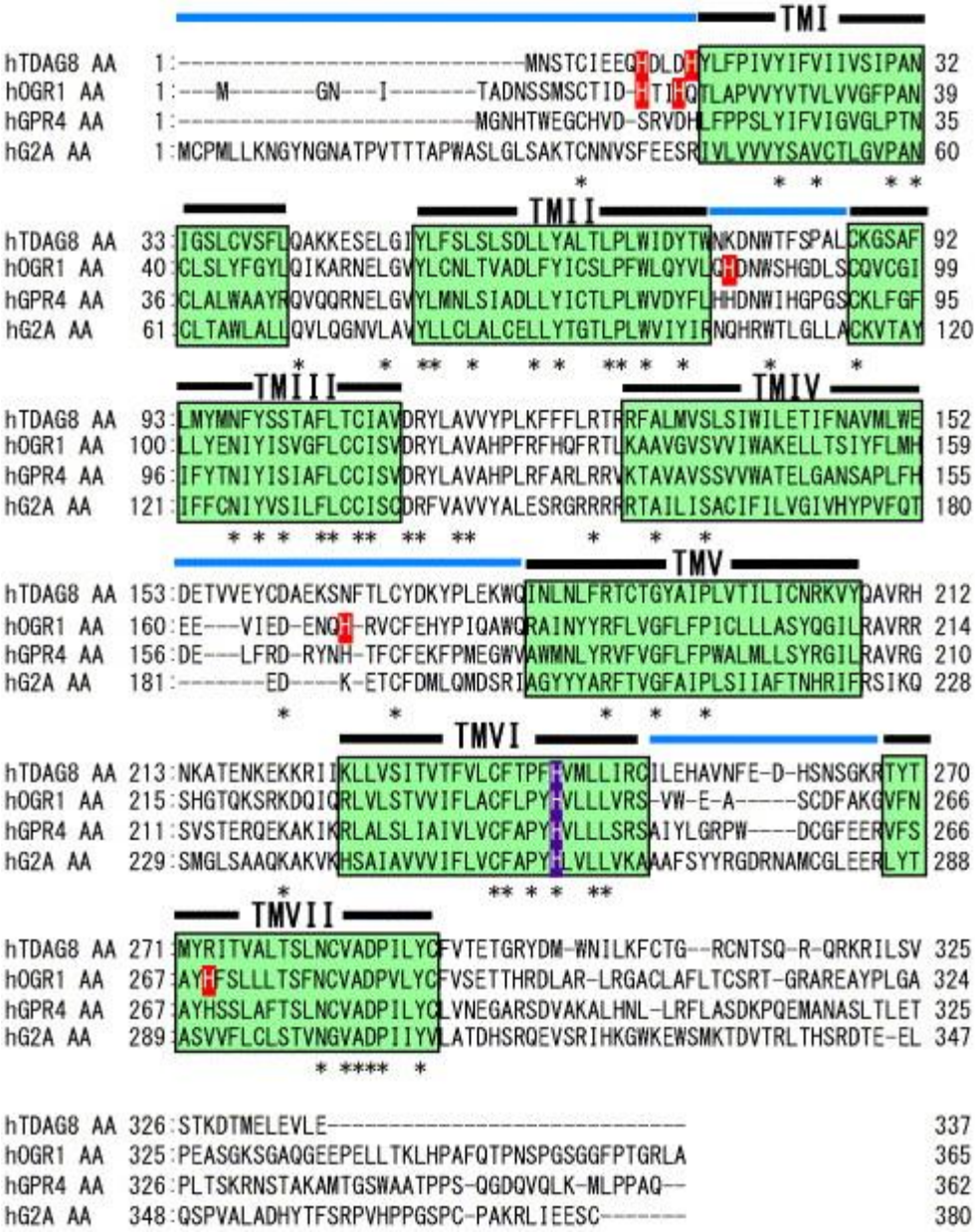
GPCR agonists are physiological, pathological, and therapeutic regulators of ASM contractile state

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).

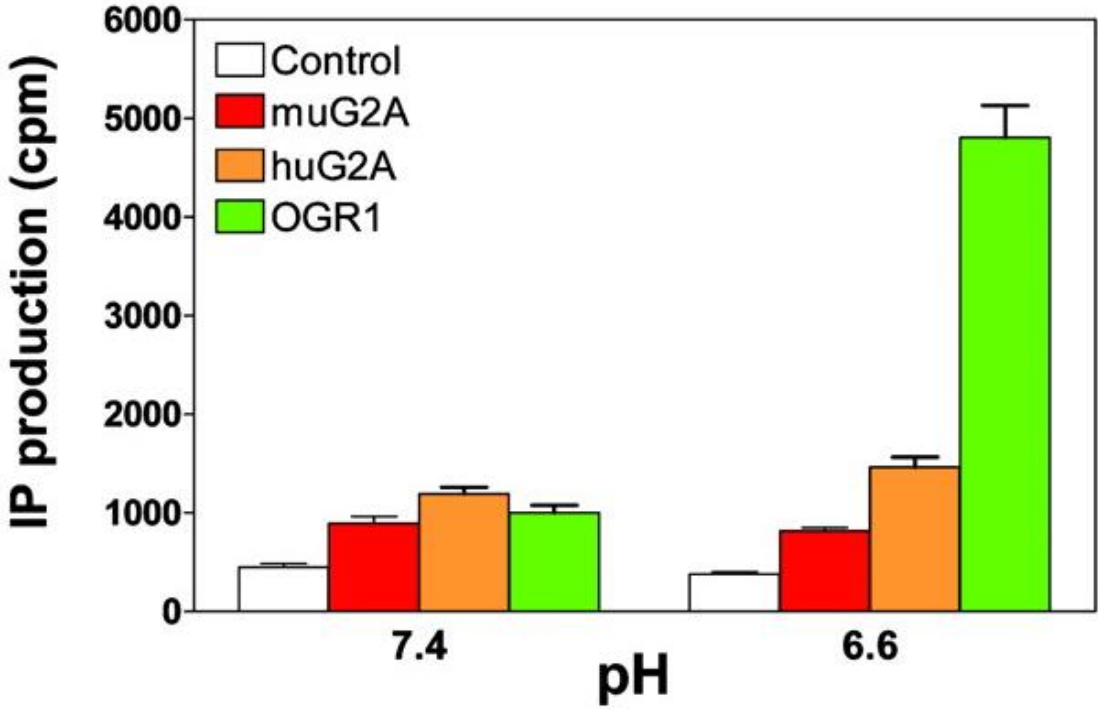
Proton-sensing GPCRs

- 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



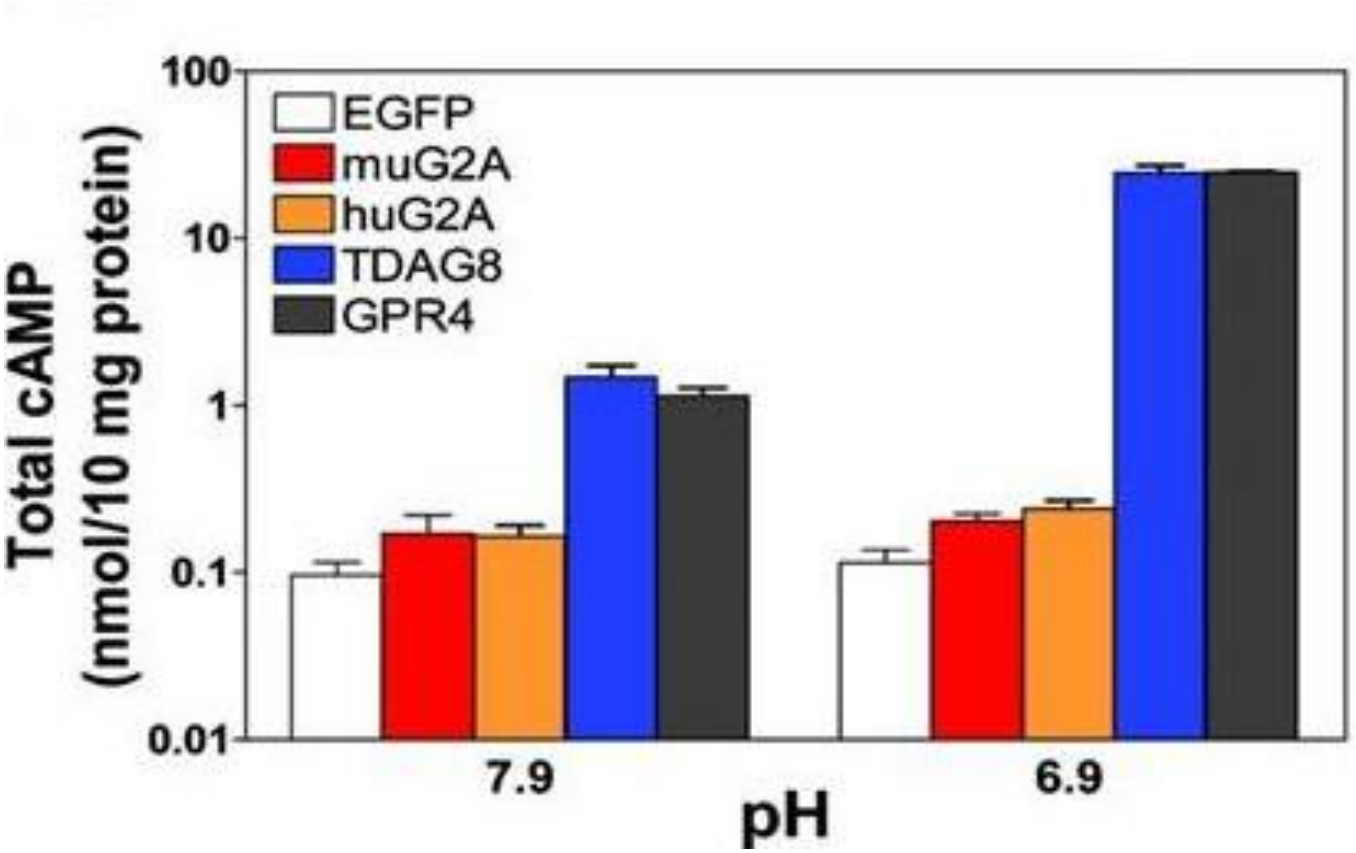
Sequence alignment identifies OGR1, GPR4, TDAG8, and G2A as family of GPCRs

