

10-8-2014

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

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Department of Medicine

Jefferson University Hospital



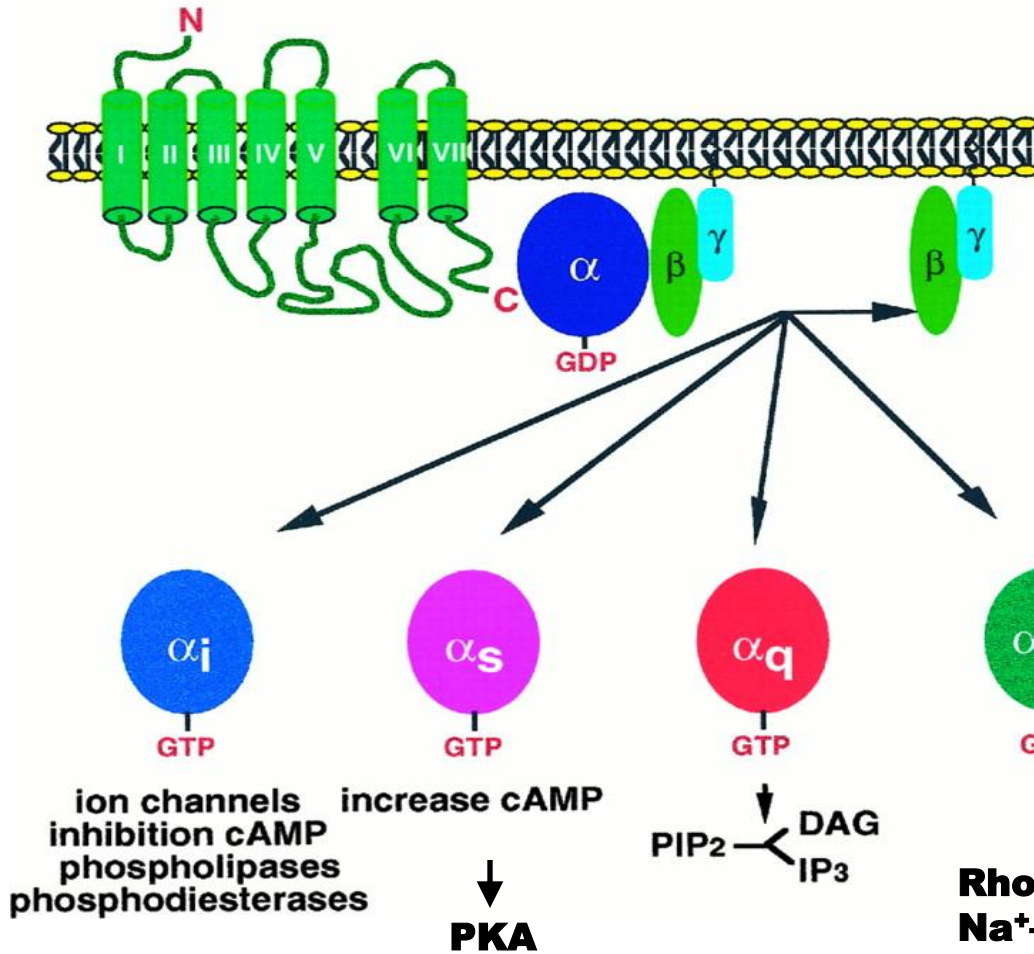
Raymond Penn, PhD
Division of Pulmonary and Critical Care Medicine
Jane and Leonard Korman Lung Center
Department of Medicine, Thomas Jefferson University

**“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

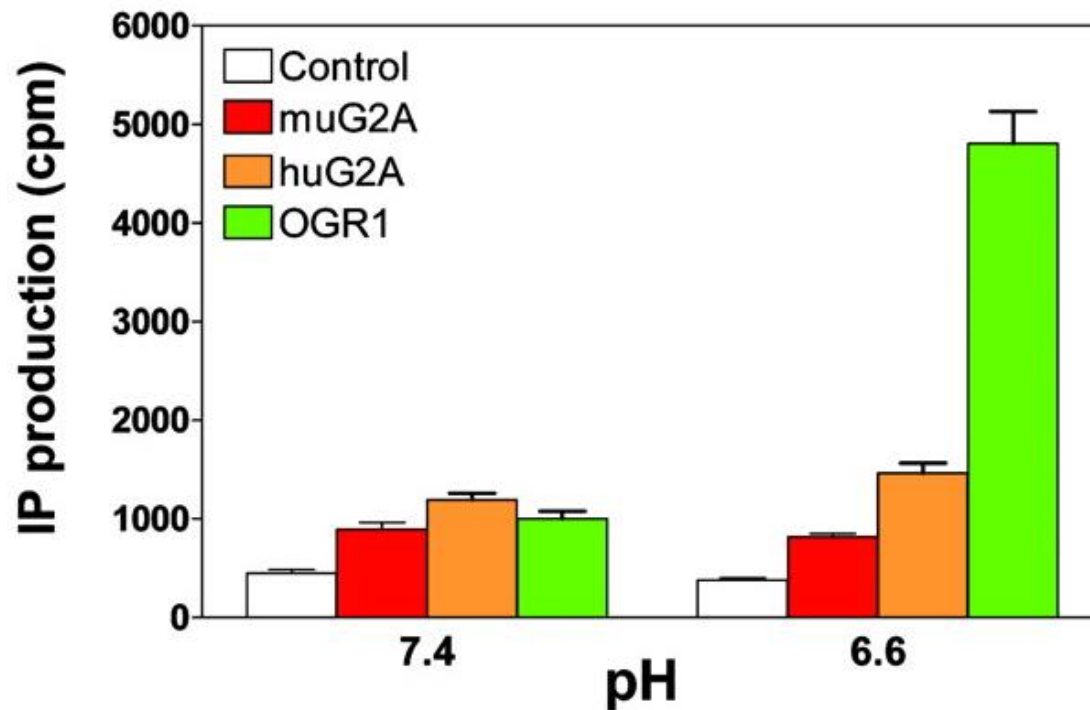

```

                                     TM I
hTDAG8 AA 1:-----MNSTCIEEQDLDYLFPIVYIFVVIIVSIPAN 32
hOGR1 AA 1:--M-----GN--I-----TADNSSMSCTID--TTFQTLAPVVVYTVLVVGF PAN 39
hGPR4 AA 1:-----MGNHTWEGCHVD--SRVDH--LFPFSLYIFVIGVGLPTN 35
hG2A AA 1:MCPMLLKNGYNGNATPVTTTAPWASLGLSAKTCNNVSFEESRIVLVVVVYSAVCTLGVPAN 60
                                     * * * *
                                     TM II
hTDAG8 AA 33:IGSLCVSFLQAKKESELGIYLFSLSLSDLLYALTLPLWIDYTWKNDNWFSPALCKGSFAF 92
hOGR1 AA 40:CLSLYFGYLDIKARNELGVYLCNLTVADLFYICSLPFWLOQYVLDGDNWSHGDLSCQVCGI 99
hGPR4 AA 36:CLALWAAYRQVQQRNELGVYLMNLSIADLLYICTLPLWVDYFLHHDNWIHGPGSKLFGF 95
hG2A AA 61:GLTAWLALLQVLOGNVLAVYLLCLALCELLYTGTLPLWVIYIFRNOHRWTLGLLACKVTAY 120
                                     * ** * * * * * * * *
                                     TM III TM IV
hTDAG8 AA 93:LMYMNFYSSTAFLTCIAVDRYLAVVYPLKFFFLRTRRFALMVSLSIWILETIFNAVMLWE 152
hOGR1 AA 100:LLYENIYISVGLCCISVDRYLAVAHPPRFHQFRTLKAAVGVSVVIWAKELLTSIYFLMH 159
hGPR4 AA 96:IFYTNIYISIAFLCCISVDRYLAVAHPLRFARLRVKTAVAVSSVWATELGANSAPLFH 155
hG2A AA 121:IFFCNIVYSILFLCCISQDRFVAVVYALESRGRRRRRTAILISACIFILVGIHVHPVFQT 180
                                     * * * * * * * * * *
                                     TM V
hTDAG8 AA 153:DETVVEYCDAEKSNFTLCYDKYPLEKWQIINLNLFRCTGYAIPLVTLICNRKYYDAVRH 212
hOGR1 AA 160:EE--VIED--ENG--RVCFEHYPIQAWQRAINYRFLVGLFPIGCLLLASYQGILRAVRR 214
hGPR4 AA 156:DE--LFRD--RYNH--TFCFEKFPMEGWAVMNLRYRVFVGLFPWALMLLSYRGILRAVRG 210
hG2A AA 181:-----ED--K--ETCFDMLQMSRITAGYYYARFTVGFAPLSIIAFTNHRIFRSIKQ 228
                                     * * * *
                                     TM VI
hTDAG8 AA 213:NKATENKEKRIIKLLVSIIVTFVLCFTPFVMLLIRCIIEHAVNFE-D-HSNSGKRITYT 270
hOGR1 AA 215:SHGTQKSRKDIQRLVLSIVVIFLACFLPYVLLLVRS-VW-E-A-----SCDFAKGVFN 266
hGPR4 AA 211:SVSTERQEKAKIKRLALSIAIVLVCFAPYVLLLSRSIAYLGRPW----DCGFEEVFS 266
hG2A AA 229:SMGLSAAQKAKVKHSAIAVVVIFLVCFAPYVLLLVKAAAFSYRGRDNAMCGLEERLYT 288