Expressed OGR1 exhibits constitutive and pH-dependent IP production

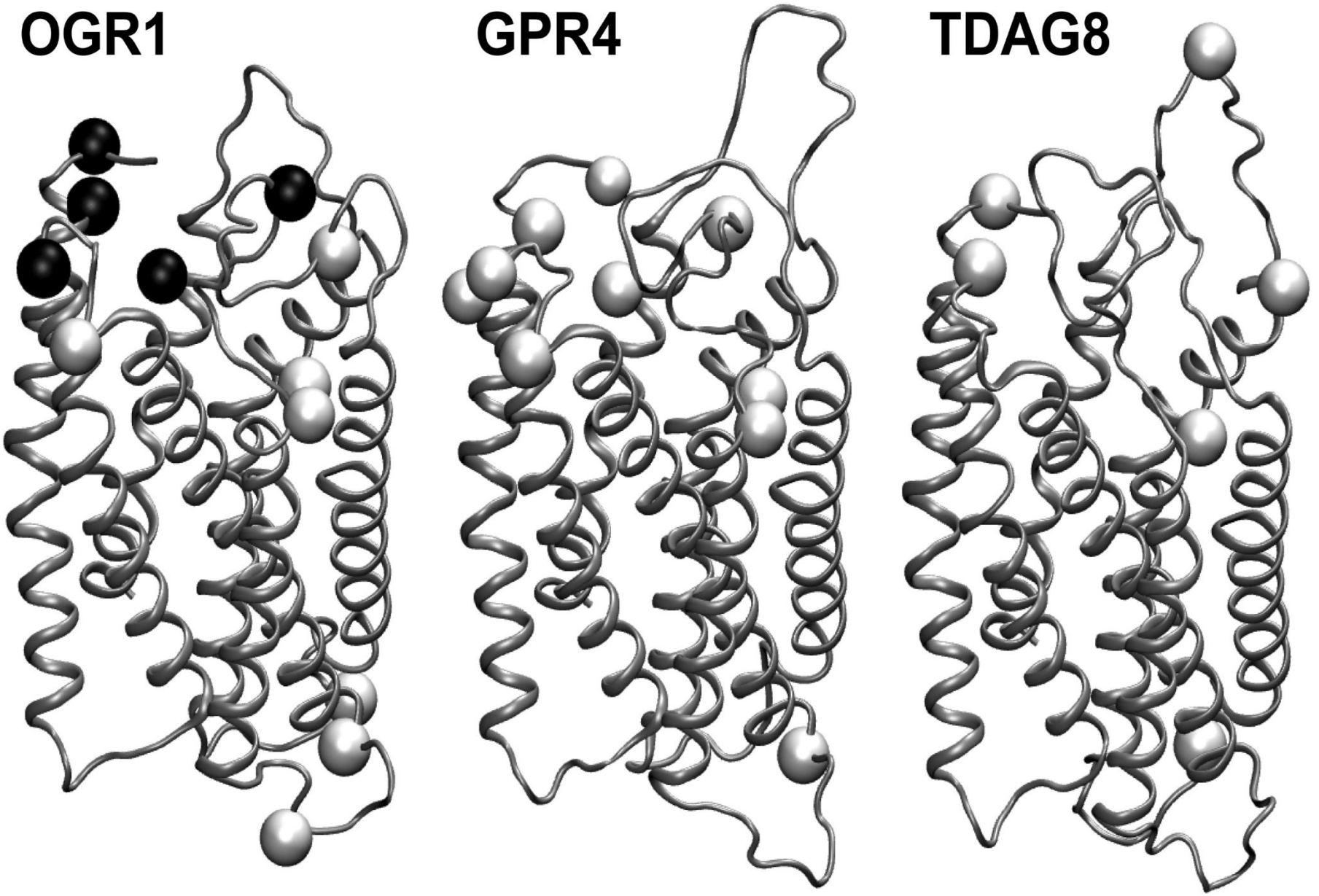


Radu C G et al. PNAS 2005;102:1632-1637

pH-dependent accumulation of intracellular cAMP by GPR4, TDAG8

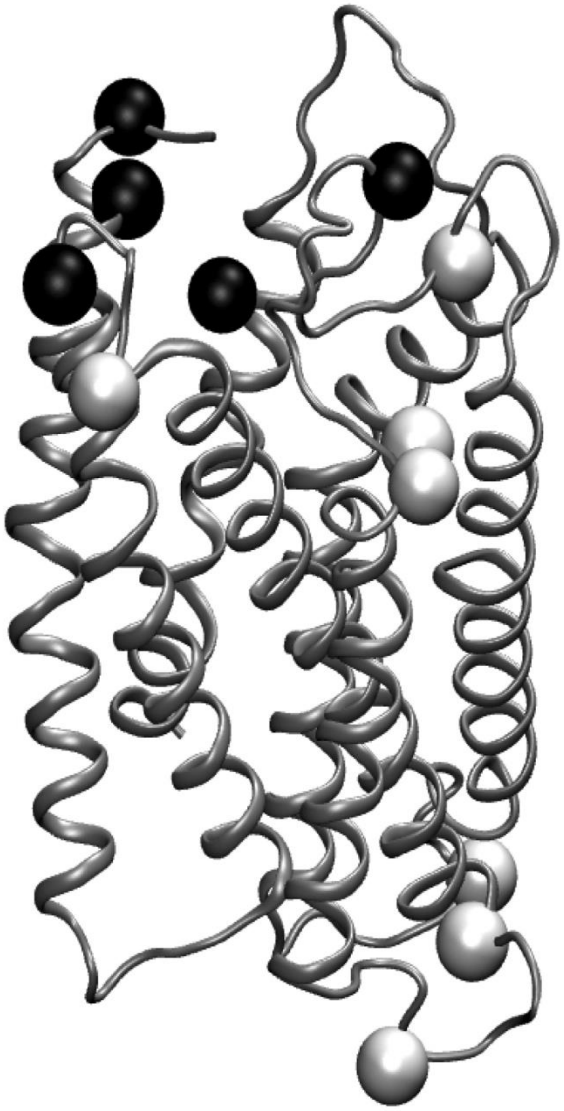


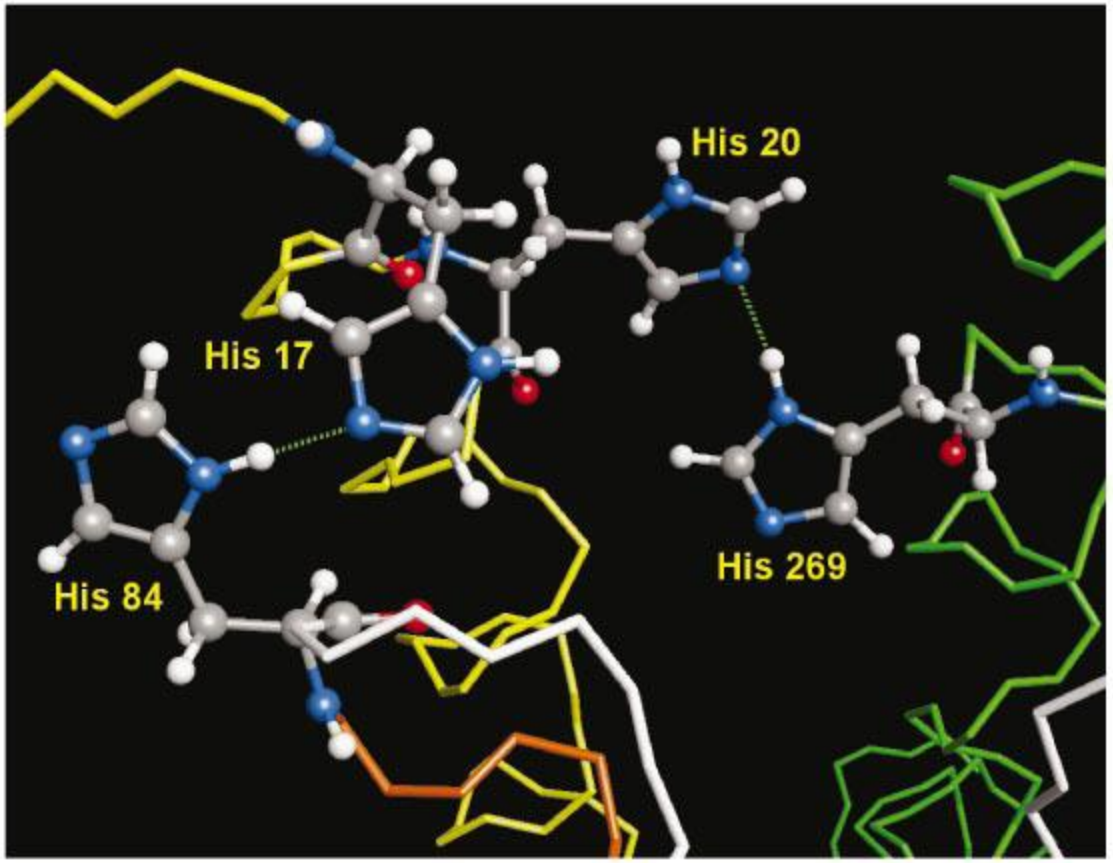
How do protons activate these GPCRs?



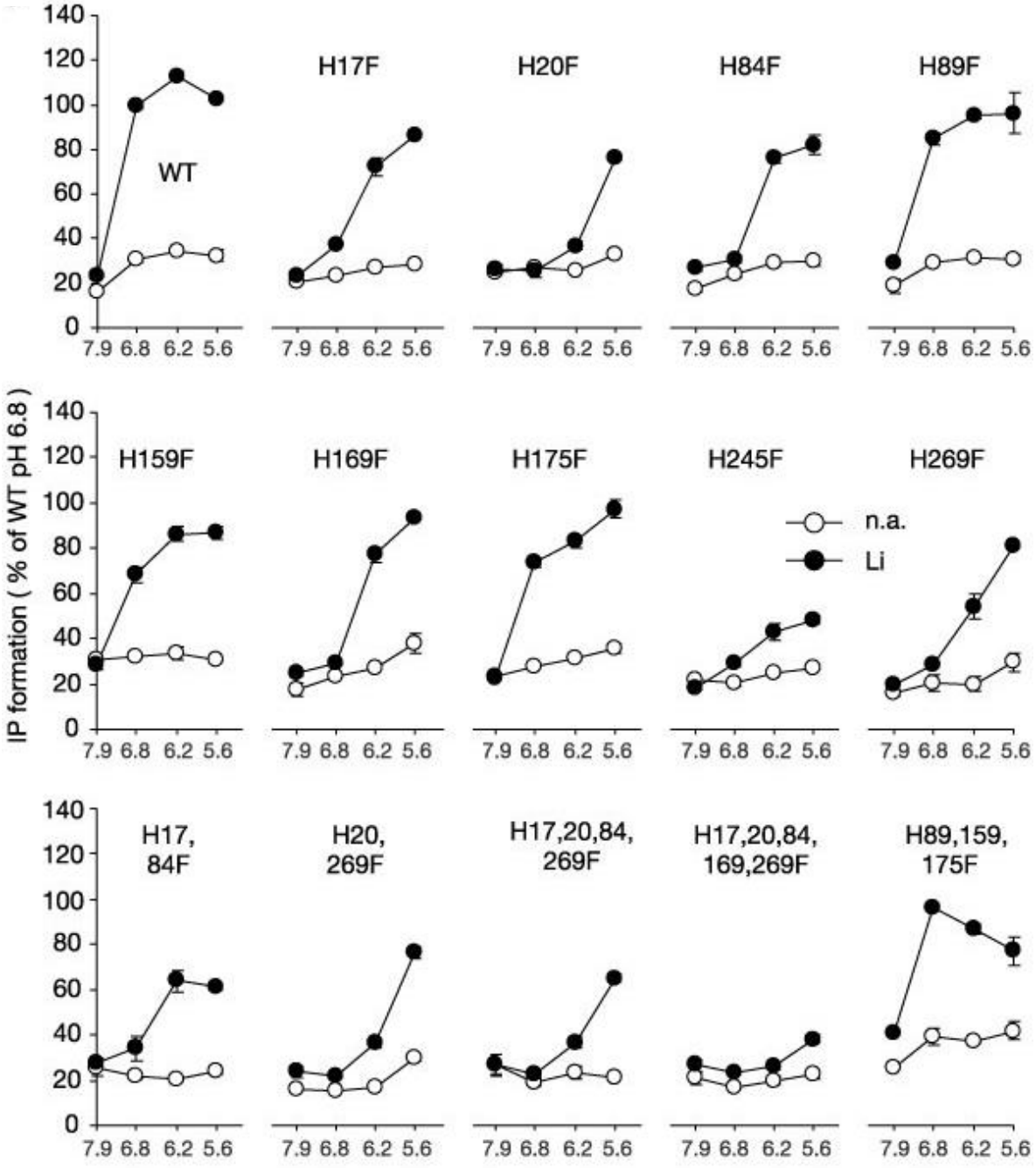
Predicted structure, proton-sensing histidine residues in OGR1, GPR4, and TDAG8