                                     * * * * *
                                     TM VII
hTDAG8 AA 271:MYRITVALTSLNCVADPILYCFVTETGRYDM-WNILKFCGTG--RCNTSQ-R-QRKRILSV 325
hOGR1 AA 267:AYHFSLLLTSFNCVADPVLVYCFVSETTHRDLAR-LRGACLAFLTCSRT-GRAREAYPLGA 324
hGPR4 AA 267:AYHSSLAFTSLNCVADPILYCLVNEGARSVDKALHNL-LRFLASDKPQEMANASLTLET 325
hG2A AA 289:ASVVFLCLSTVNGVADPIIYVLA TDHSRQEVSR IHKGWKEWSMKTDVTRLTHSRDTE-EL 347
                                     * * * * *
hTDAG8 AA 326:STKDTMELEVL----- 337
hOGR1 AA 325:PEASGKSGAQGEPELLTKLHPAFQTPNSPGSGGFPTGRLA 365
hGPR4 AA 326:PLTSKRNSTAKAMTGSWAATPPS-QGDQVQLK-MLPPAQ-- 362
hG2A AA 348:QSPVALADHYTFSRPVHPPGSPC-PAKRLIEESC----- 380

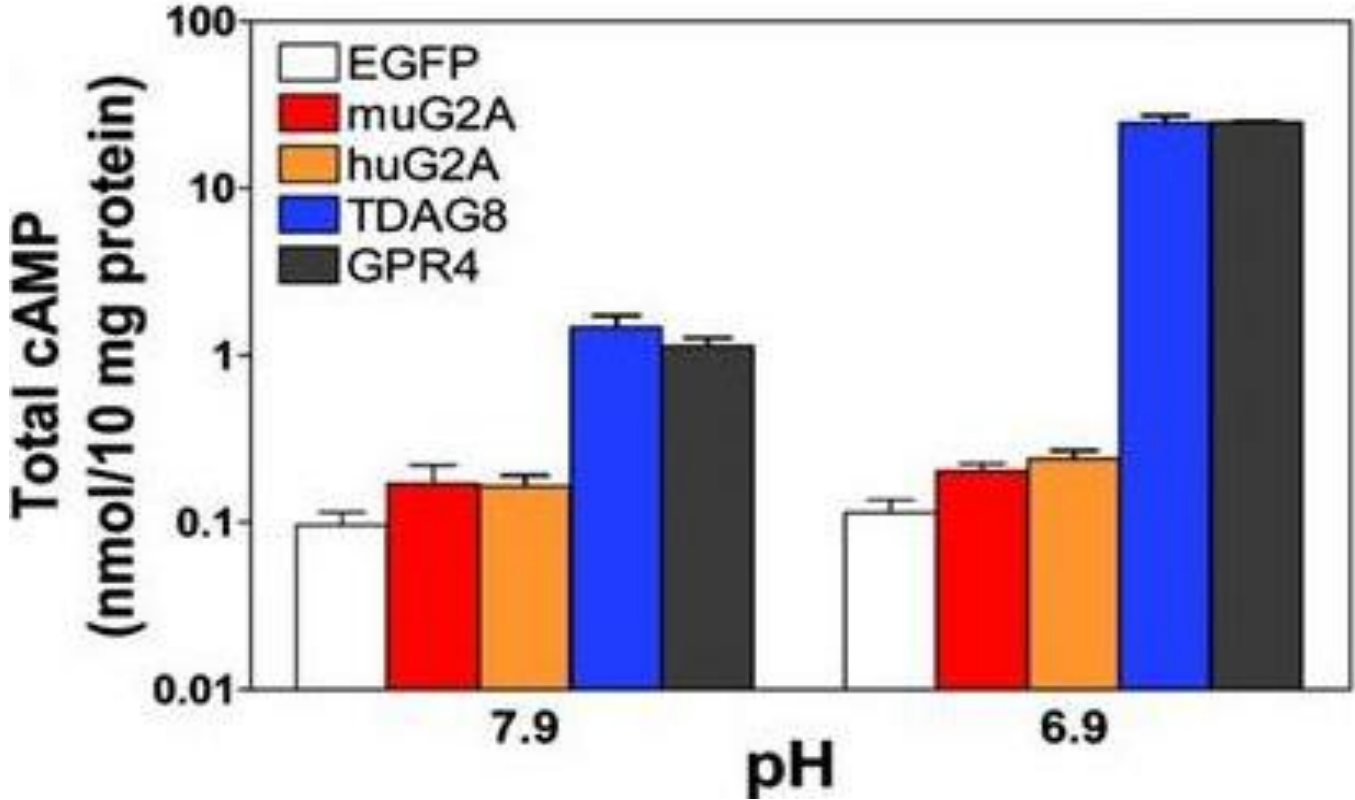
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Sequence alignment identifies OGR1, GPR4, TDAG8, and G2A as family of GPCRs

Expressed OGR1 exhibits constitutive and pH-dependent IP production

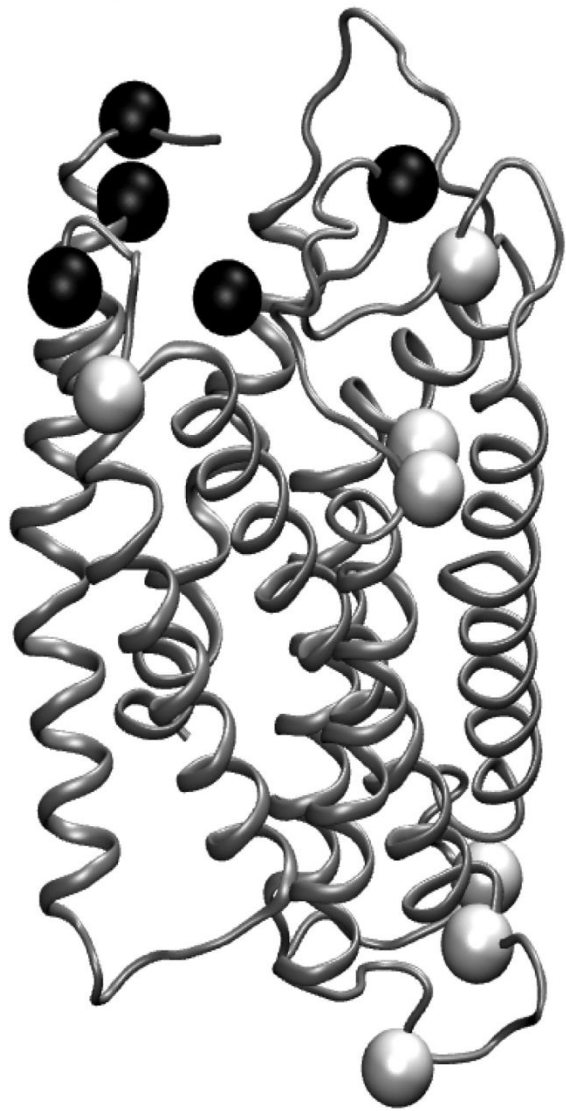


pH-dependent accumulation of intracellular cAMP by GPR4, TDAG8

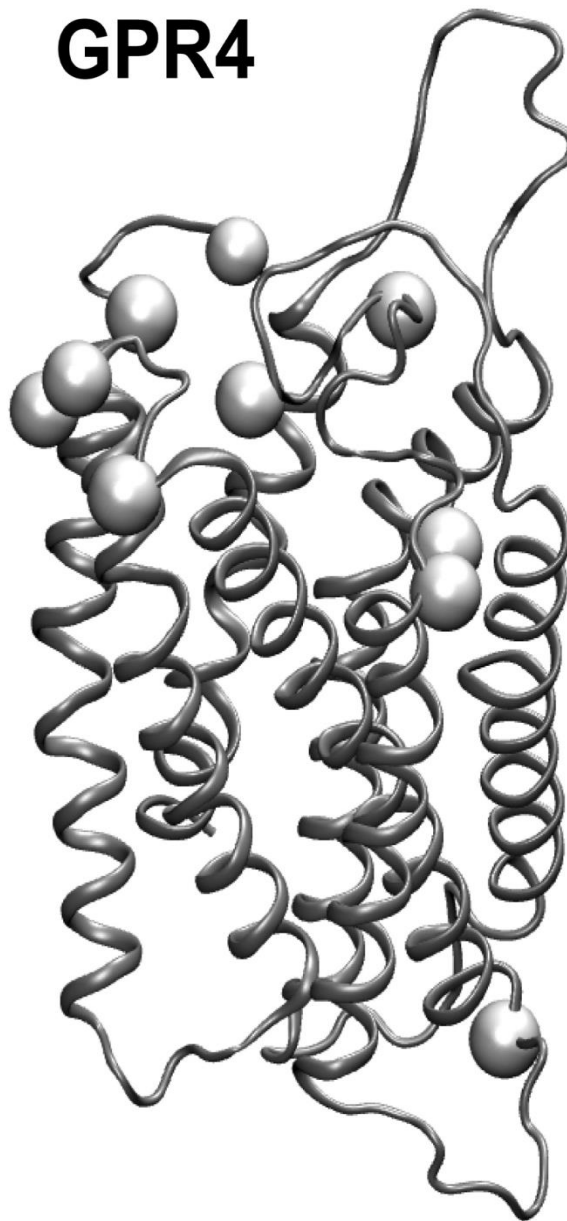


How do protons activate these GPCRs?

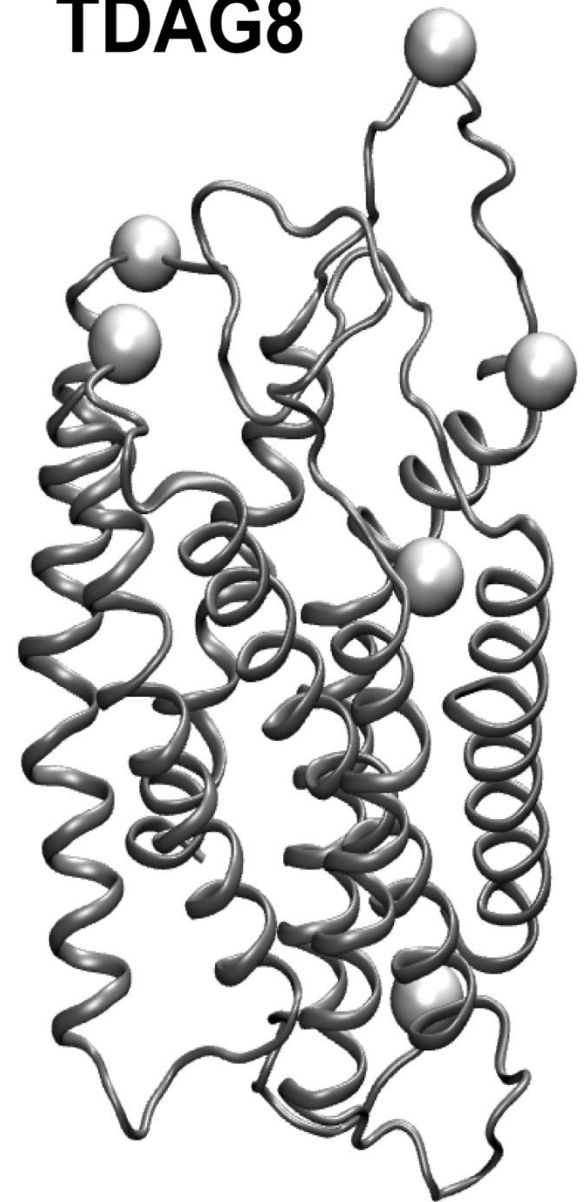
OGR1



GPR4

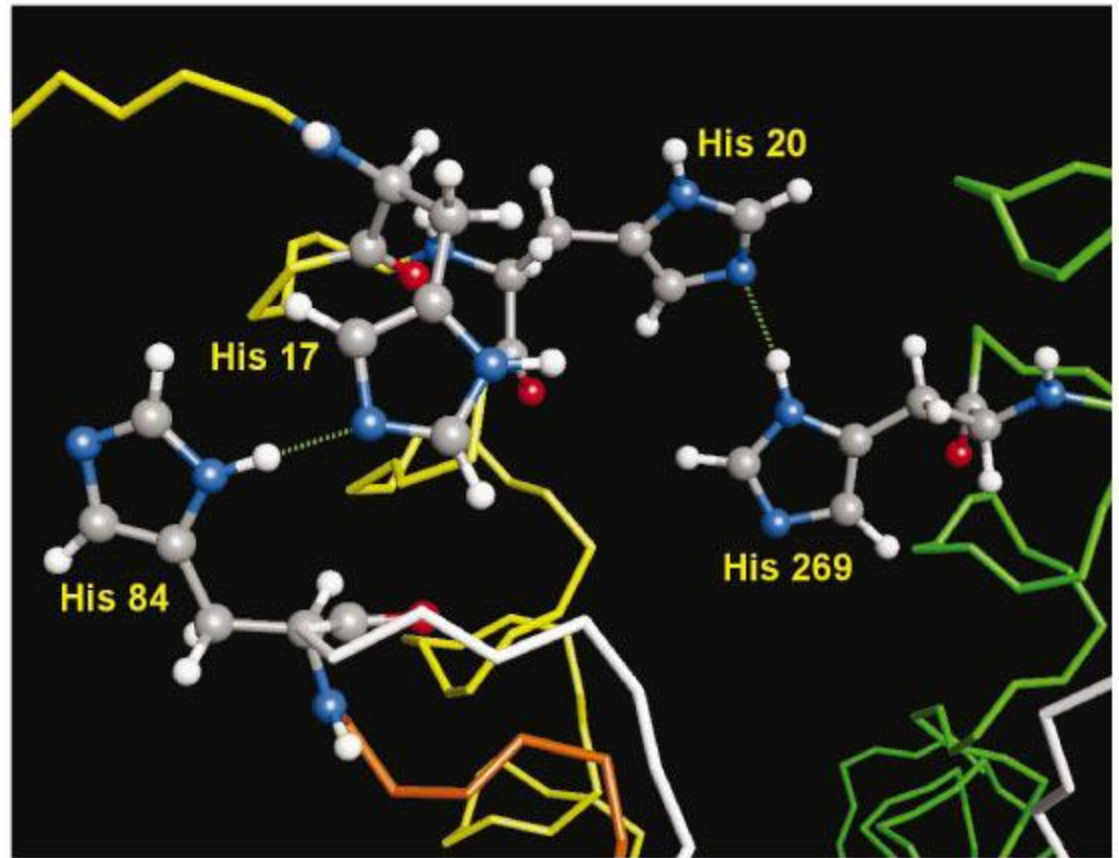