OGR1



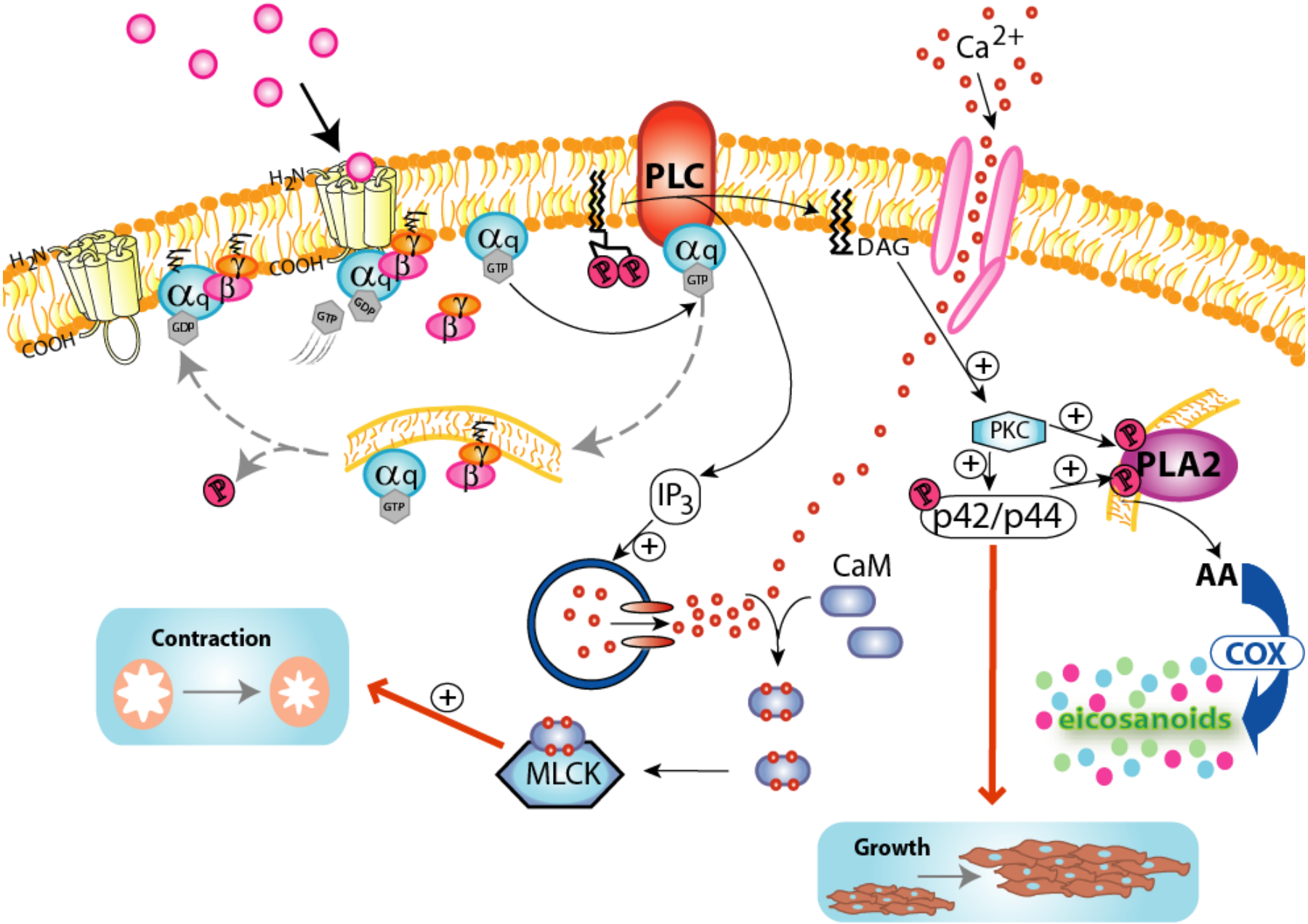


Mutation of putative proton-sensing histidines inhibits pH sensing by OGR1

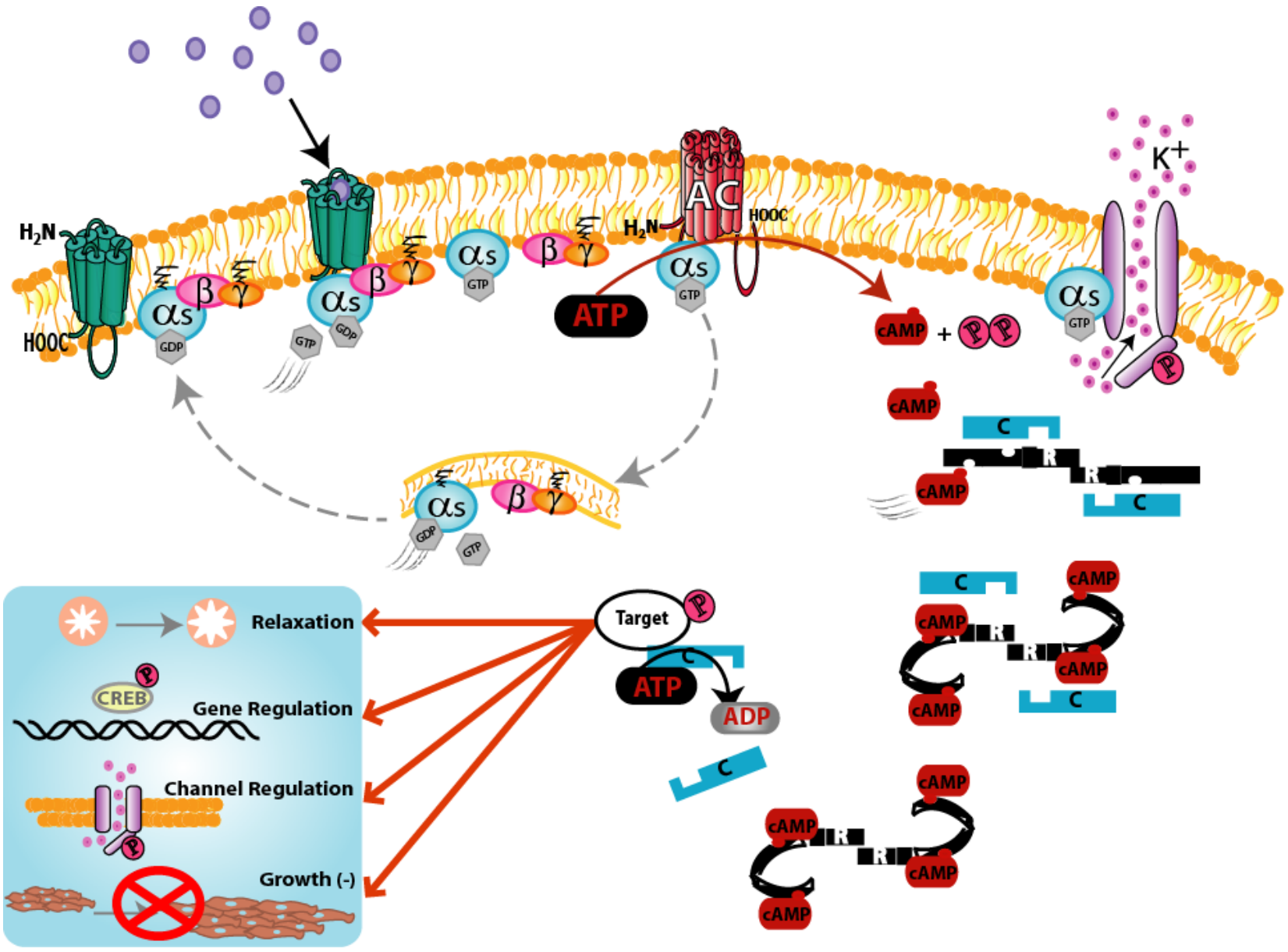


Ludwig et al. *Nature* 2003

G_q-coupled receptor signaling in ASM

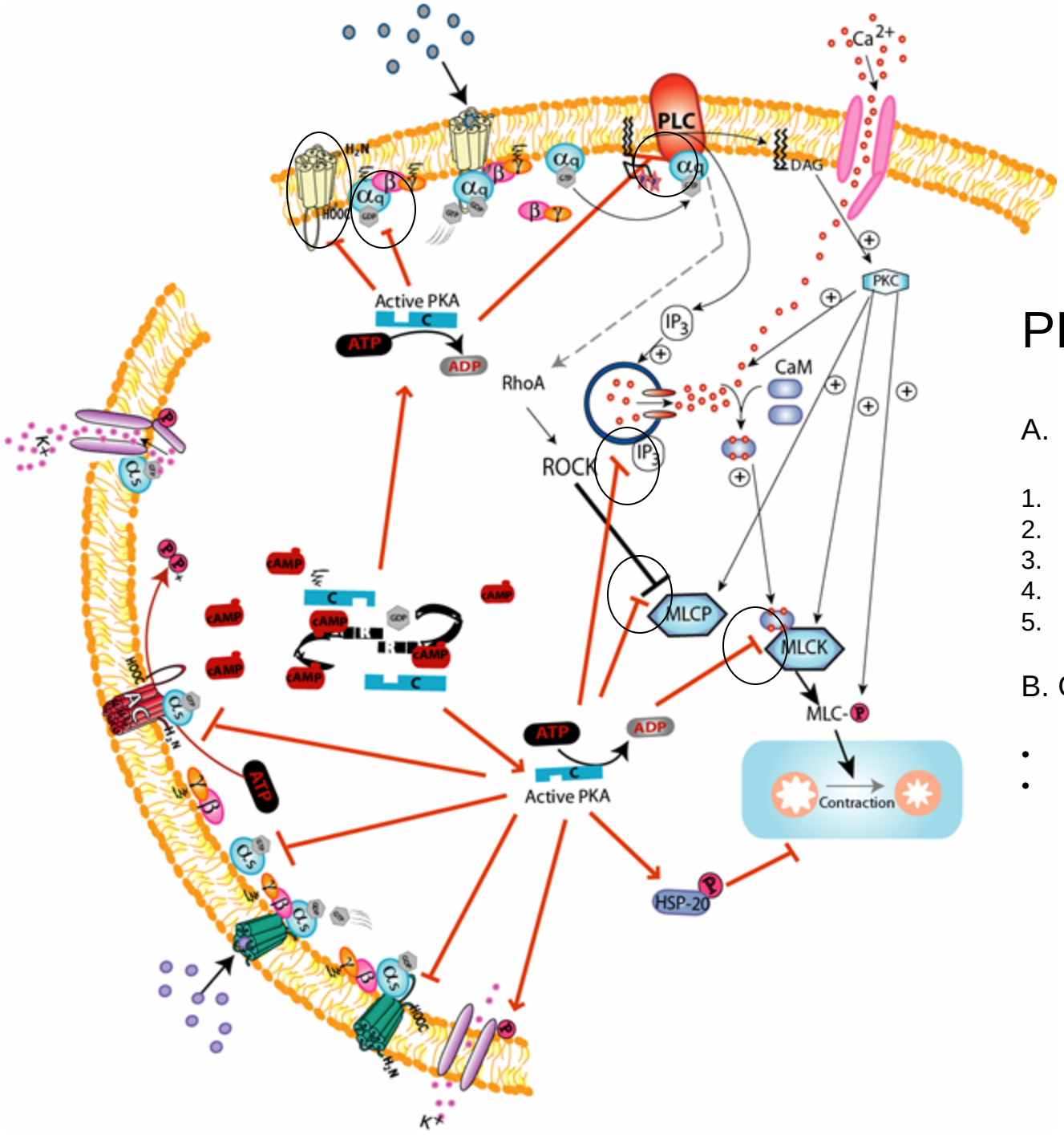


G_s-coupled receptor signaling



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 Ca $^{2+}$ release entry:

1. Gq-GPCR
2. Gq
3. PLC
4. Phospholamban/IP3R
5. K+ and Ca^{2+} channels

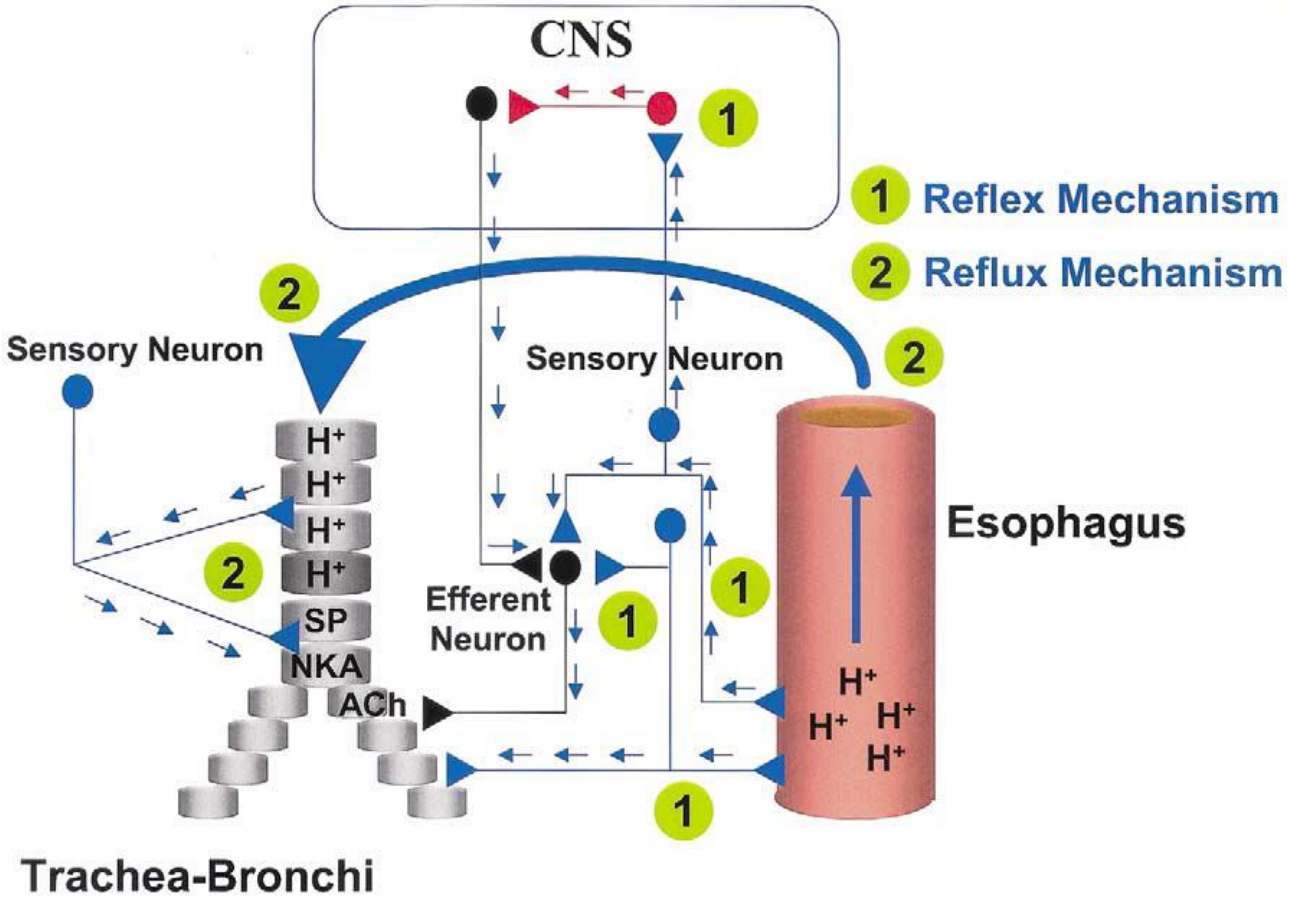
B. Calcium Sensitization:

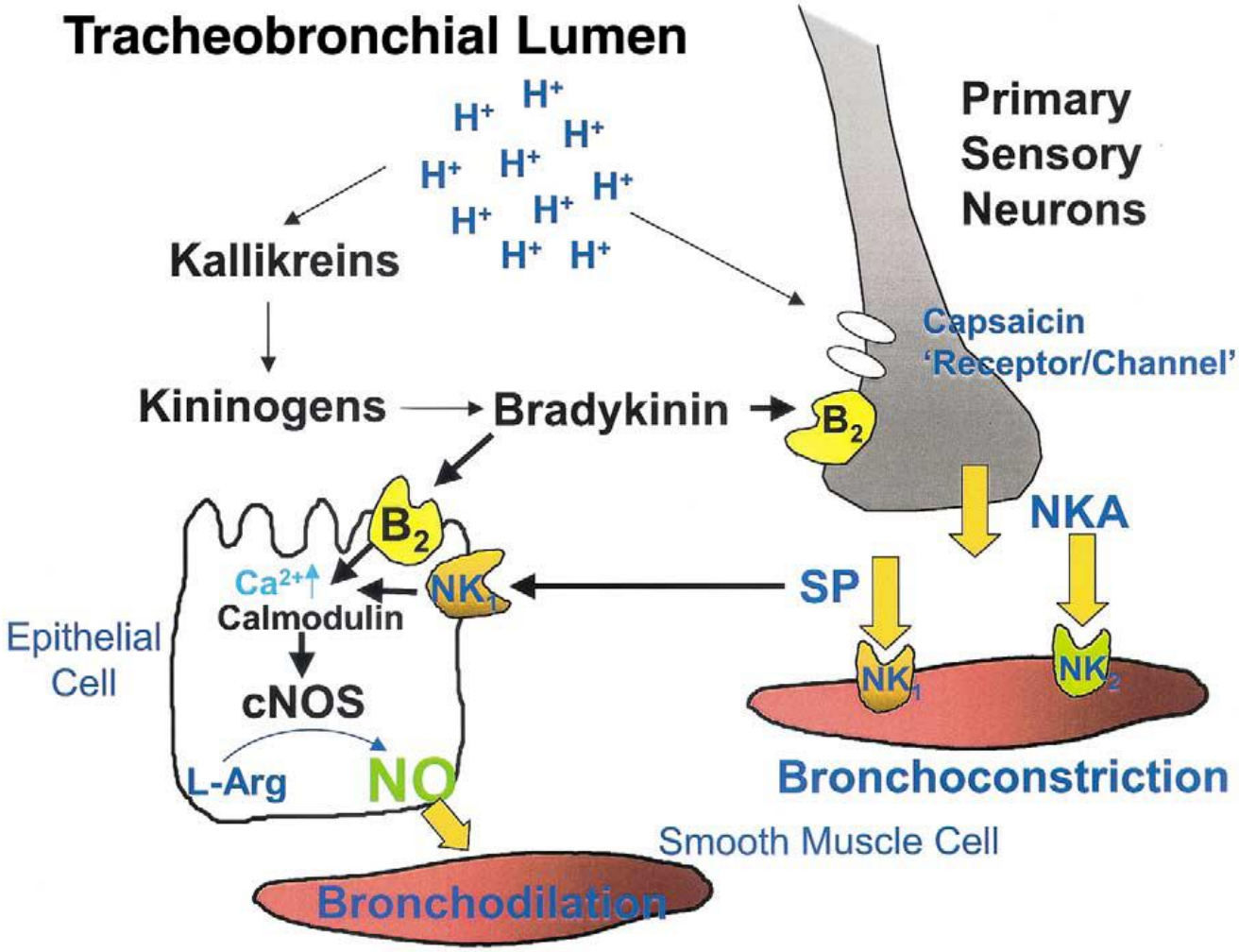
- Calcium/calmodulin/MLCK
- Rho Kinase reg of MLCP

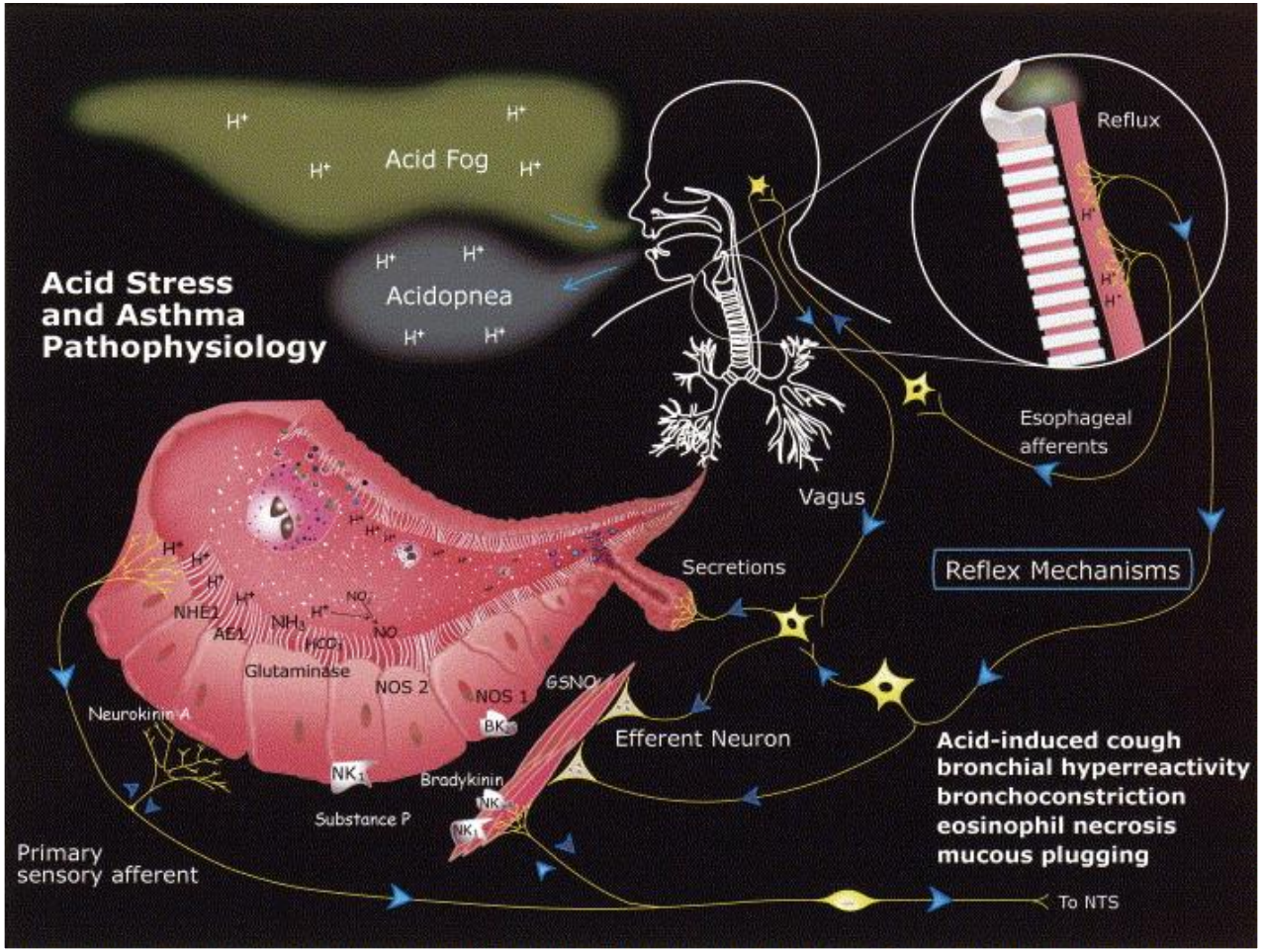
Objectives

- 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
H+
Kallikreins
Kininogens
Bradykinin
Primary Sensory Neurons
Capsaicin 'Receptor/Channel'
B2
NKA
SP
NK1
NK2
Ca2+↑
Calmodulin
cNOS
L-Arg
NO
Epithelial Cell
Smooth Muscle Cell
Bronchoconstriction
Bronchodilation



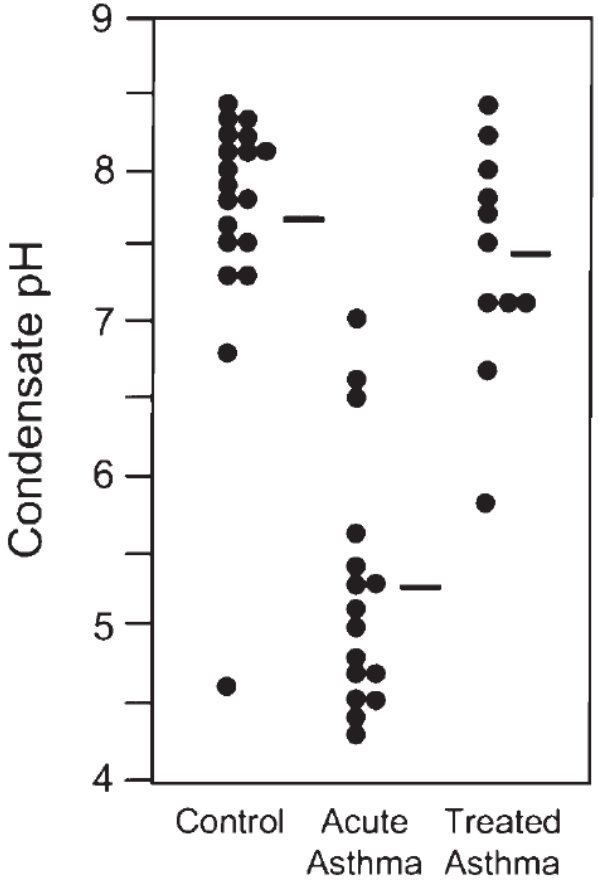
Journal of Allergy and Clinical Immunology 2004; 113:610-619

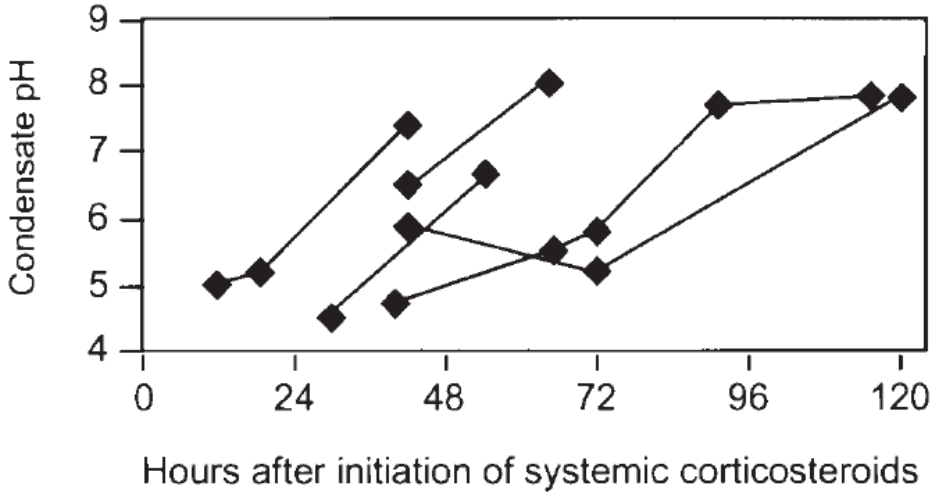
Airway pH tends to alkaline, but decreases with allergic inflammation

Respiratory fluid	Condition	Measured pH
EBC (deaerated to remove CO2)	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)

From Ricciardolo, Gaston, and Hunt *JACI* 2004

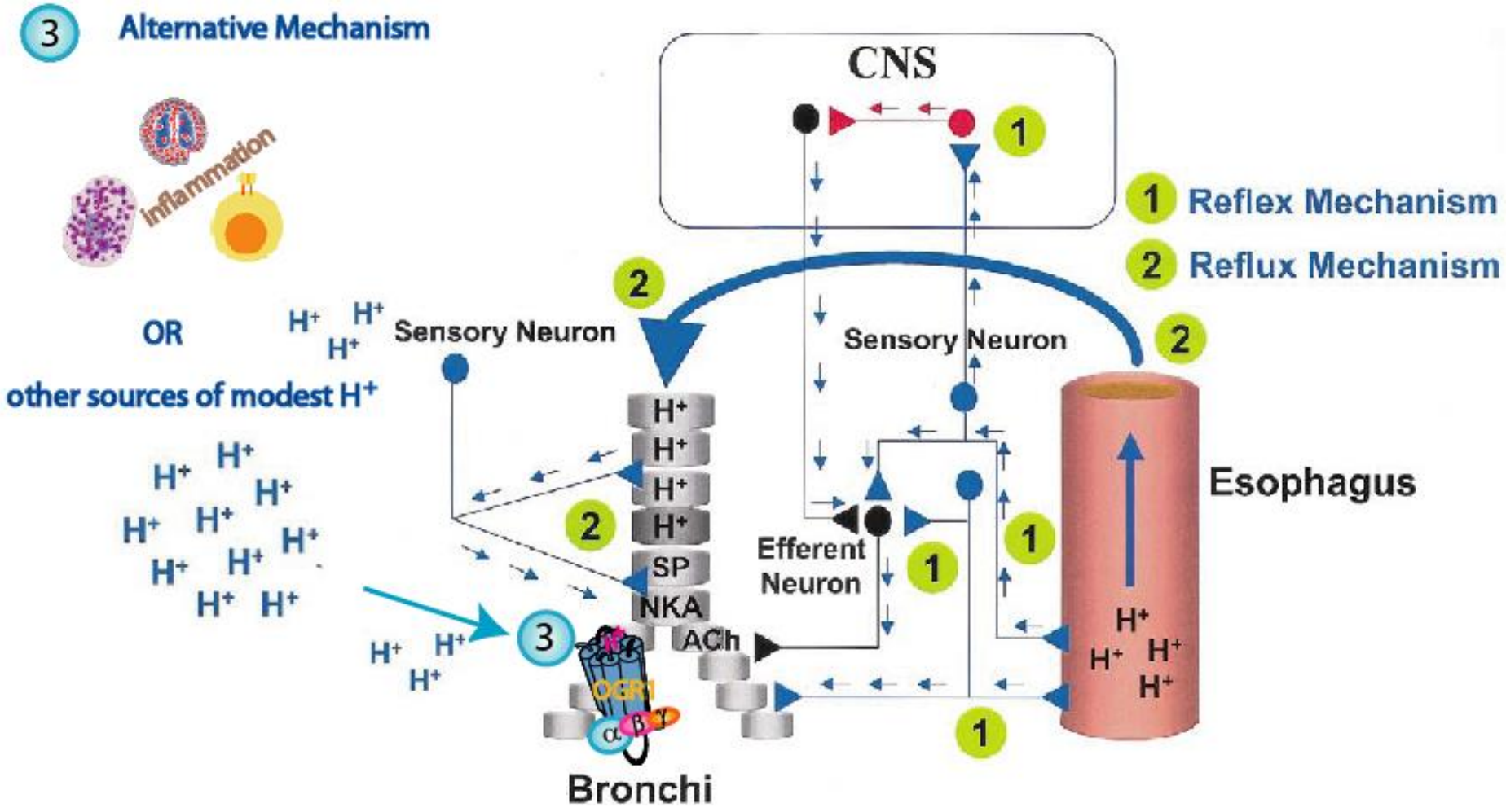
Corticosteroids reverse the reduced airway pH associated with acute asthma exacerbations



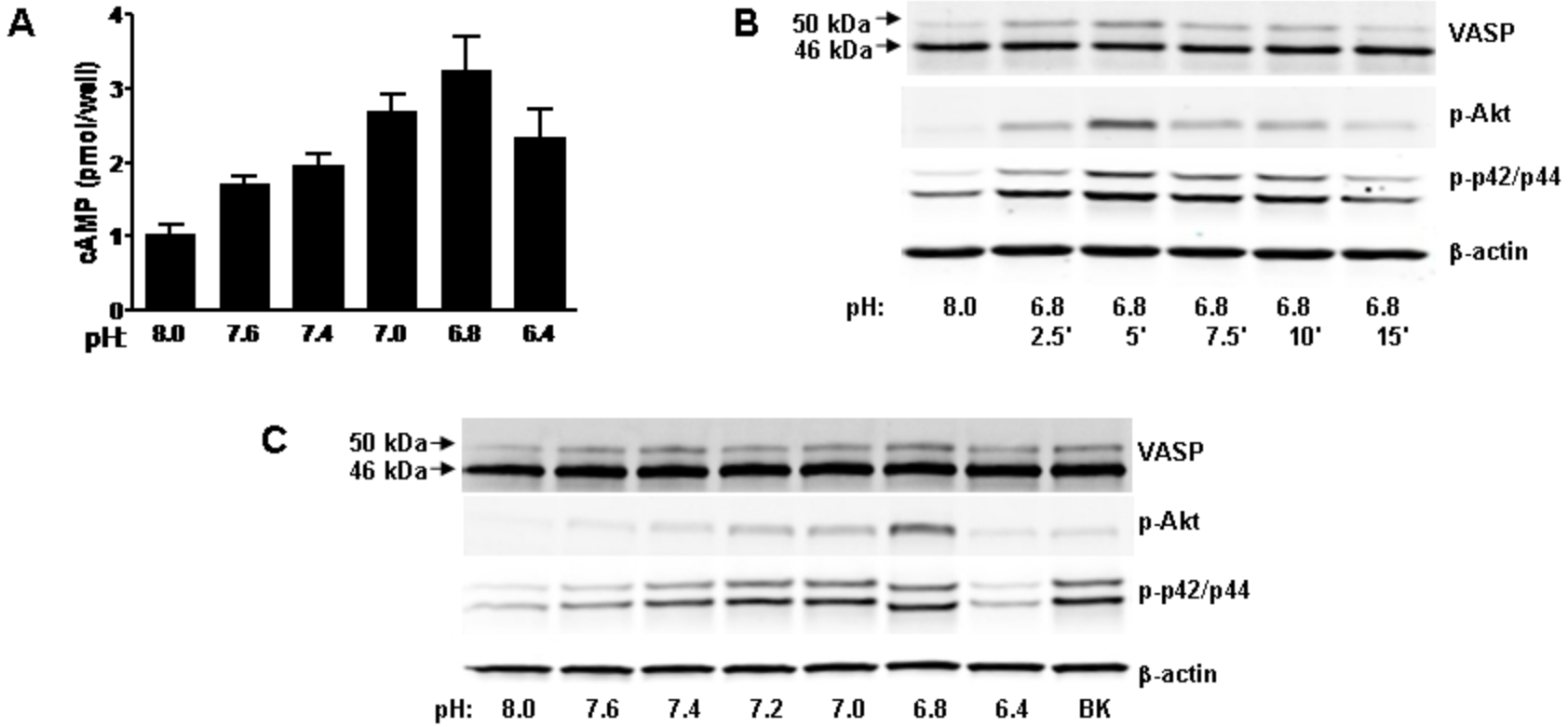


Hunt et al. *Am J Respir Crit Care Med.* 2000

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



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



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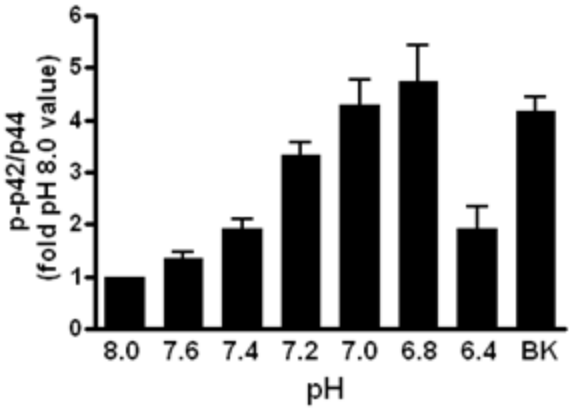
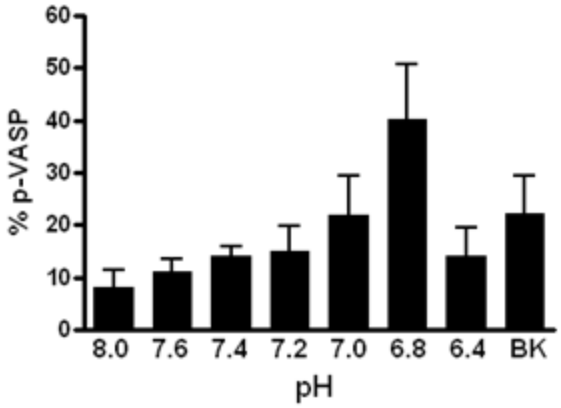
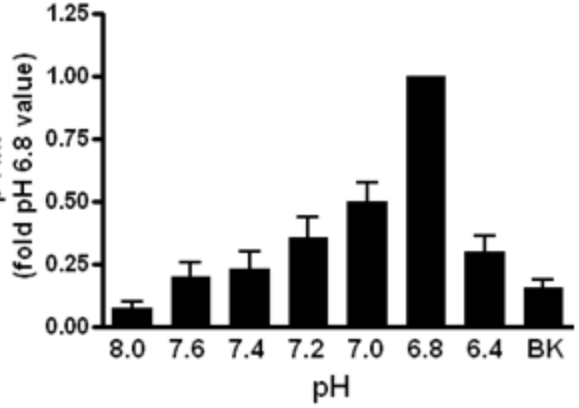
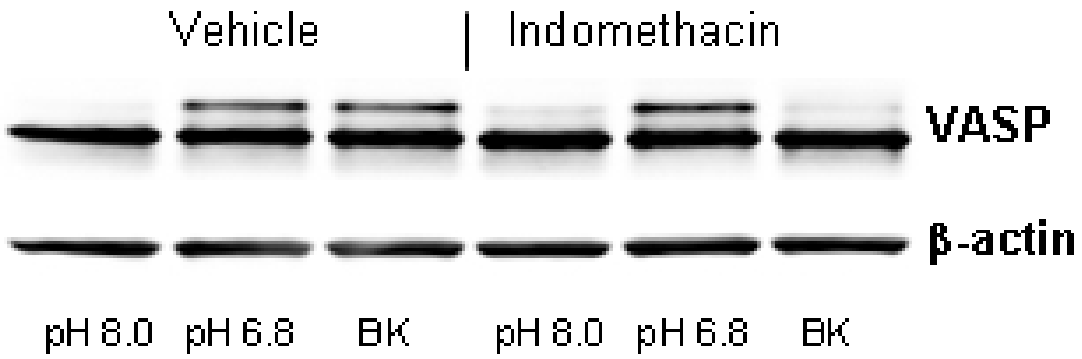