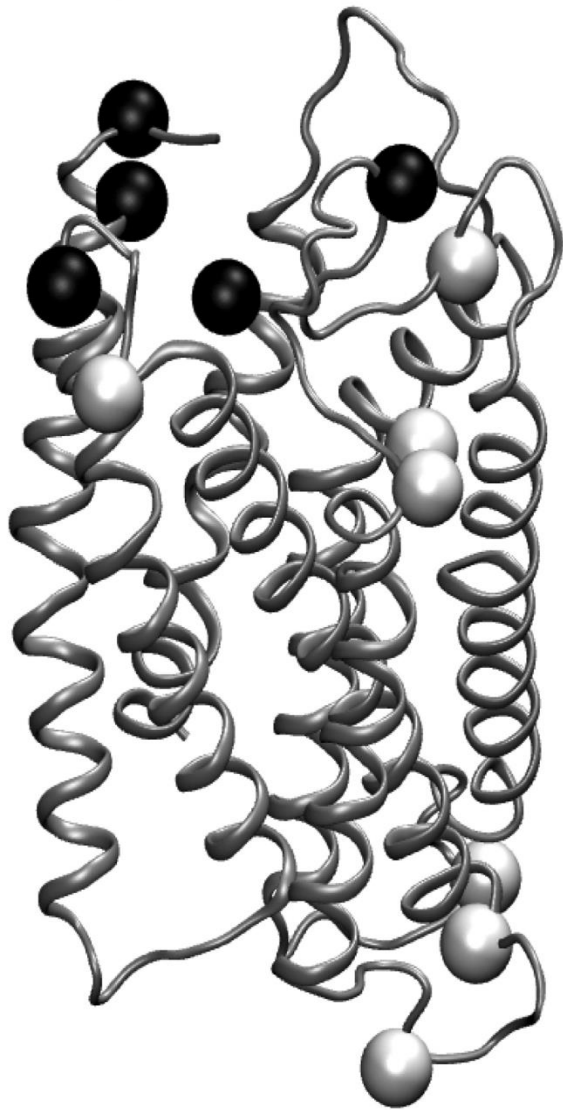


TDAG8

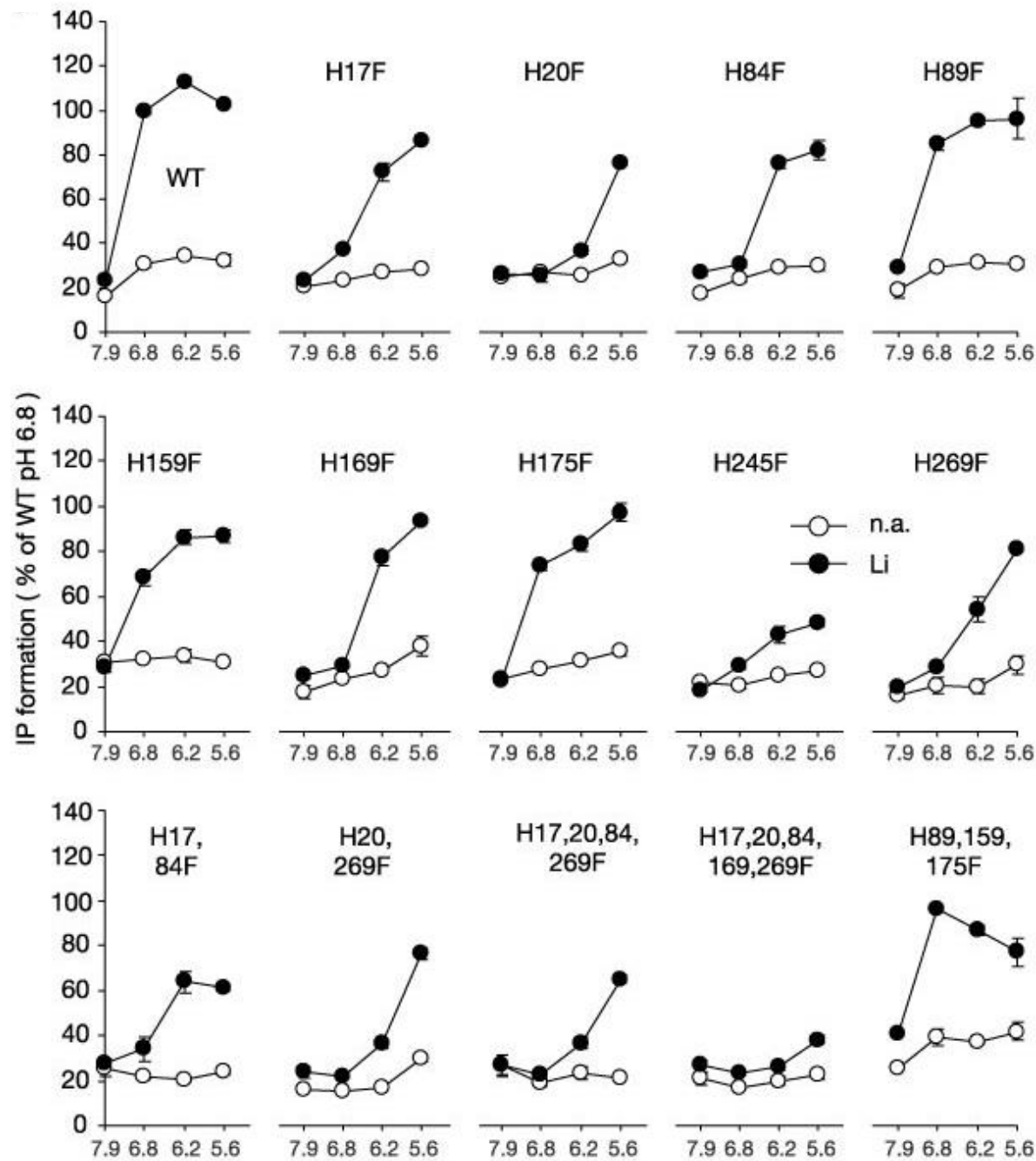


**Predicted structure, proton-sensing histidine residues in
OGR1, GPR4, and TDAG8**

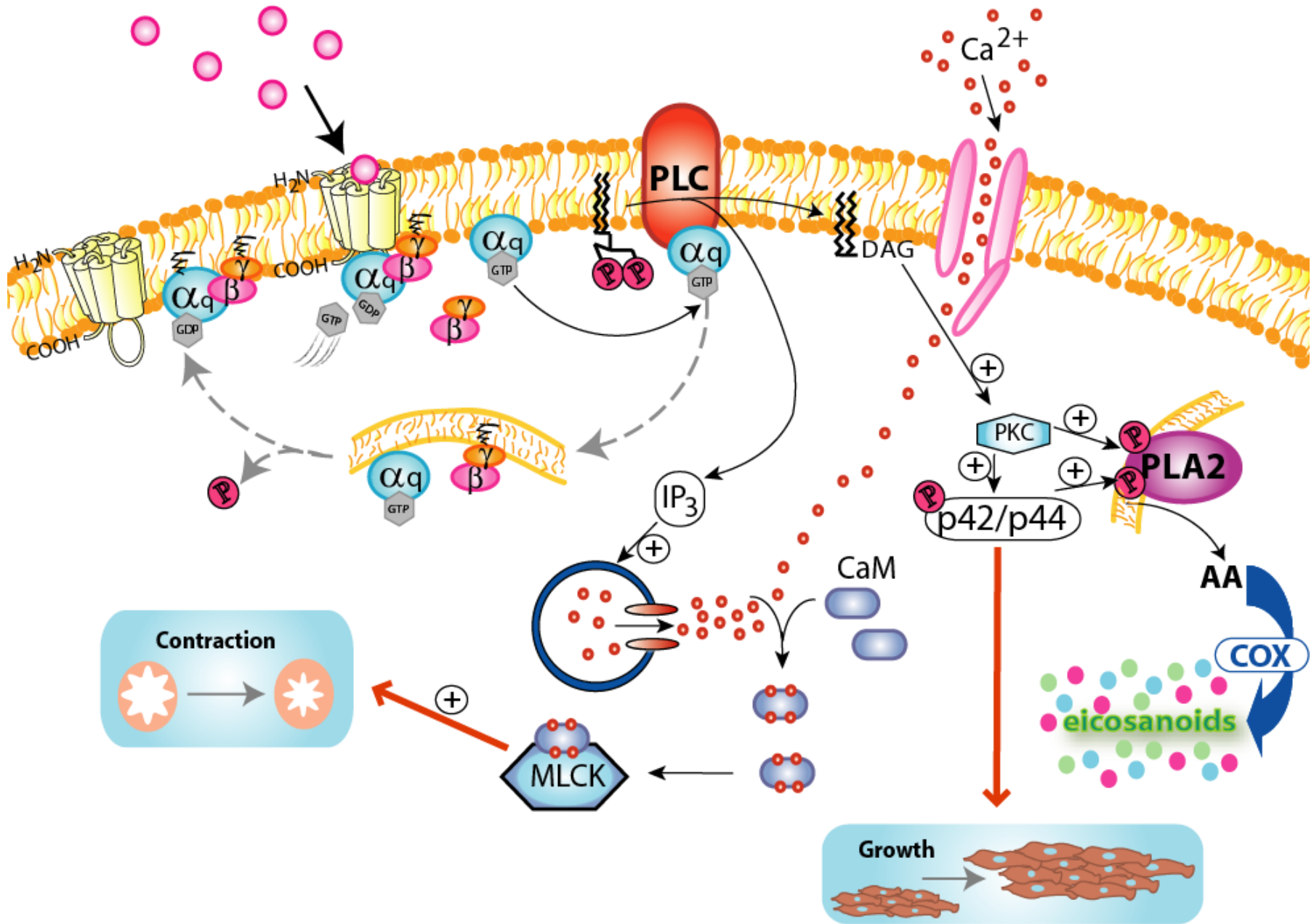
OGR1



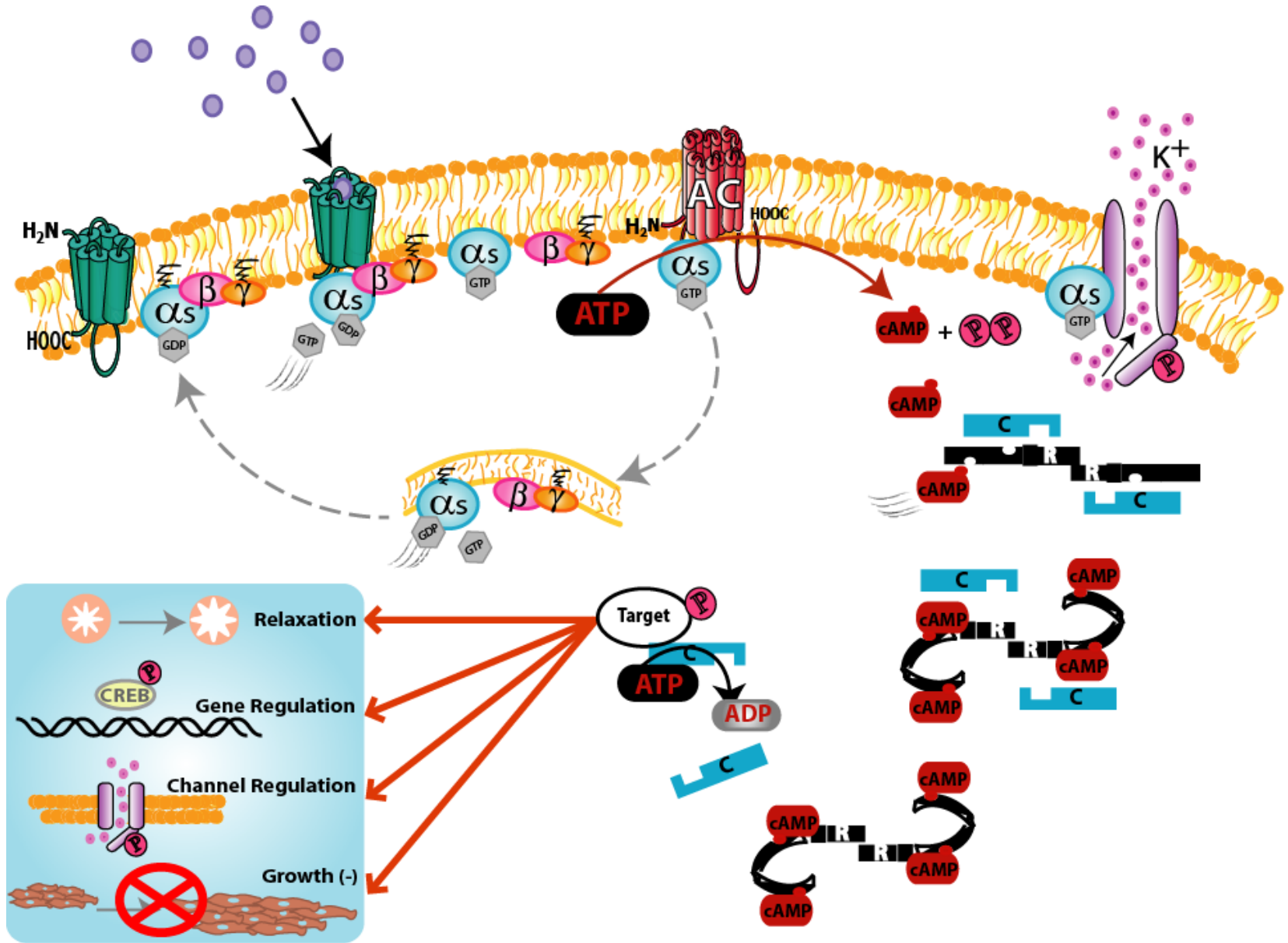
Mutation of putative proton-sensing histidines inhibits pH sensing by OGR1



G_q-coupled receptor signaling in ASM

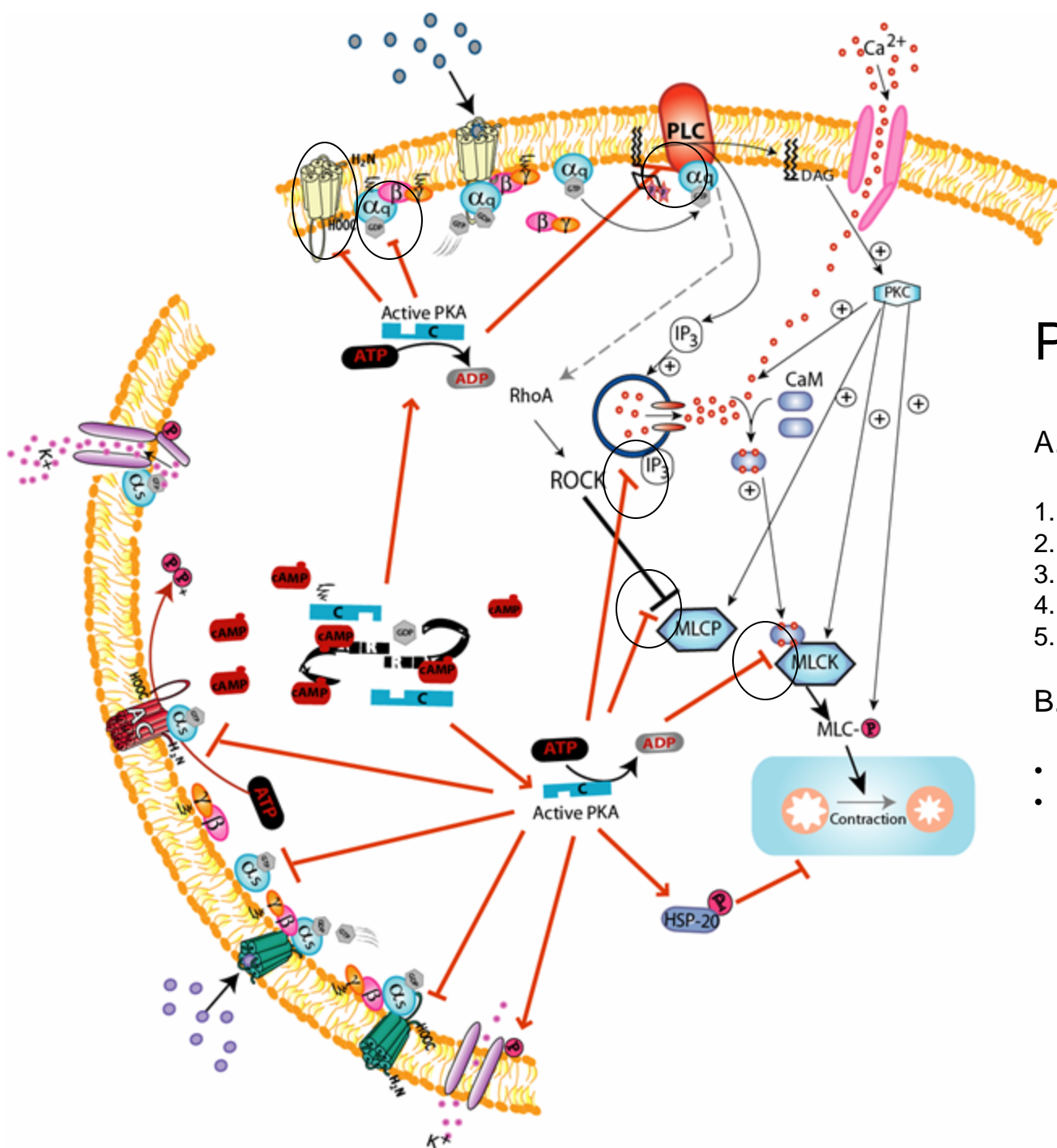


G_s-coupled receptor signaling



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

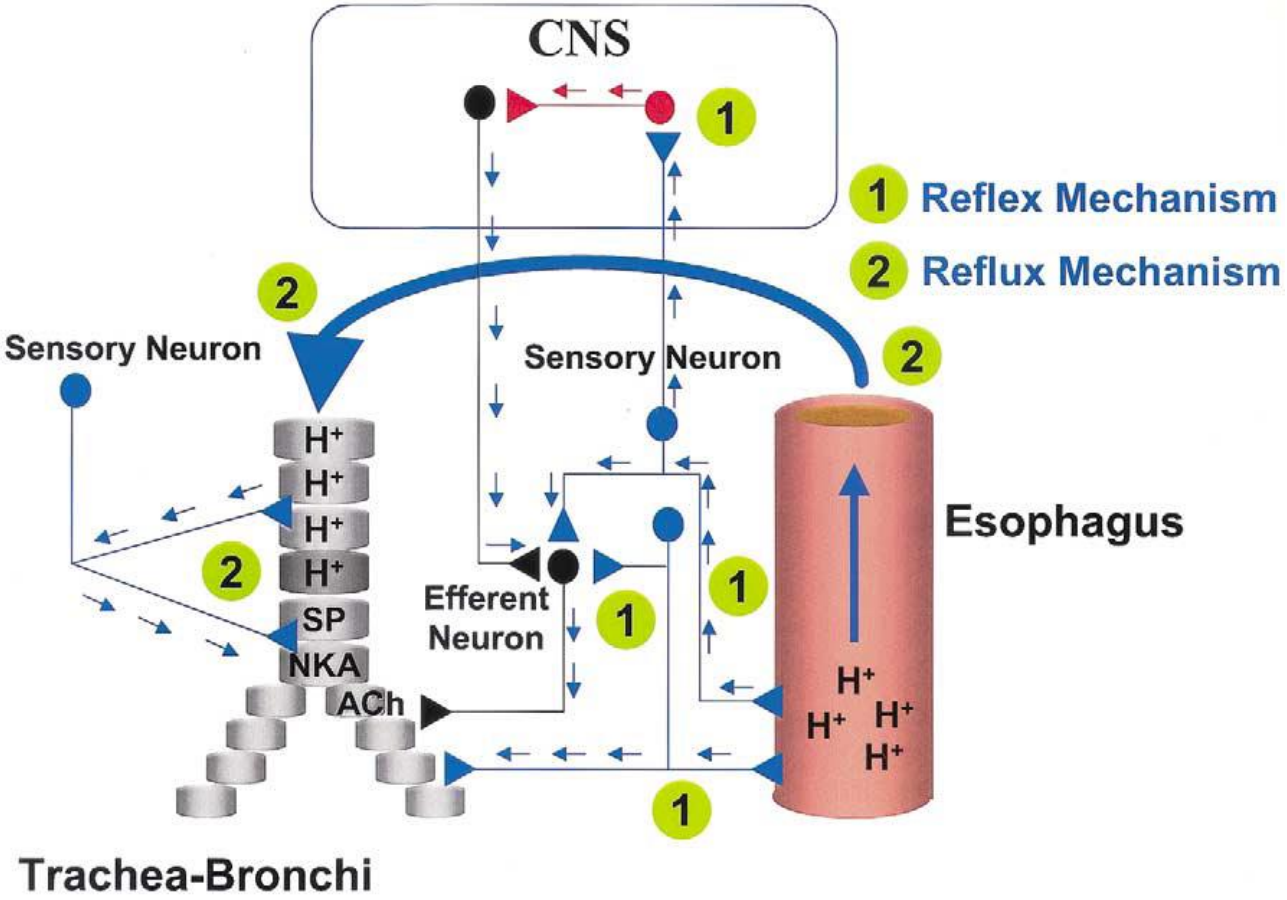
B. Calcium Sensitization:

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

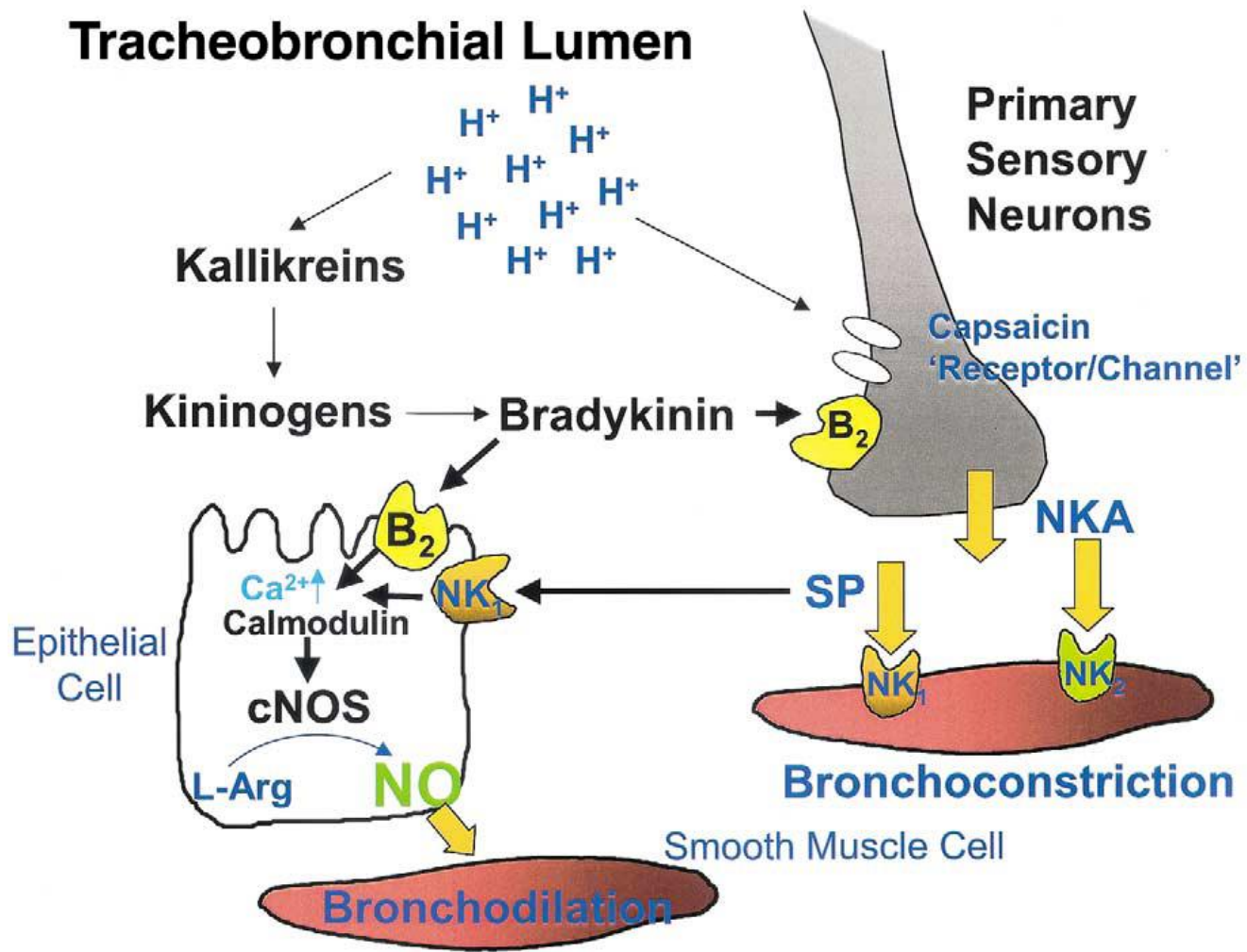
Objectives

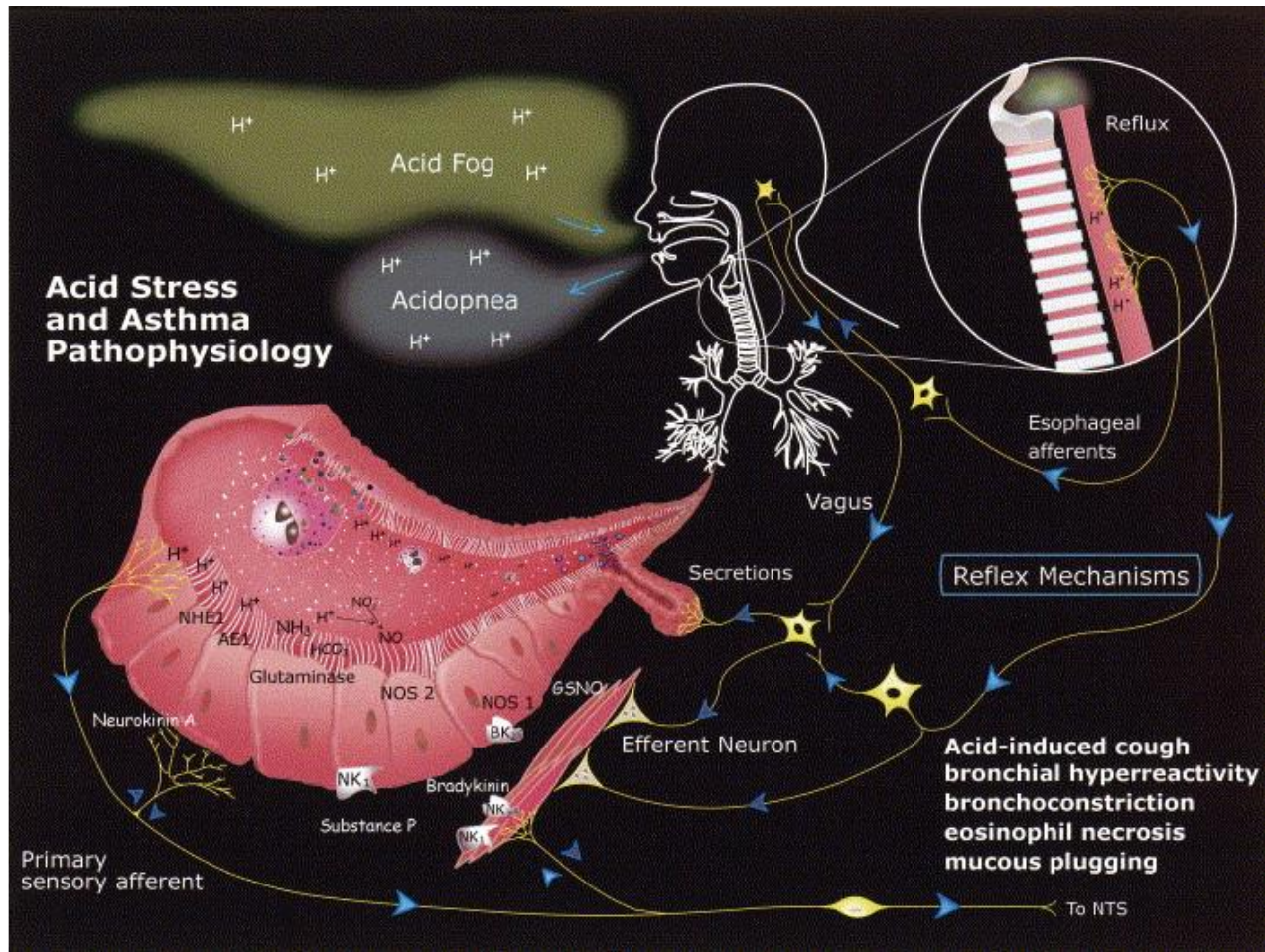
- Characterize the proton sensitive GPCRs in the ASM
- Determine the intracellular signaling mechanisms activated by proton sensitive receptors in the ASM
- Determine the functional consequences of changing pH in the microenvironment of the ASM

Current proposed mechanisms



Tracheobronchial Lumen

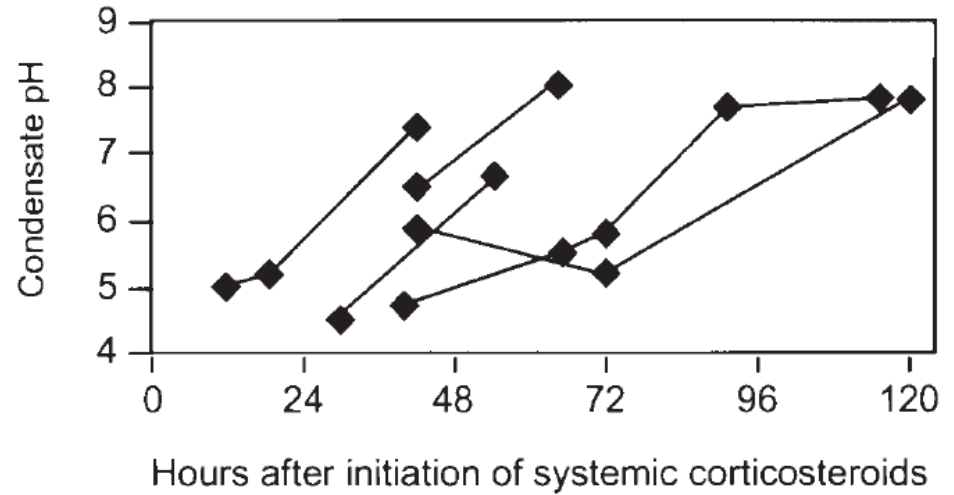
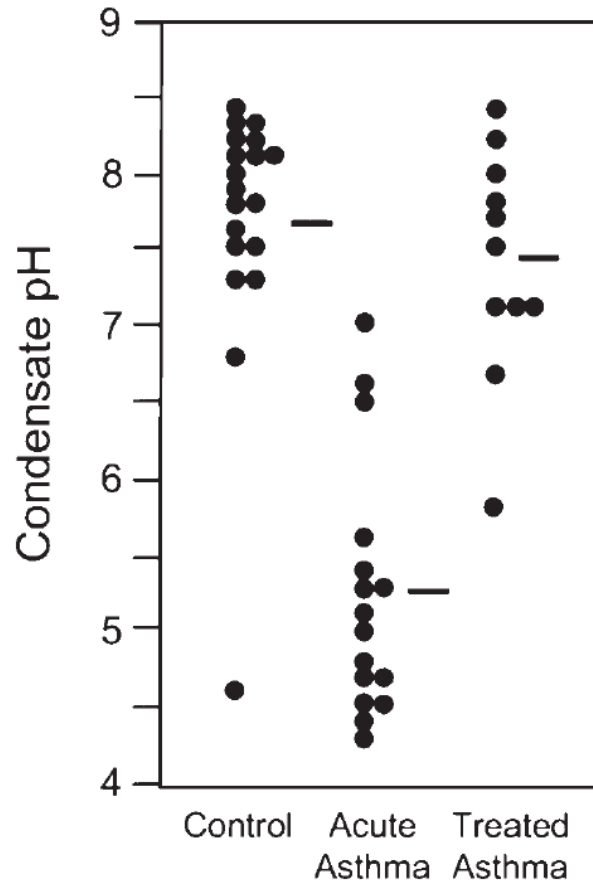




Airway pH tends to alkaline, but decreases with allergic inflammation

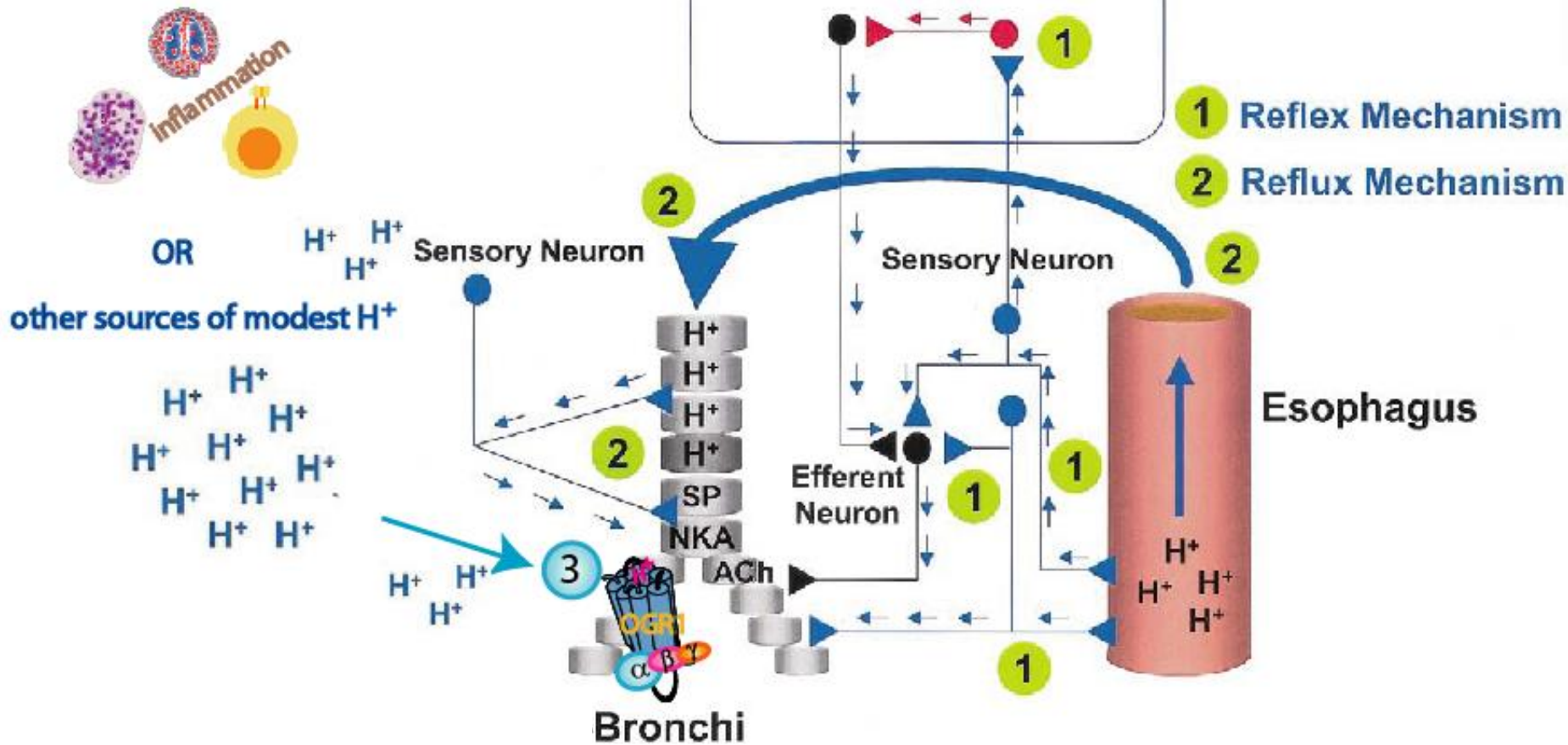