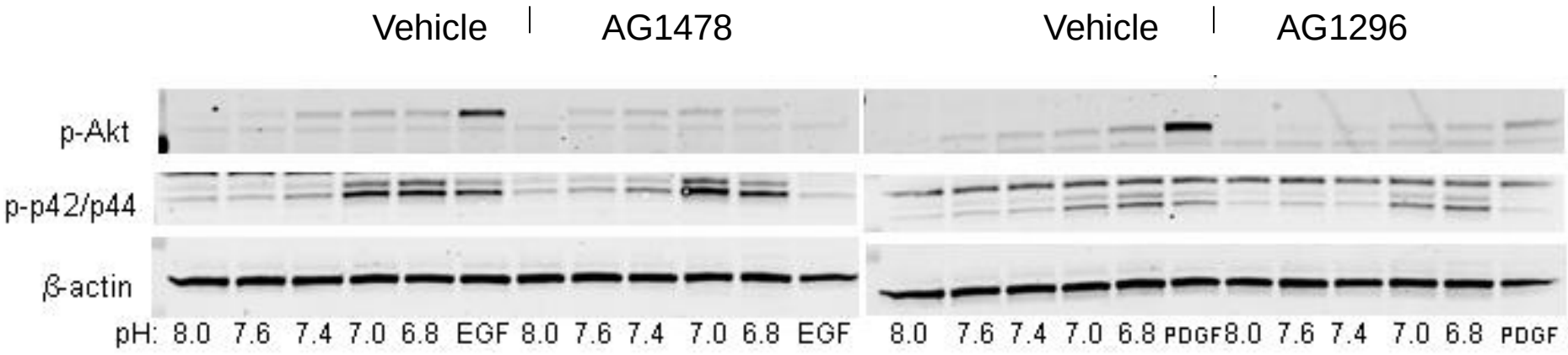




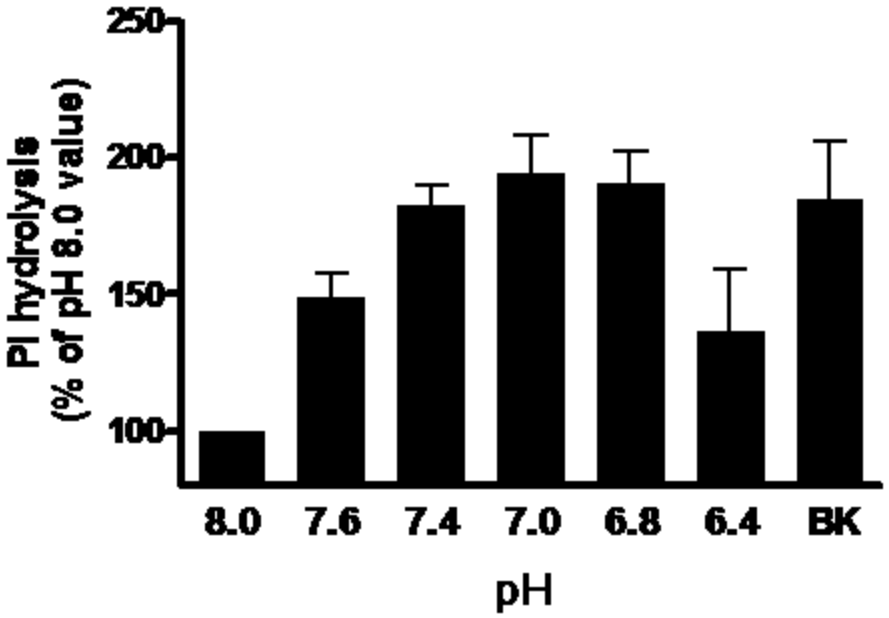
PKA activation by ↓pHo not necessarily dependent on COX: Pleiotropic signaling?

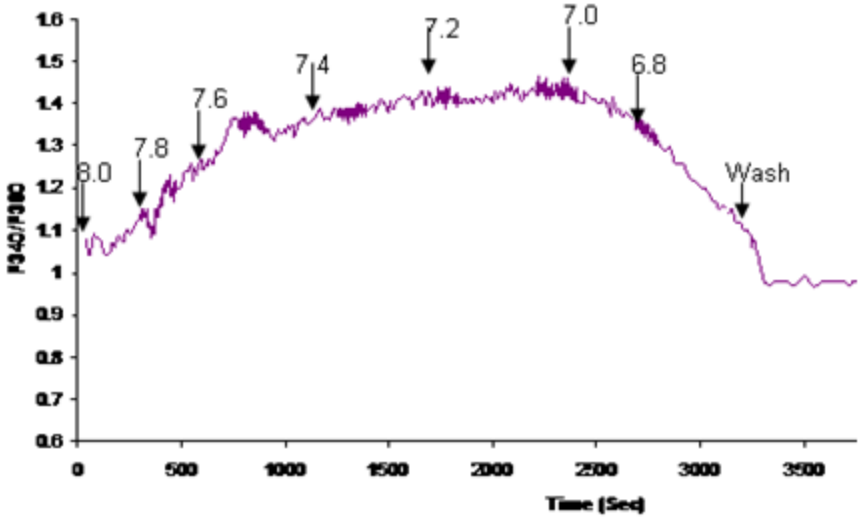


Akt, p42/p44 activation by ↓pHo is not via RTK transactivation



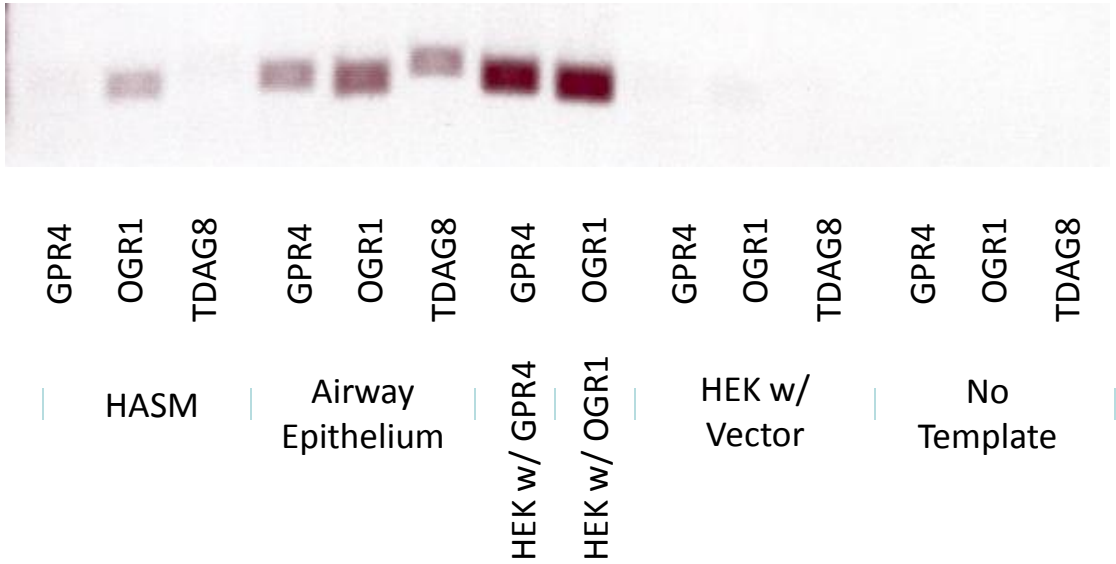
↓pHo stimulates PI hydrolysis, Ca^{2+} mobilization



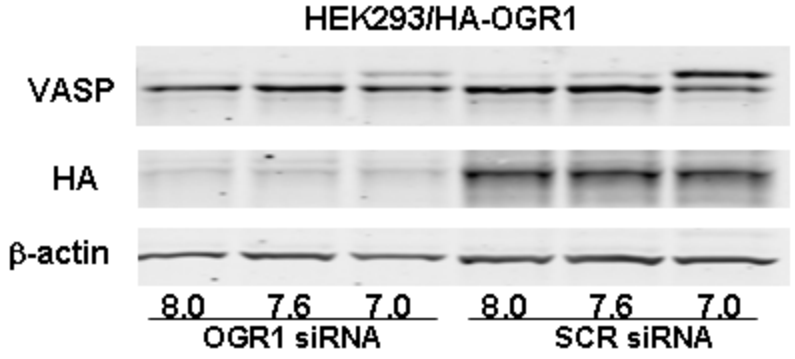


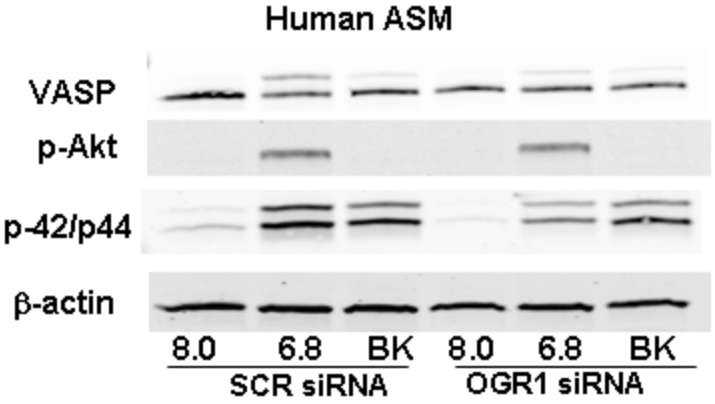
Expression of proton sensitive receptors in ASM

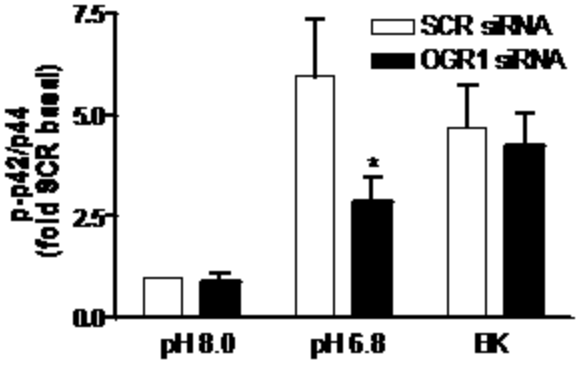
PCR of GPCRs in HASM and Airway Epithelium

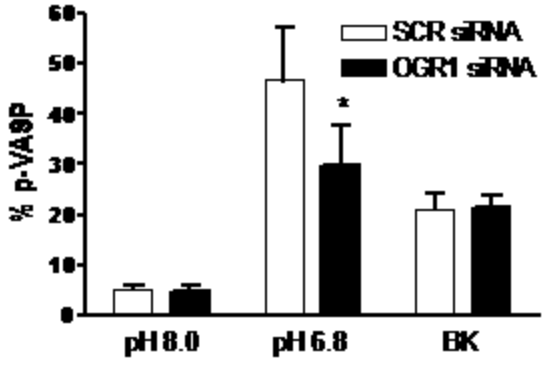


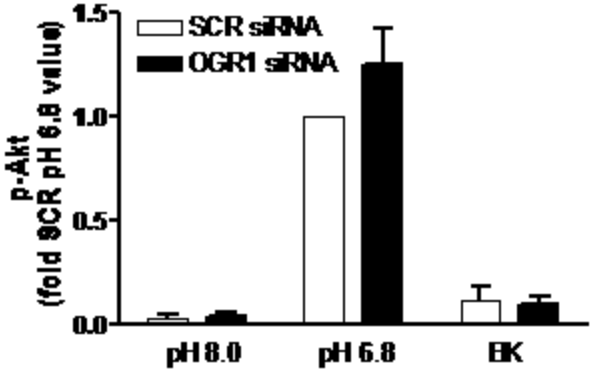
OGR1 knockdown reduces PKA, p42/p44 activation by ↓pHo



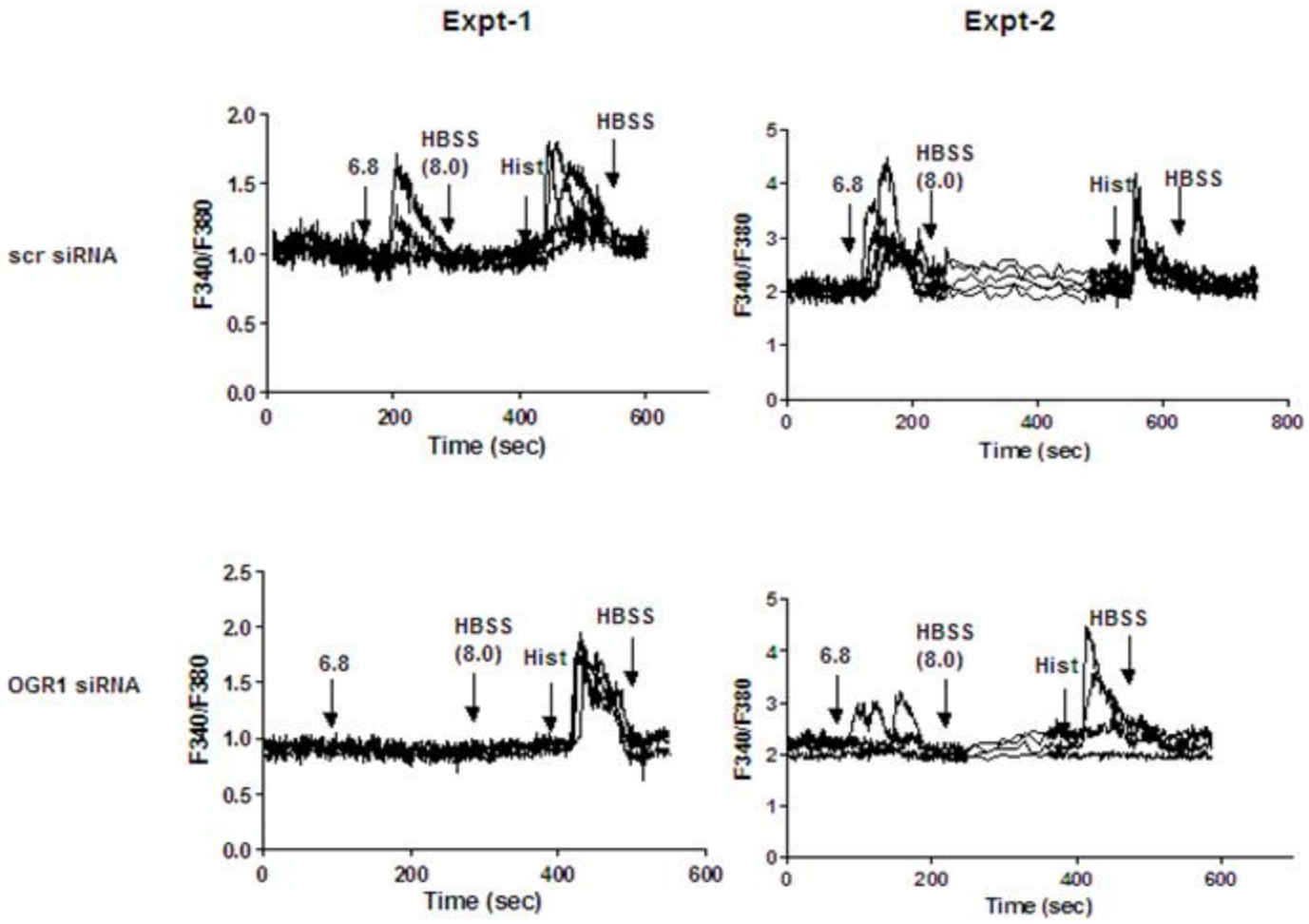








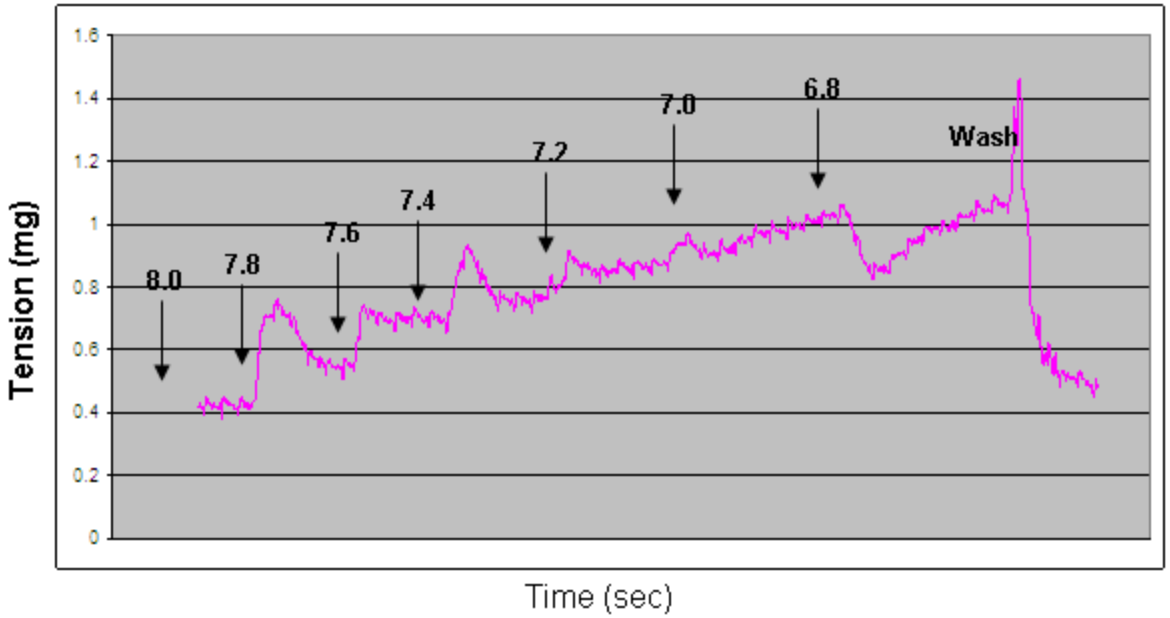
OGR1 knockdown reduces Ca^{2+} mobilization by ↓pHo

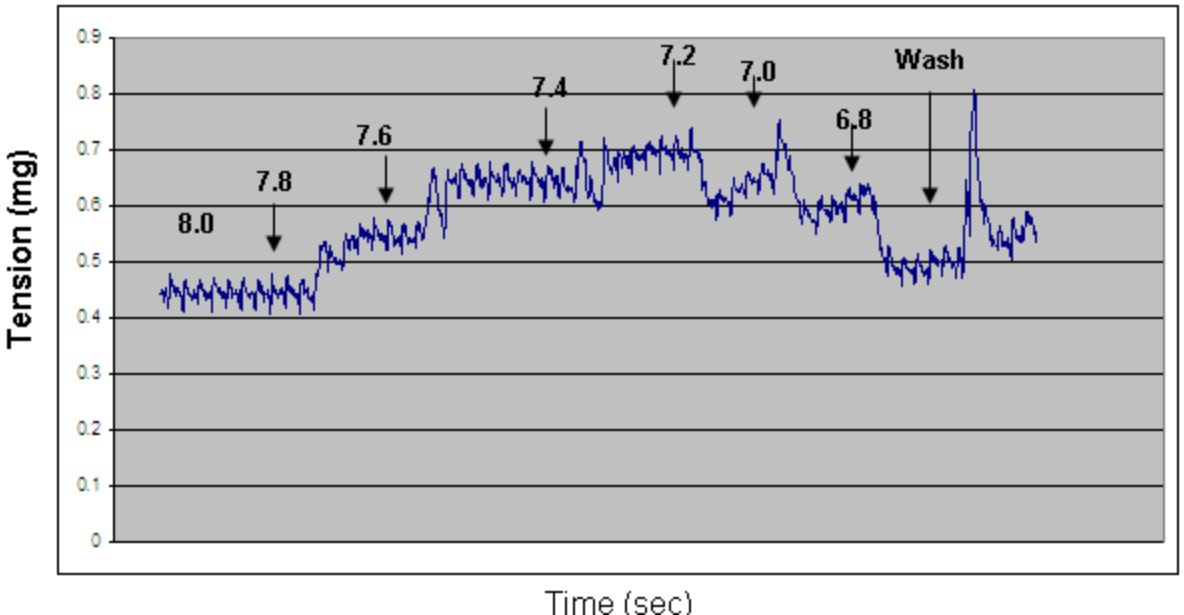


Signaling looks good.

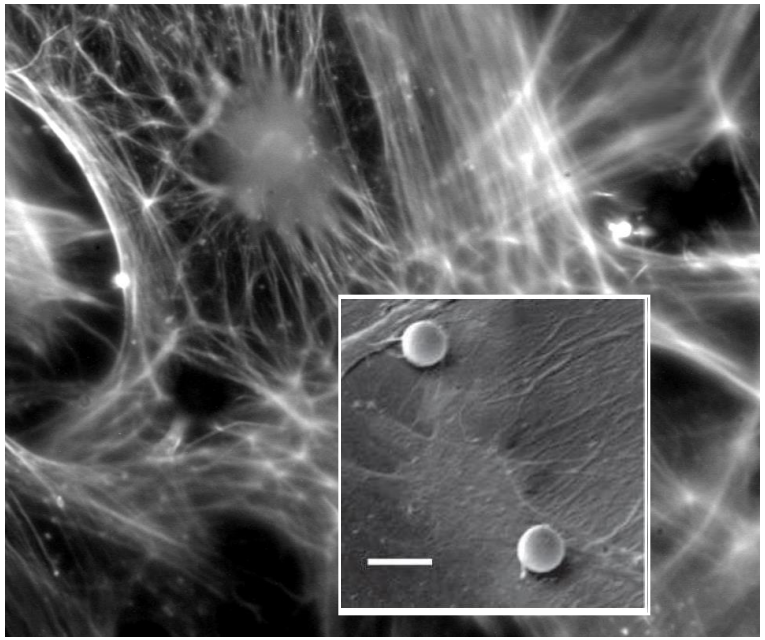
What about function?

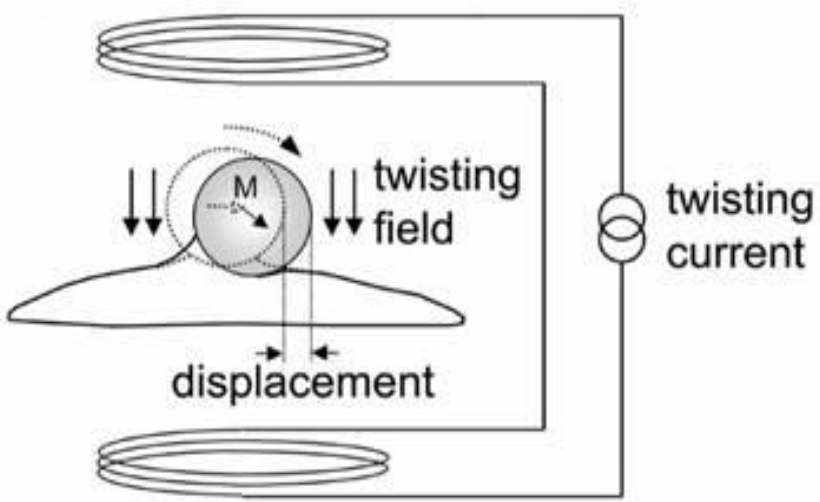
ASM tissue contracts in a pH dose-dependent manner



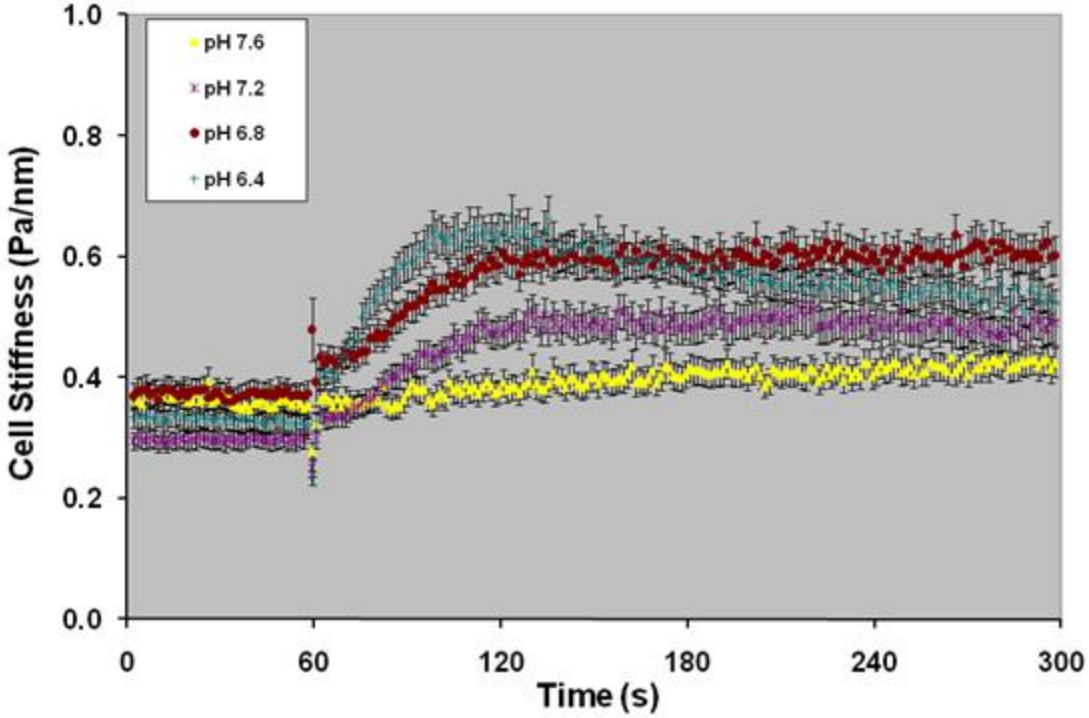


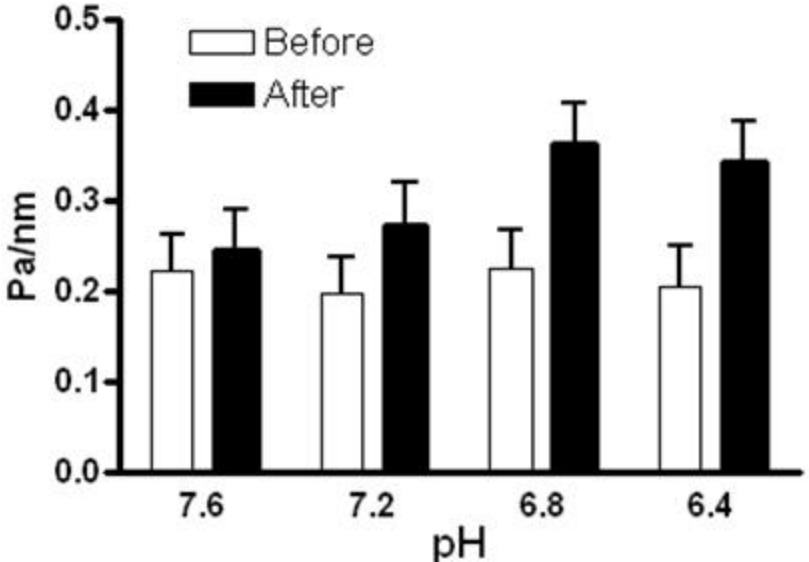
ASM cell contraction: Magnetic Twisting Cytometry (MTC)