Respiratory fluid	Condition	Measured pH
EBC (deaerated to remove CO ₂)	Health	7.7±0.49
	Asthma exacerbation	5.2±0.21
	Stable mild asthma	7.6 (7.55-7.65)
	Stable moderate asthma	7.27 (CI, 7.15-7.39)
	Stable COPD	7.16 (CI, 7.09-7.23)
	COPD exacerbation	6.25
	Stable bronchiectasis	7.11 (CI, 7.04-7.19)
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

Corticosteroids reverse the reduced airway pH associated with acute asthma exacerbations

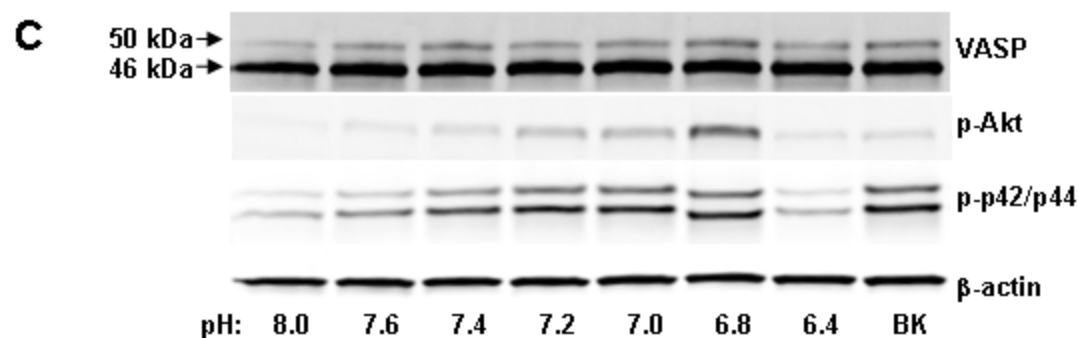
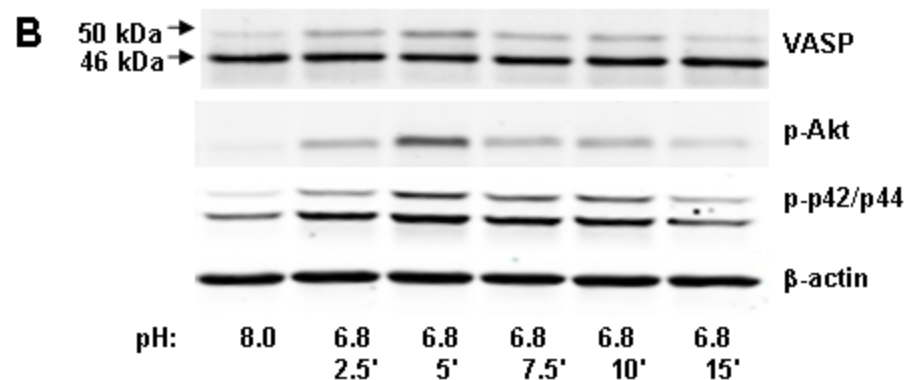
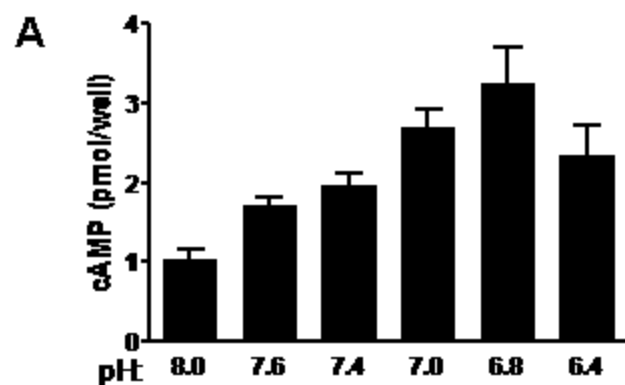


Proton-sensing GPCRs may represent another mechanisms mediating effects of more subtle decreases in airway pH

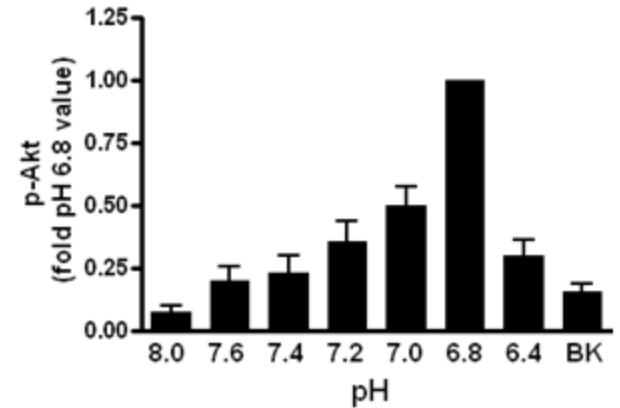
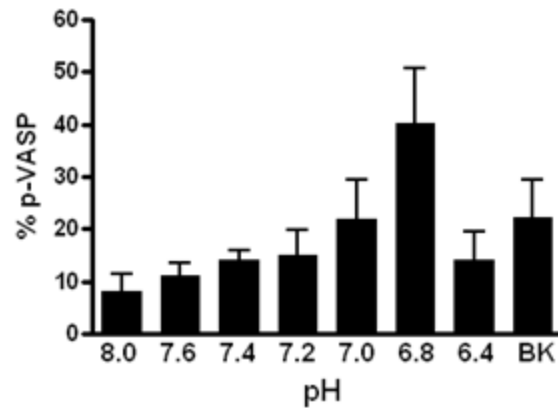
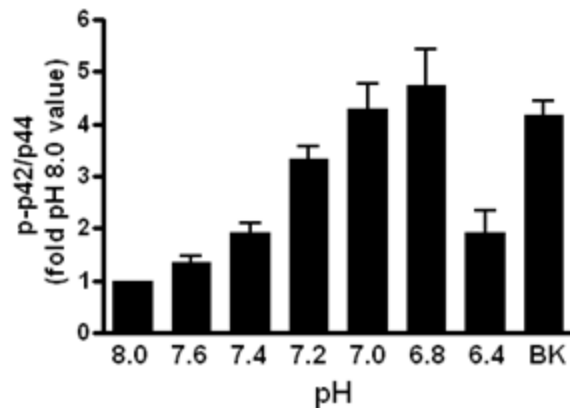
3 Alternative Mechanism



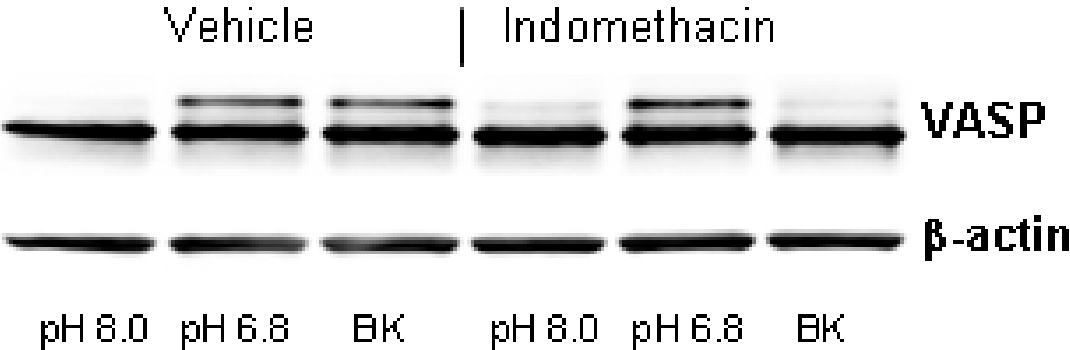
↓pHo activates cAMP/PKA, Akt, and p42/p44 in ASM



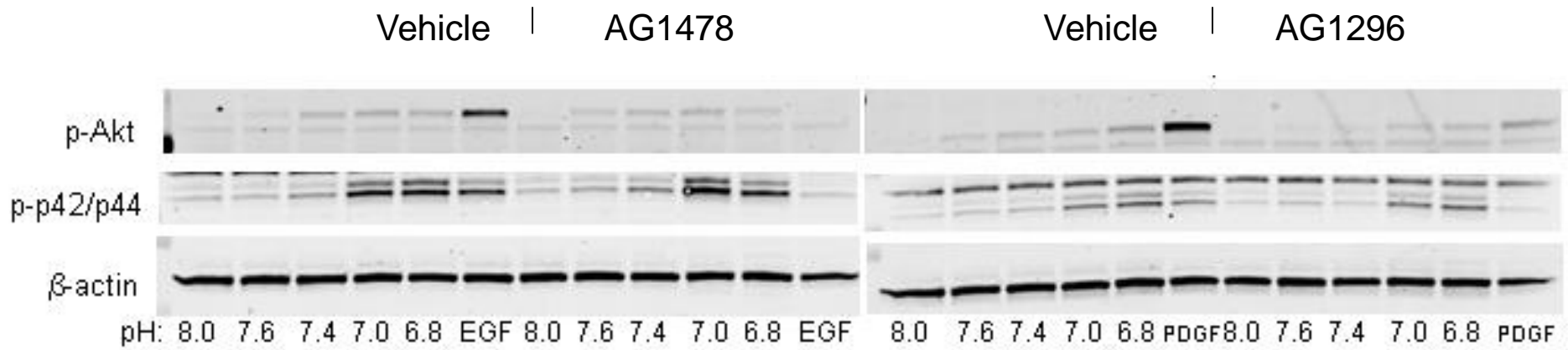
↓pHo activates cAMP/PKA, Akt, and p42/p44 in ASM



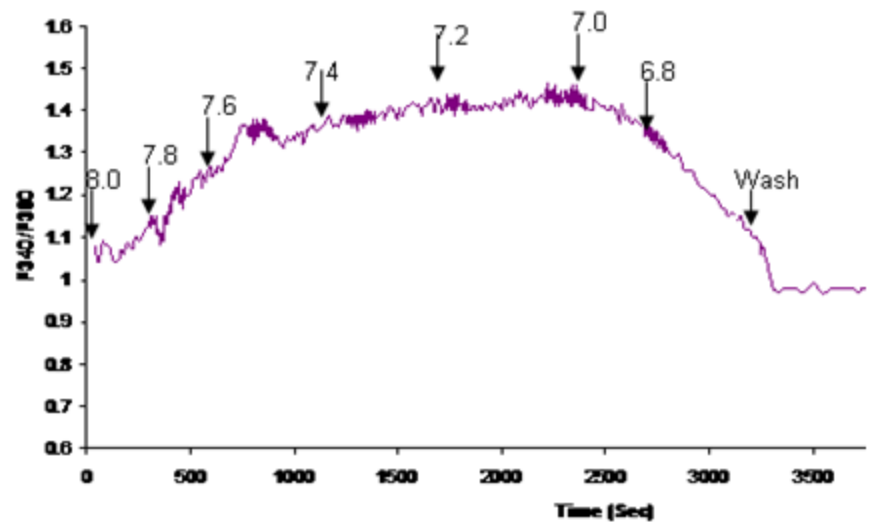
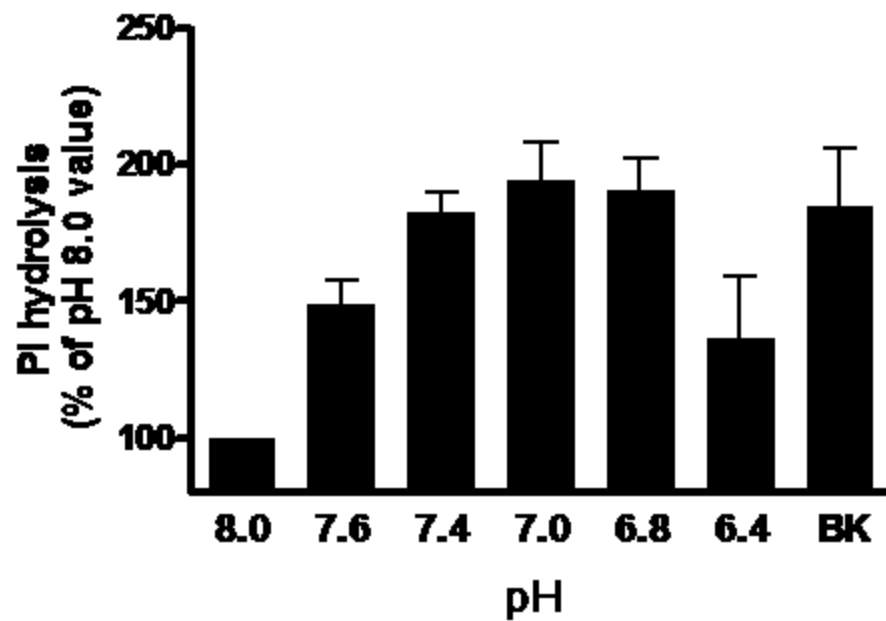
PKA activation by ↓pHo not necessarily dependent on COX:
Pleiotropic signaling?



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



↓pHo stimulates PI hydrolysis, Ca²⁺ mobilization



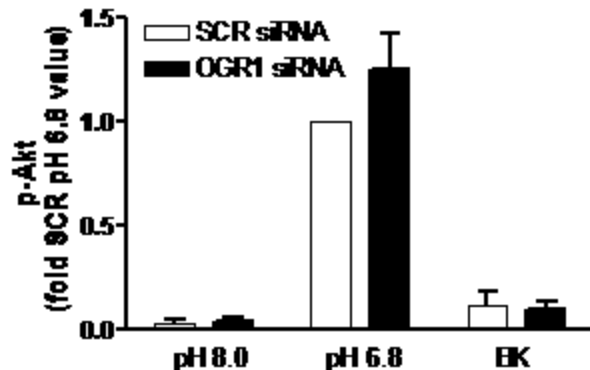
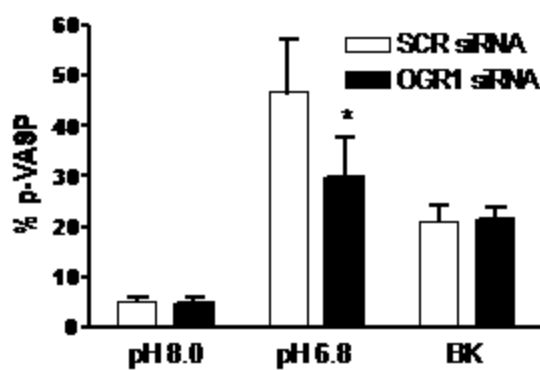
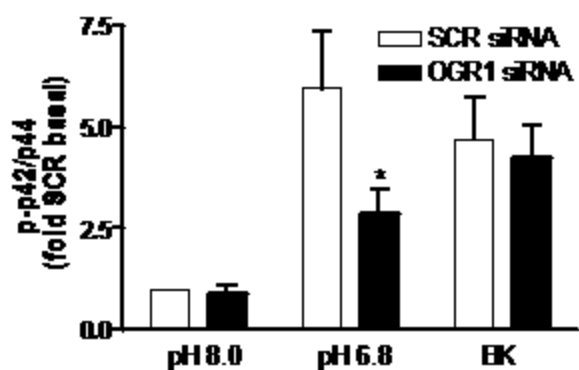
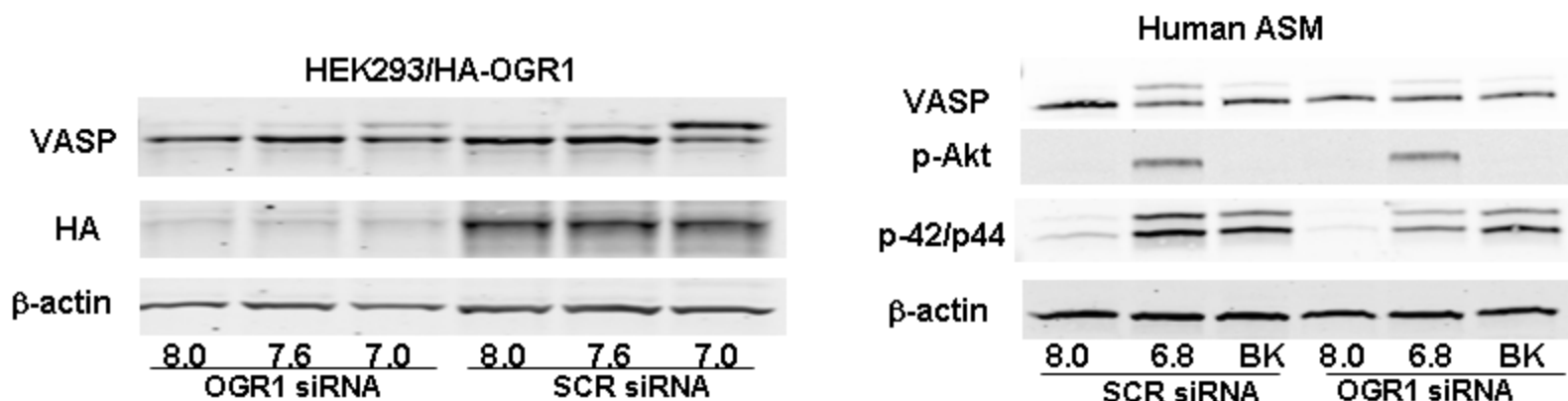
Expression of proton sensitive receptors in ASM

PCR of GPCRs in HASM and Airway Epithelium

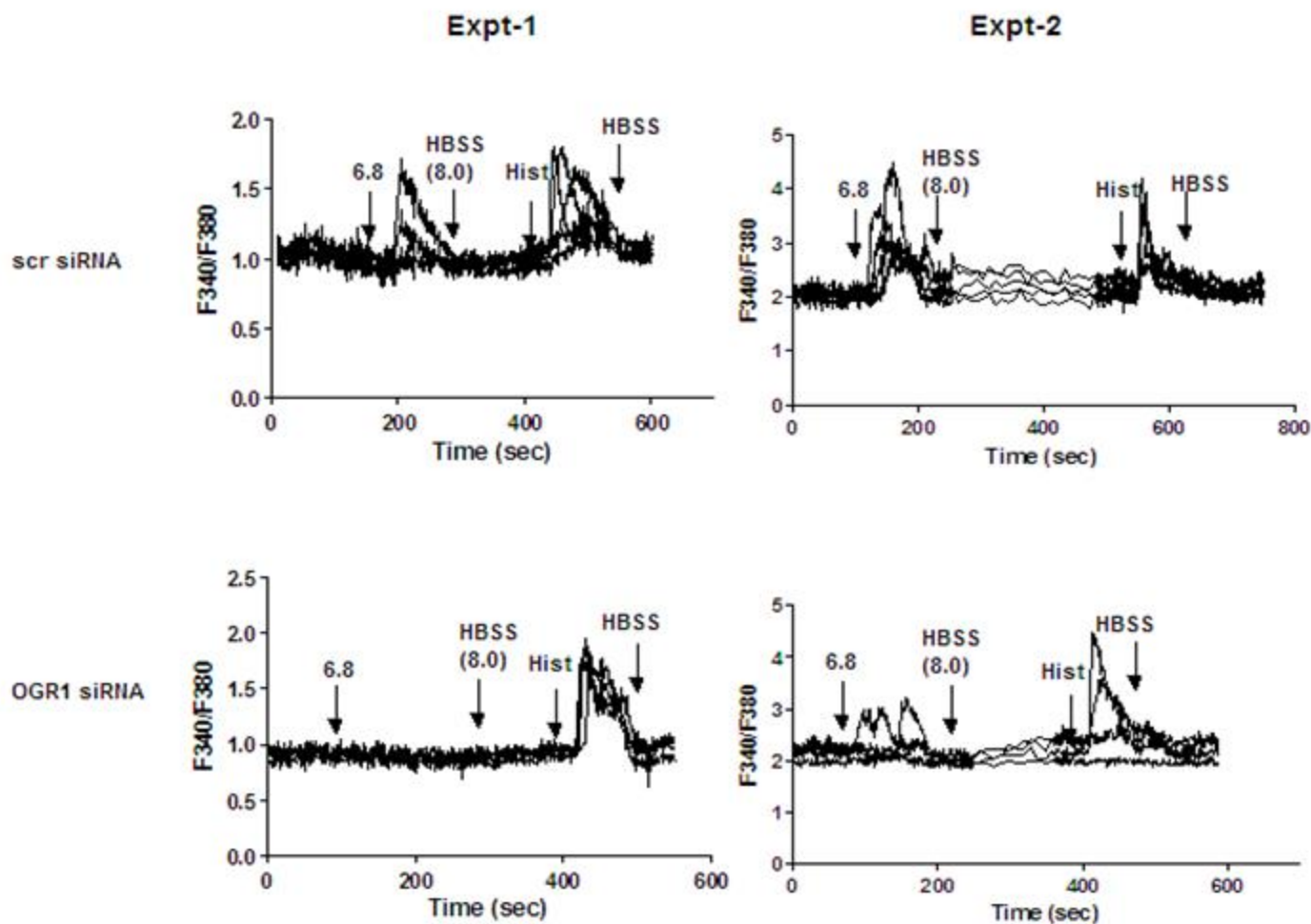


GPR4	OGR1	TDAG8	GPR4	OGR1	TDAG8	GPR4	OGR1	GPR4	OGR1	TDAG8	GPR4	OGR1	TDAG8
HASM			Airway Epithelium			HEK w/ GPR4	HEK w/ OGR1	HEK w/ Vector			No Template		

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