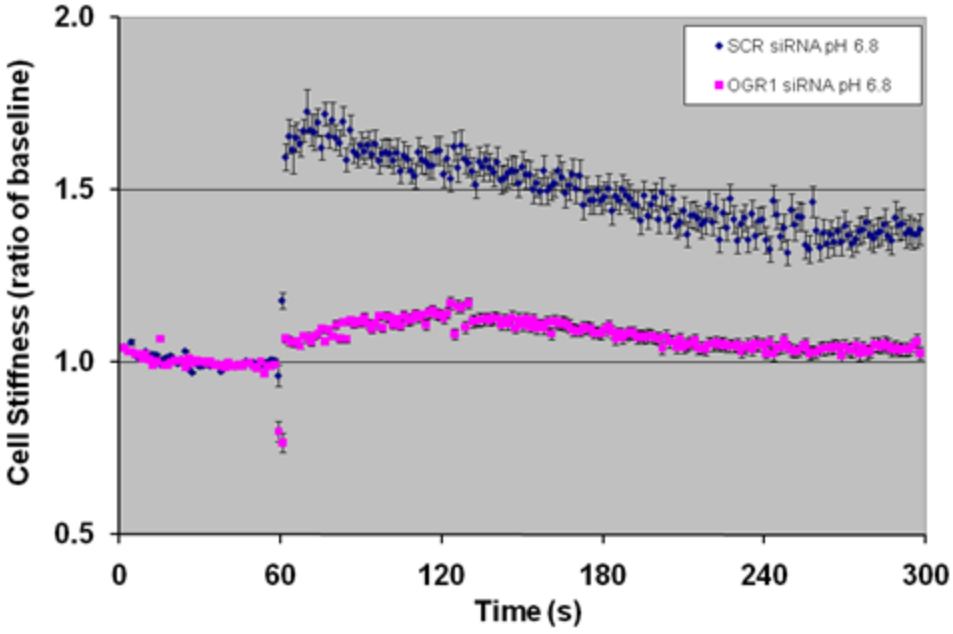


Cells contract, too

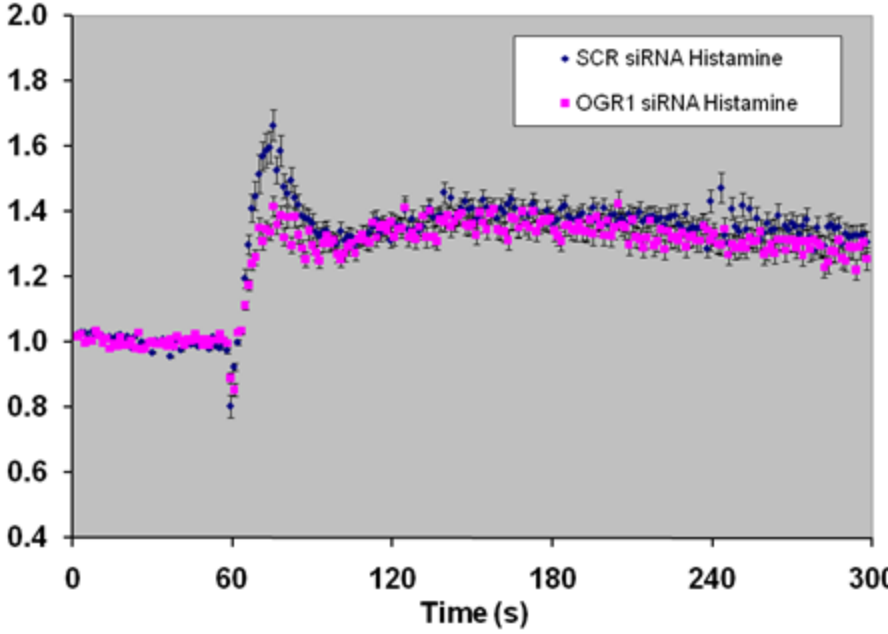




…and OGR1 knockdown inhibits this contraction

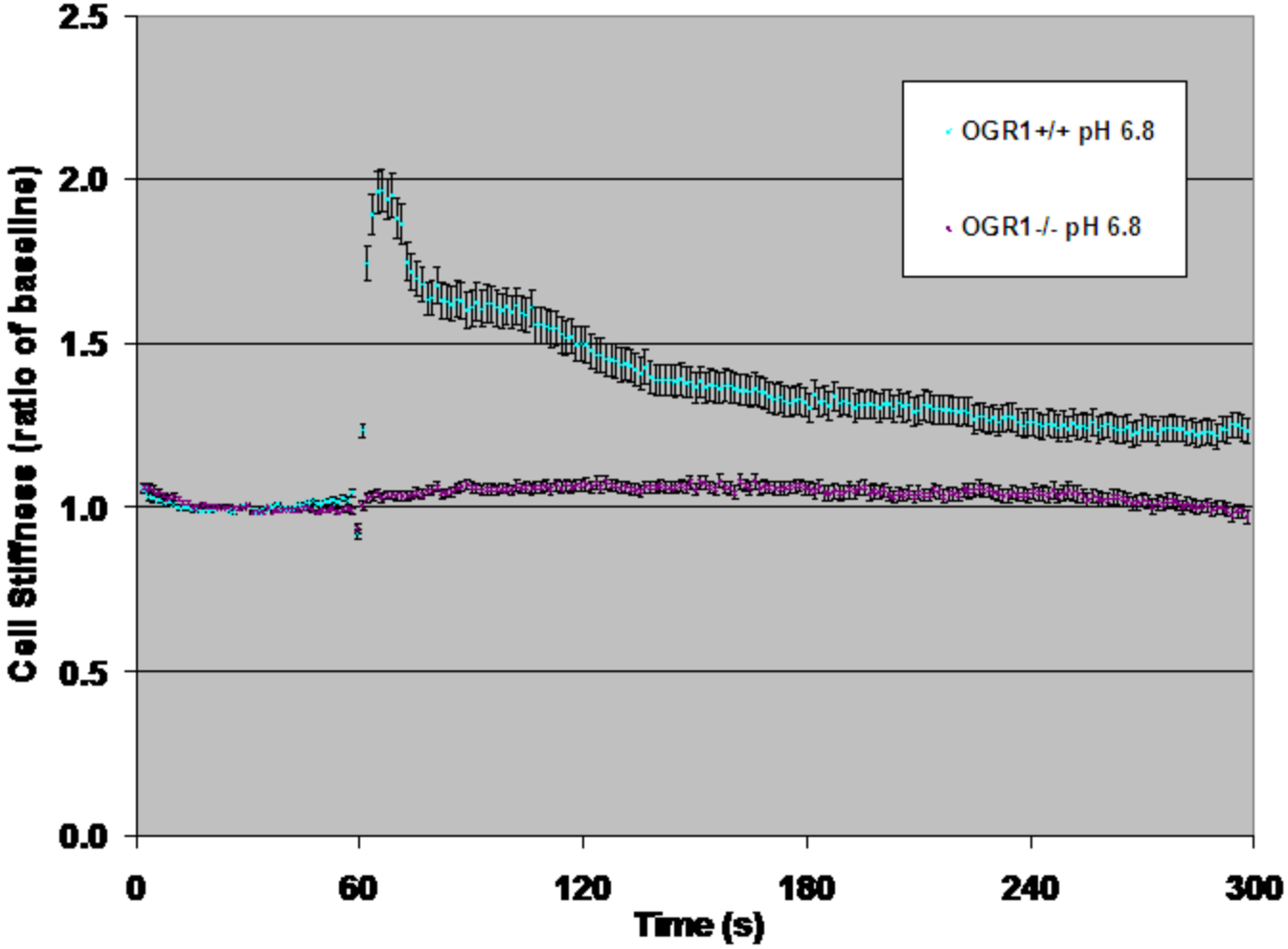


pH 6.8



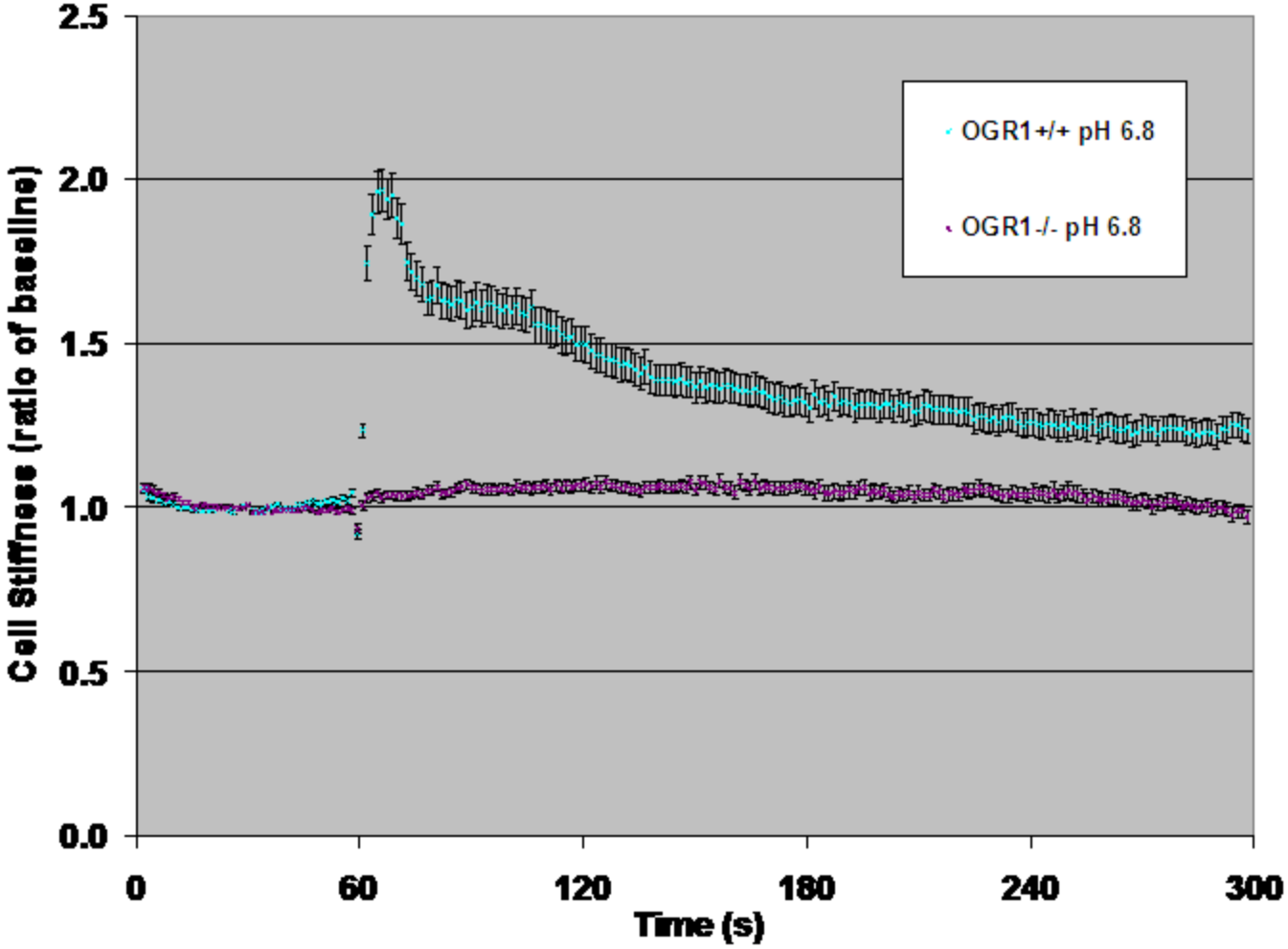
Histamine

…and in the obligatory mouse experiment

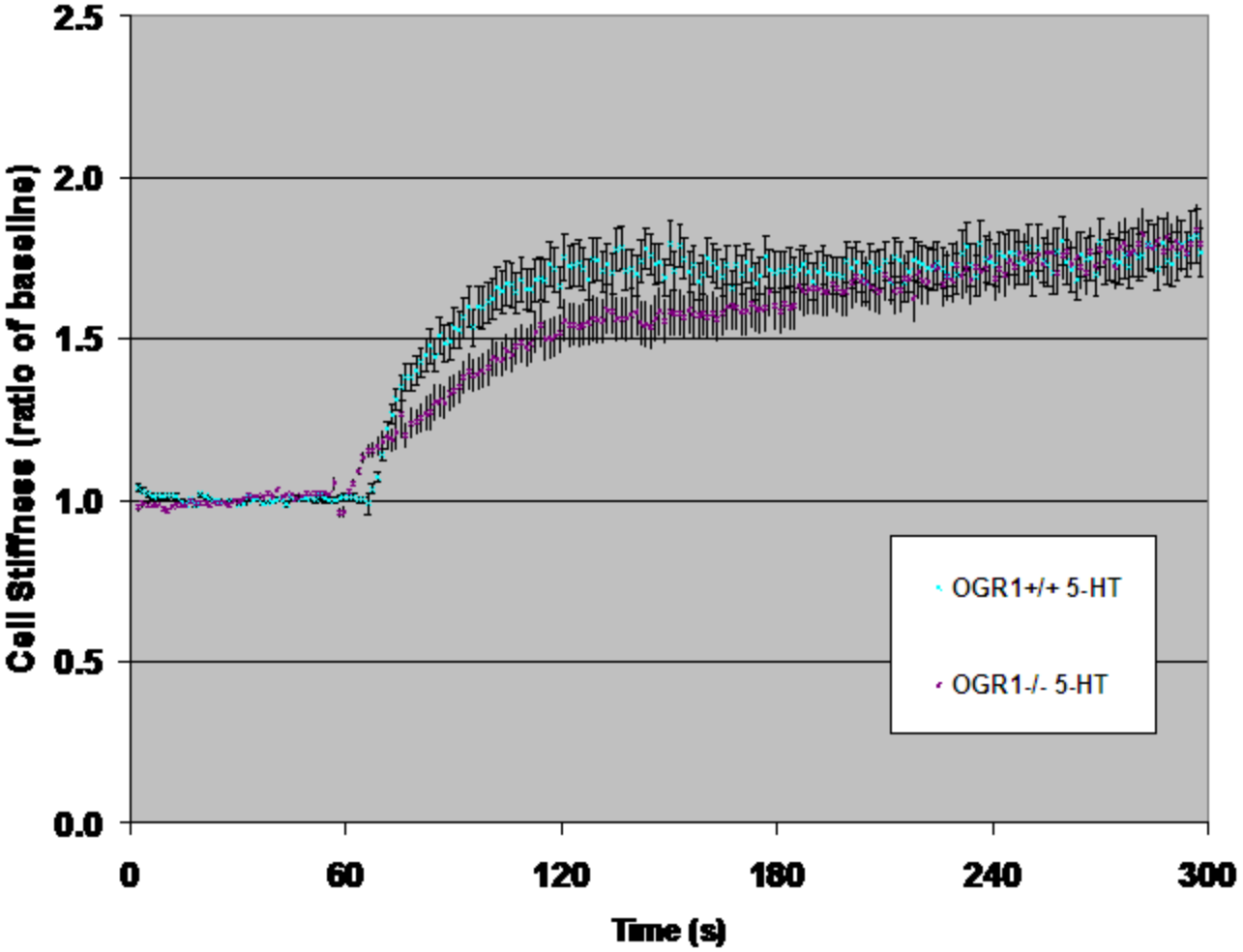


pH 8.0→6.8

…and in the obligatory mouse experiment



pH 8.0→6.8


Cell Stiffness (ratio of baseline)
2.5
2.0
1.5
1.0
0.5
0.0
0
60
120
180
240
300
Time (s)
OGR1+/+ 5-HT
OGR1-/- 5-HT

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?