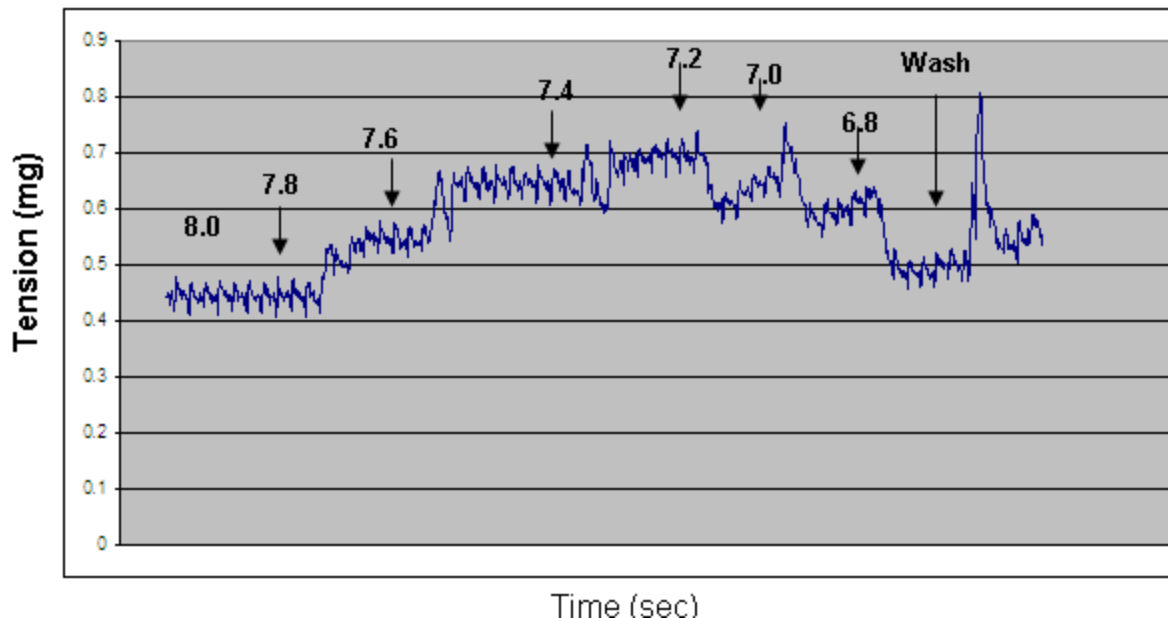
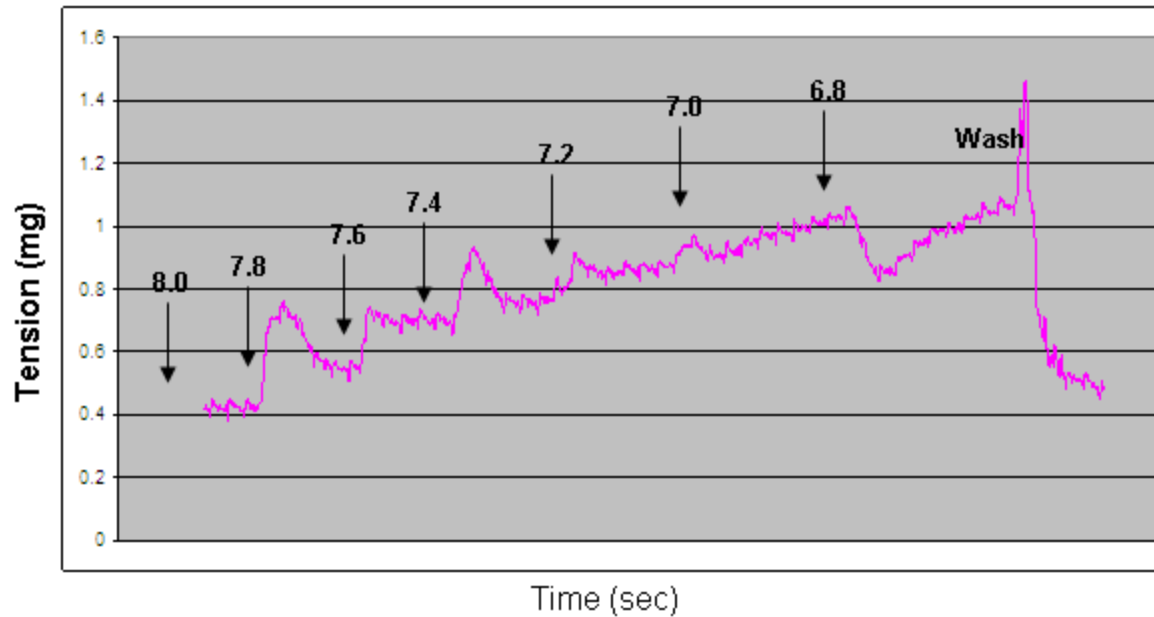
OGR1 knockdown reduces Ca^{2+} mobilization by $\downarrow\text{pH}_o$



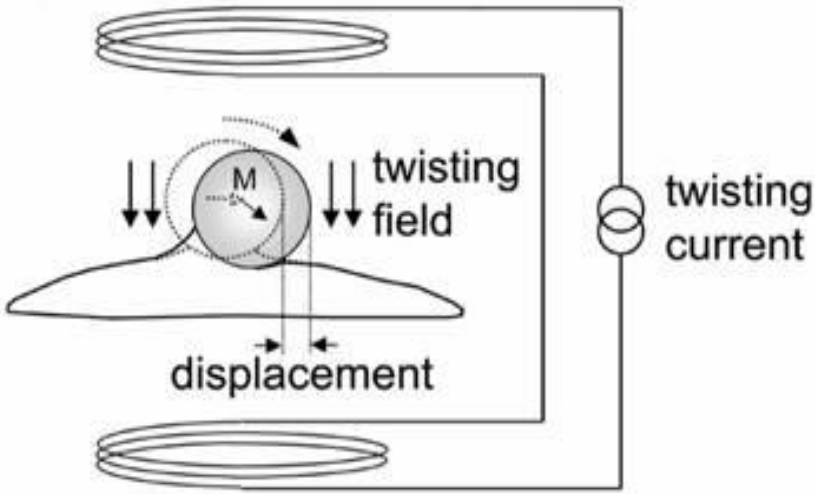
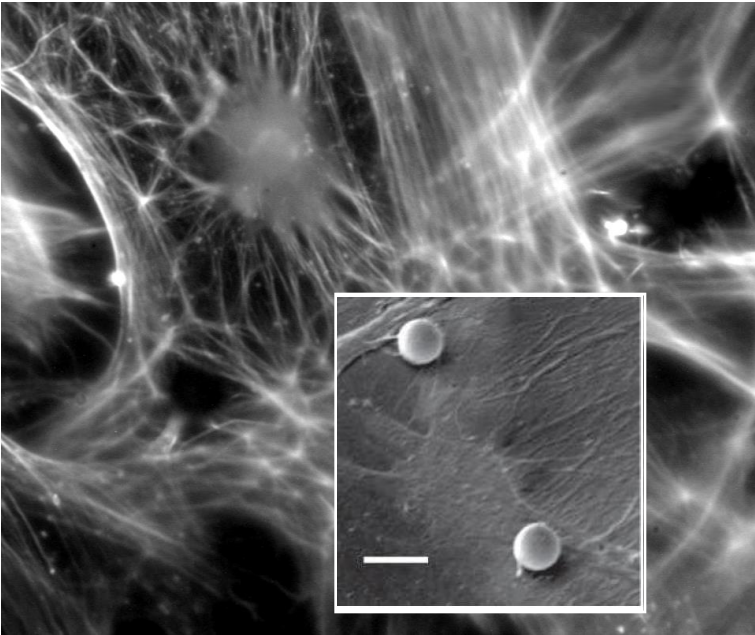
Signaling looks good.

What about function?

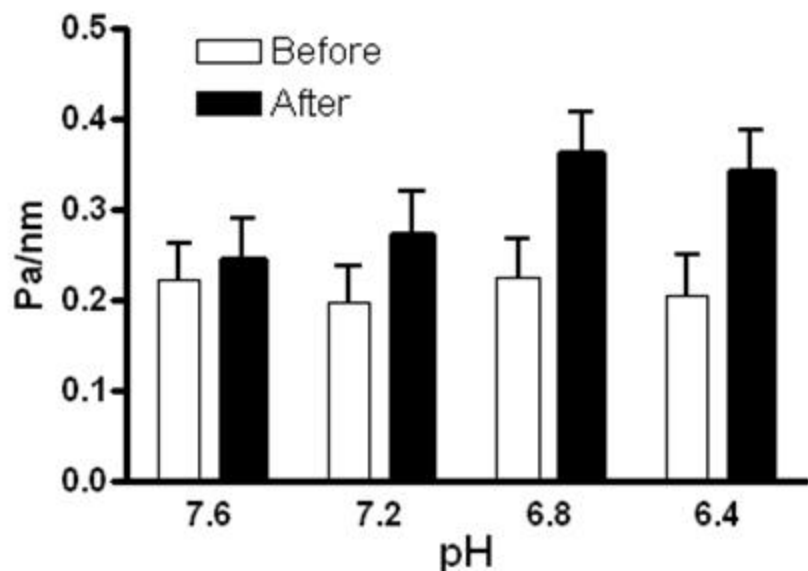
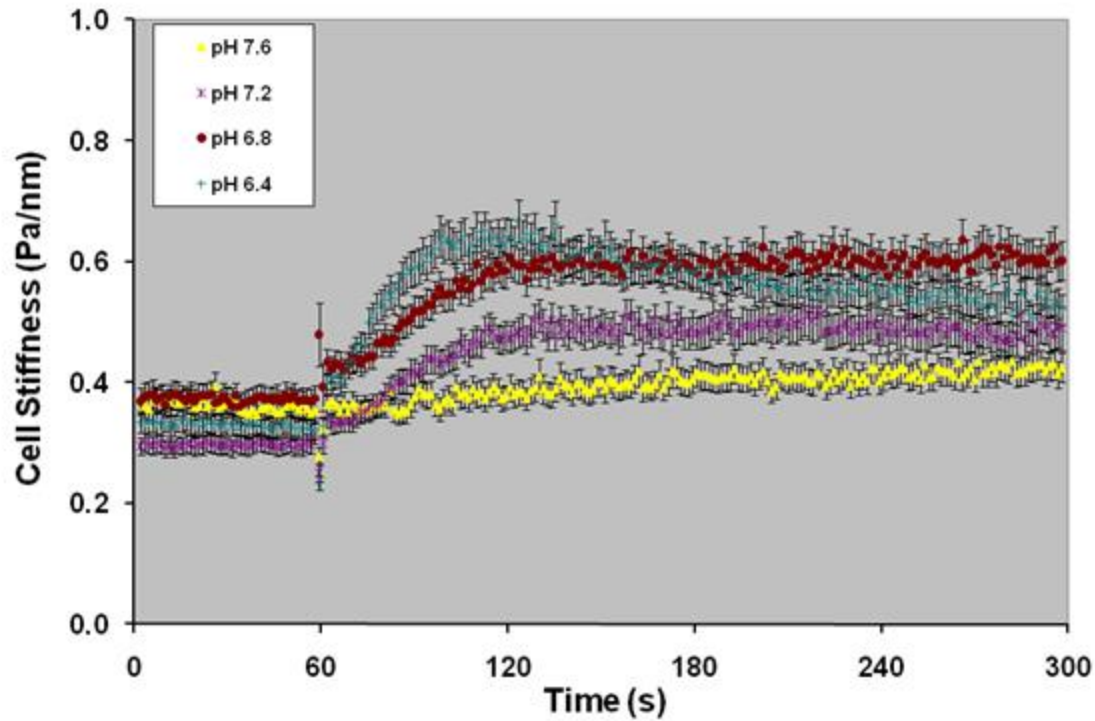
ASM tissue contracts in a pH dose-dependent manner



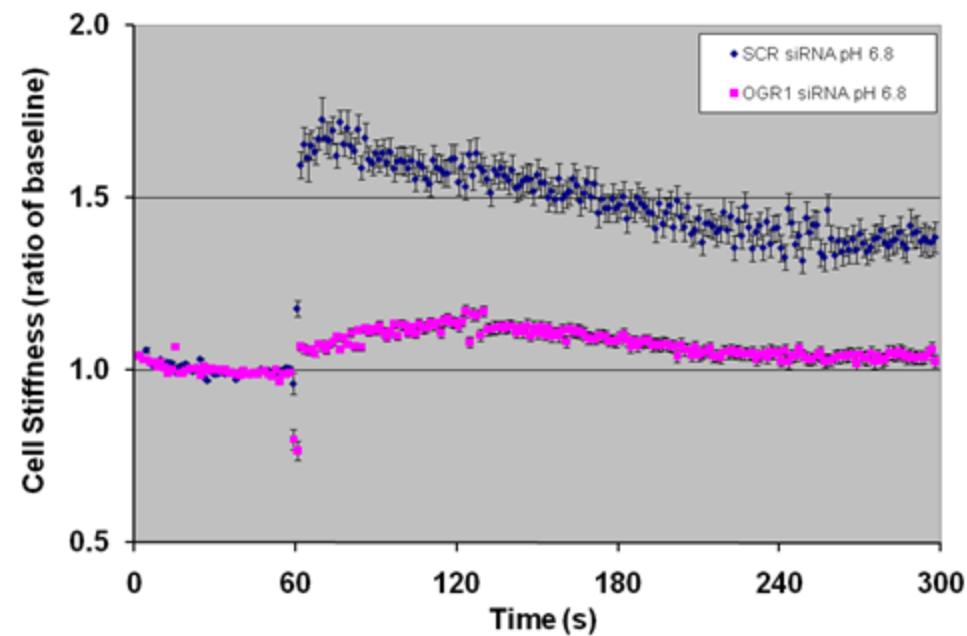
ASM cell contraction: Magnetic Twisting Cytometry (MTC)



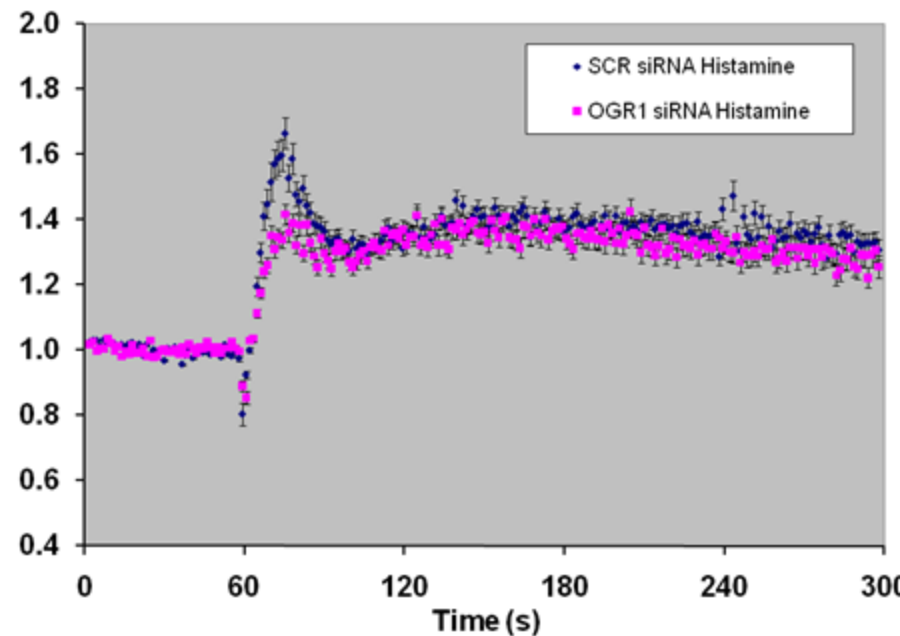
Cells contract, too



...and OGR1 knockdown inhibits this contraction