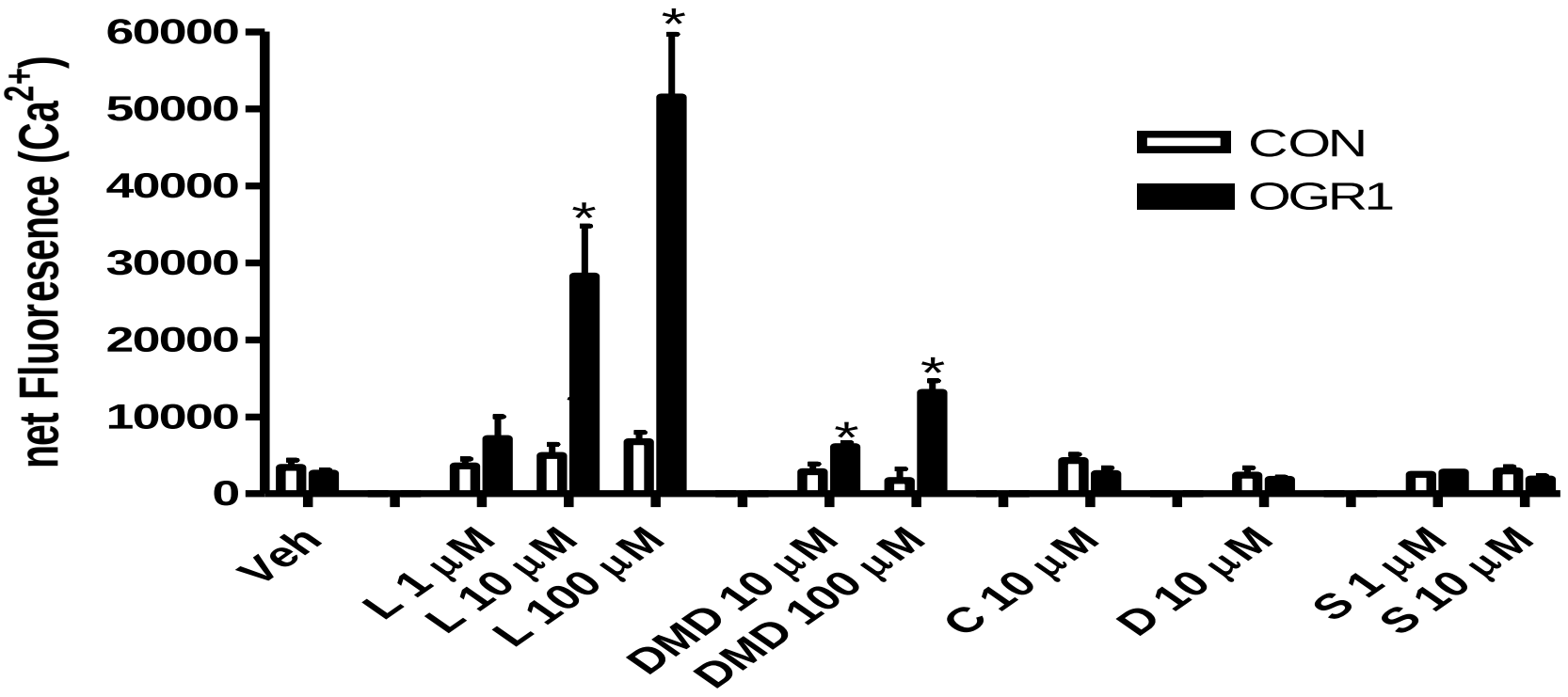
- Control pHo or pHi
- Block, activate, or bias GPCR signaling:

    a. Downstream signaling- usual suspect pathway inhibitors, COX inhibitors, antagonists of induced GPCR ligands, tyrphostins.

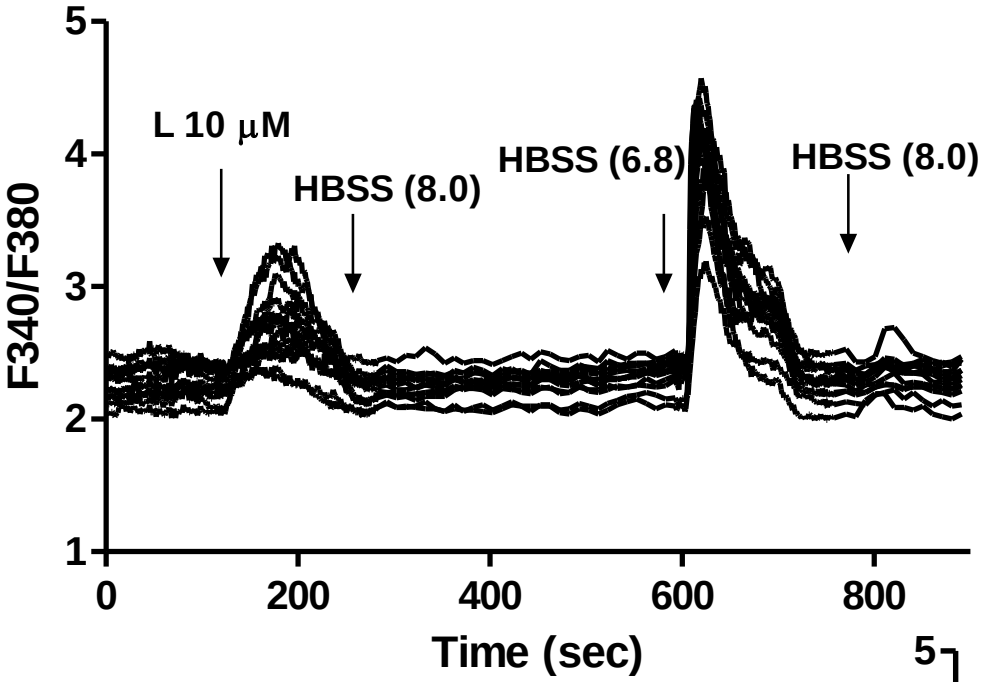
    b. At the receptor level for proton-sensing GPCRs; this quite hard given no ligands!

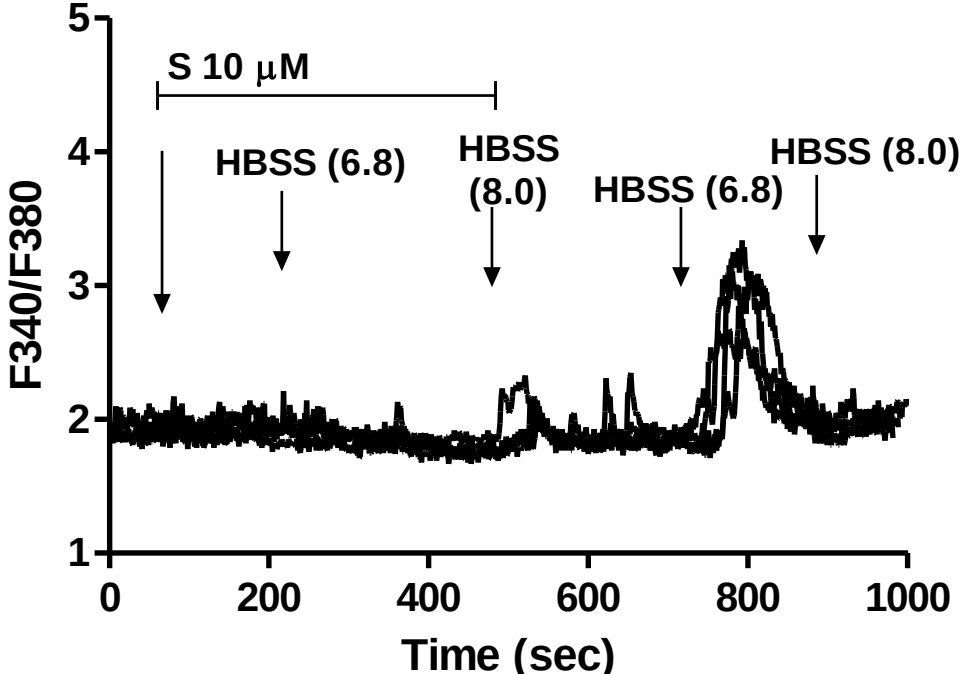
We have characterized a class of OGR1 ligands (allosteric modulators actually) and data to date that suggest we can bias OGR1 signaling

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

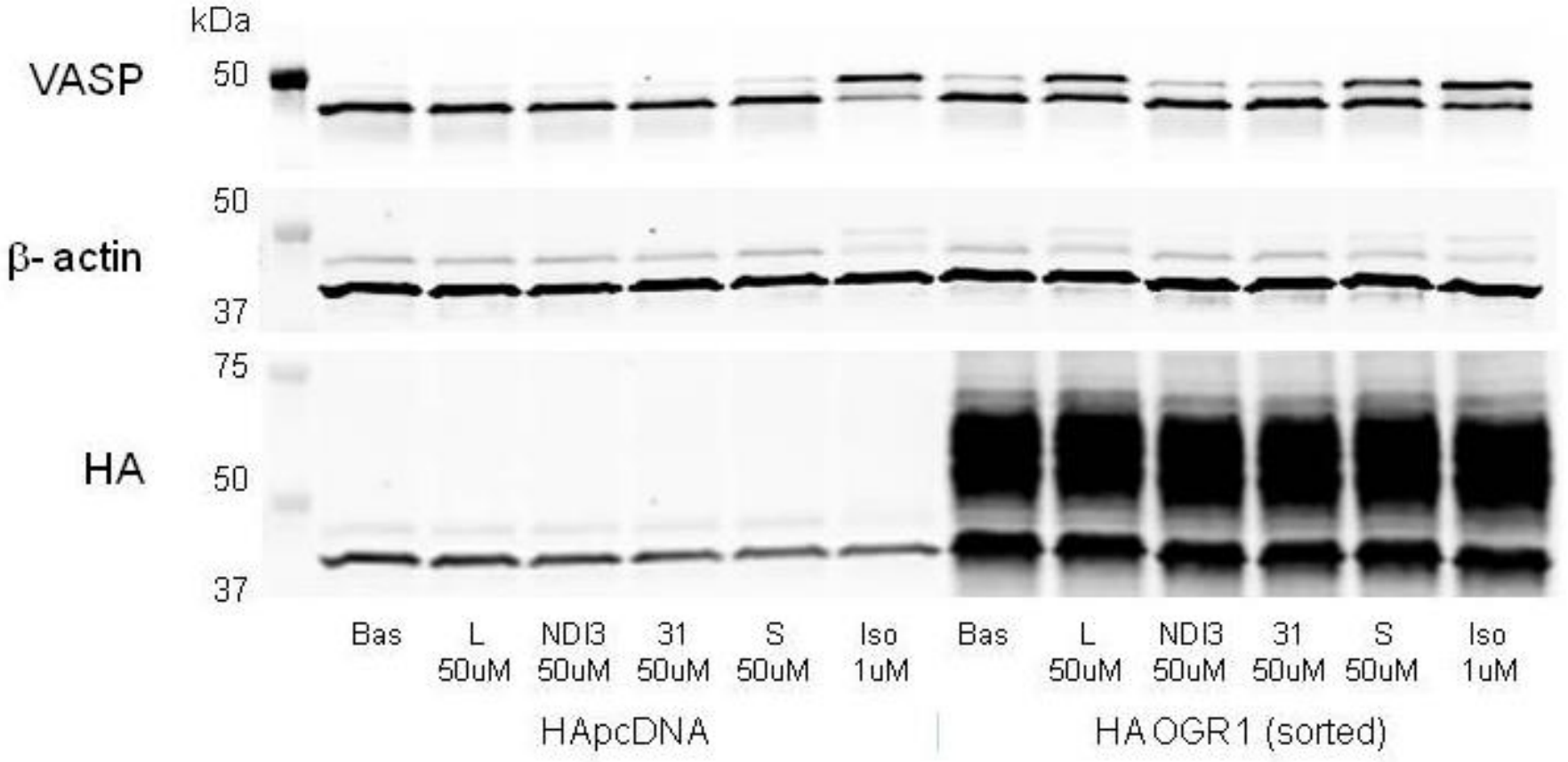


Some ligands appear balanced (L), some biased (S)

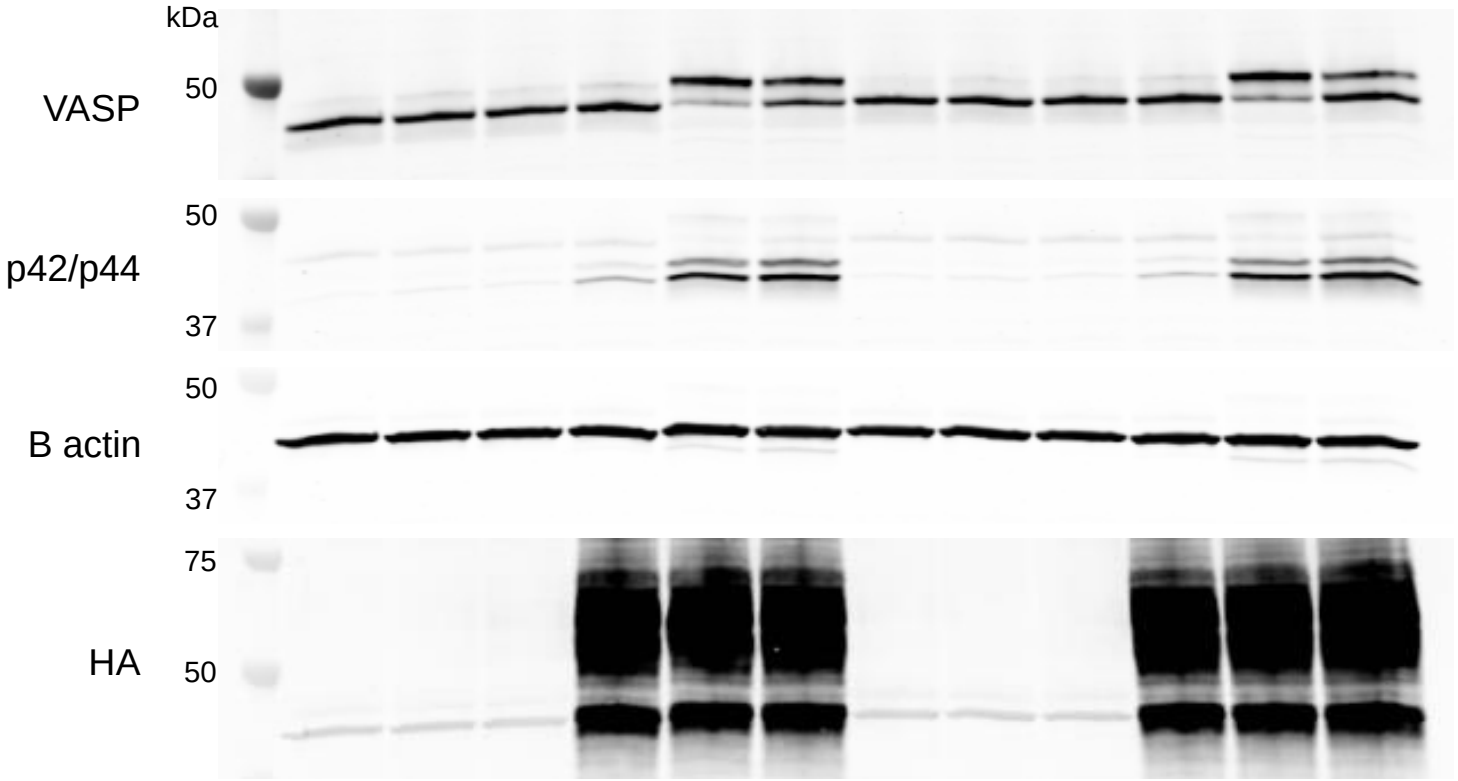




S stimulates Gs/ PKA but not Gq/Ca2+



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 broncholdilation!)

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

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