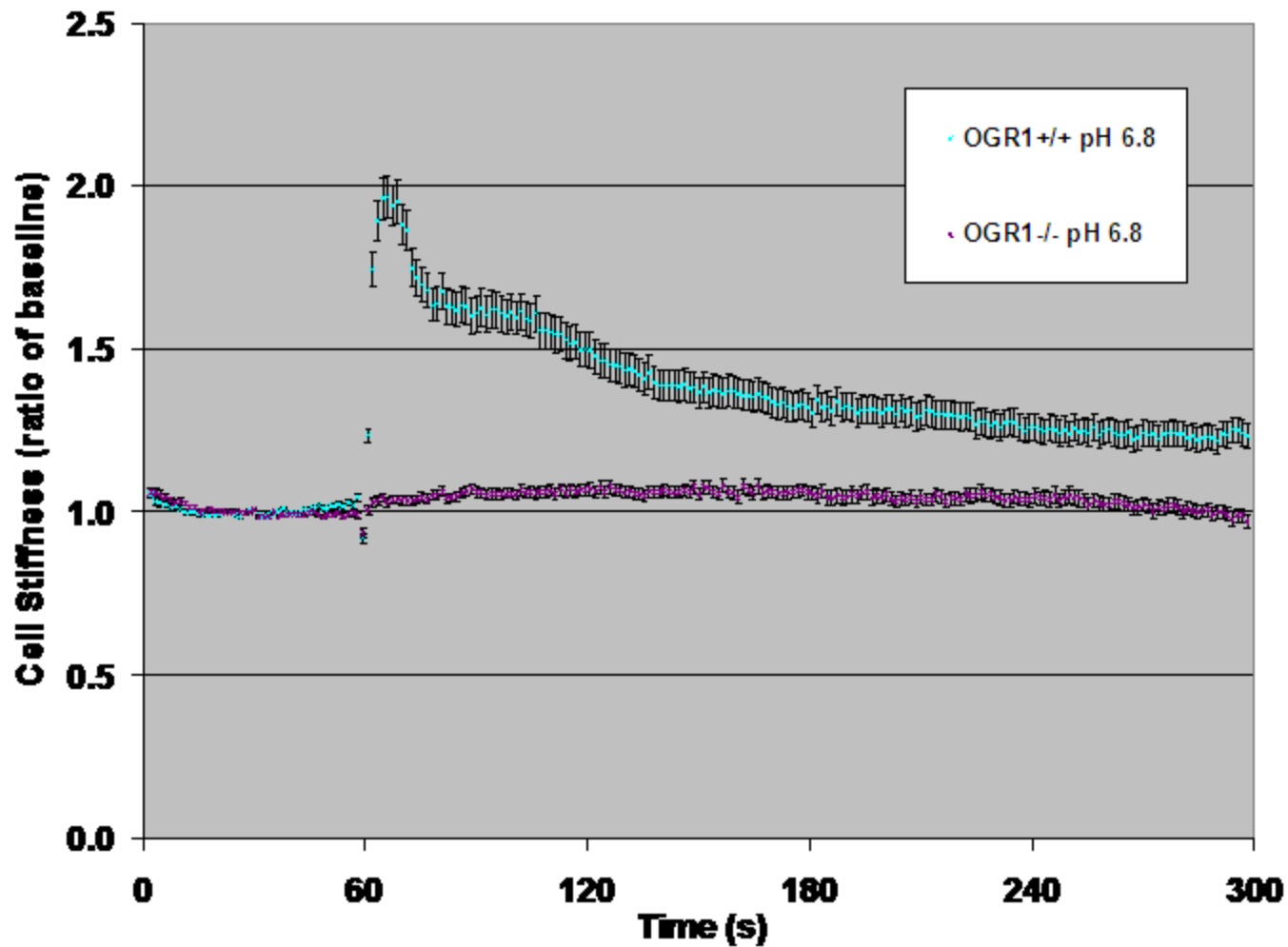


pH 6.8



Histamine

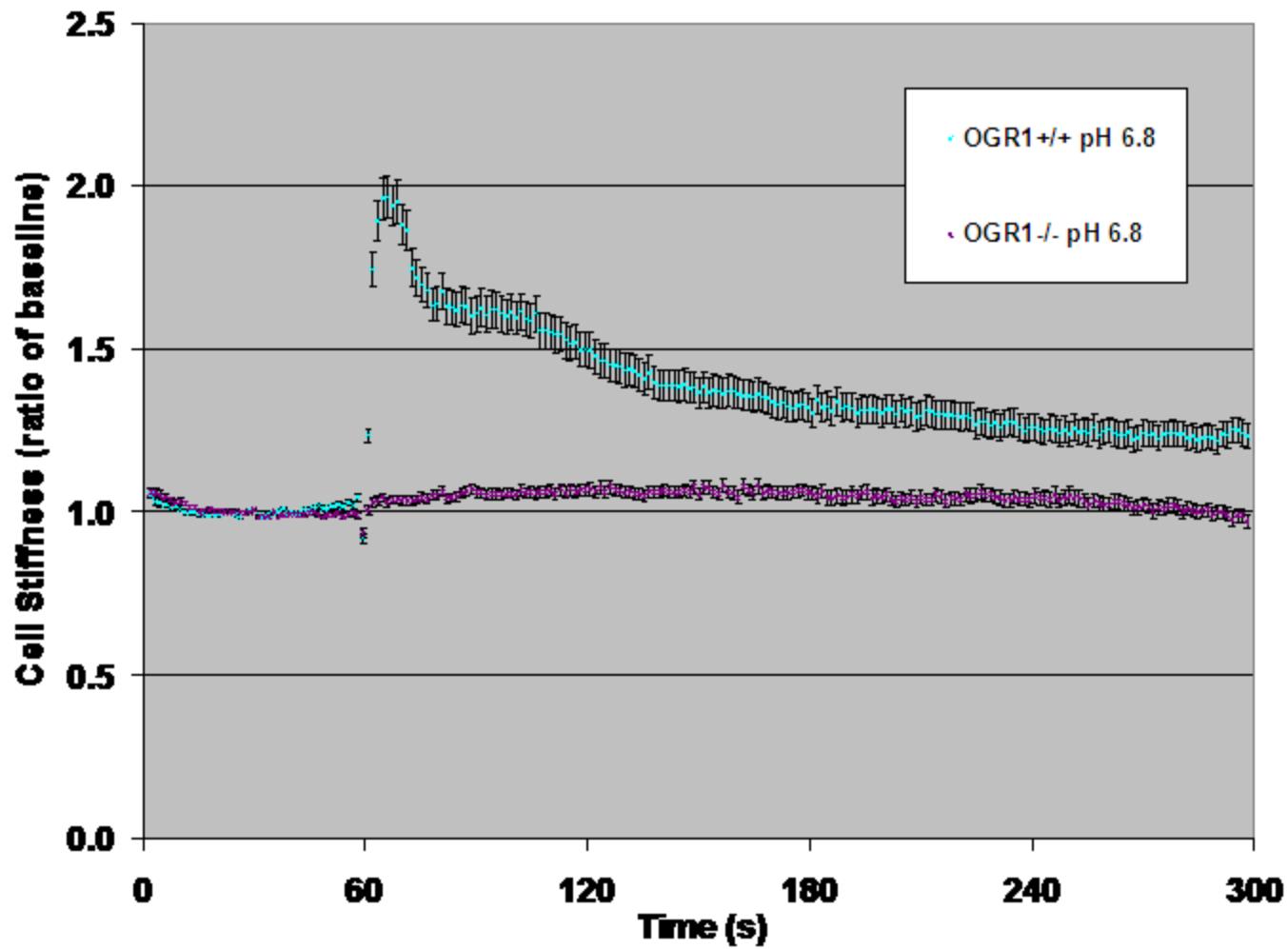
...and in the obligatory mouse experiment



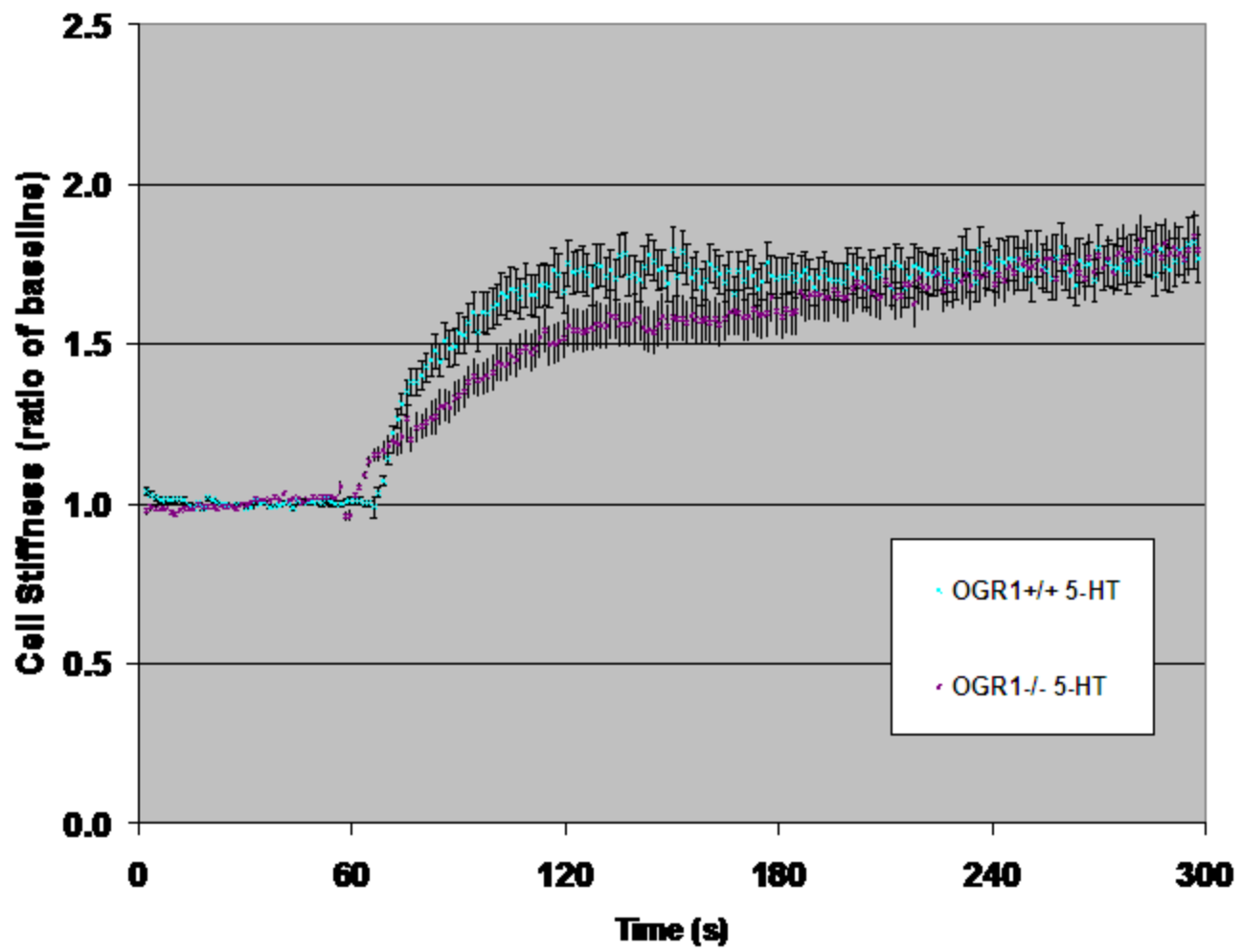
pH 8.0→6.8



...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

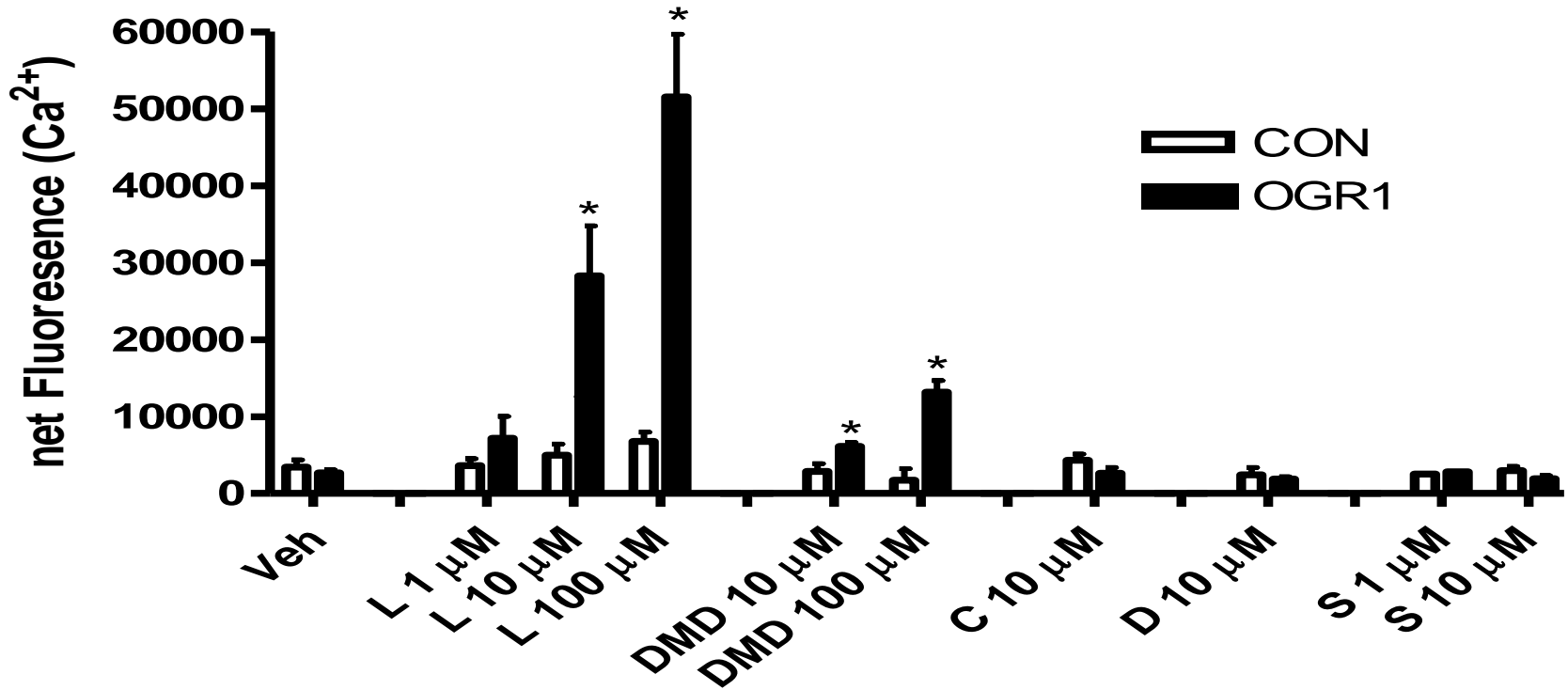
- OGR1 is the predominant proton sensitive receptor in the ASM cells
- OGR1 knockdown in human ASM inhibits acid-induced PKA and Ca²⁺ mobilization
- OGR1 knockdown in human ASM inhibits acid-induced contraction
- OGR1 knockout in murine ASM inhibits acid-induced contraction

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

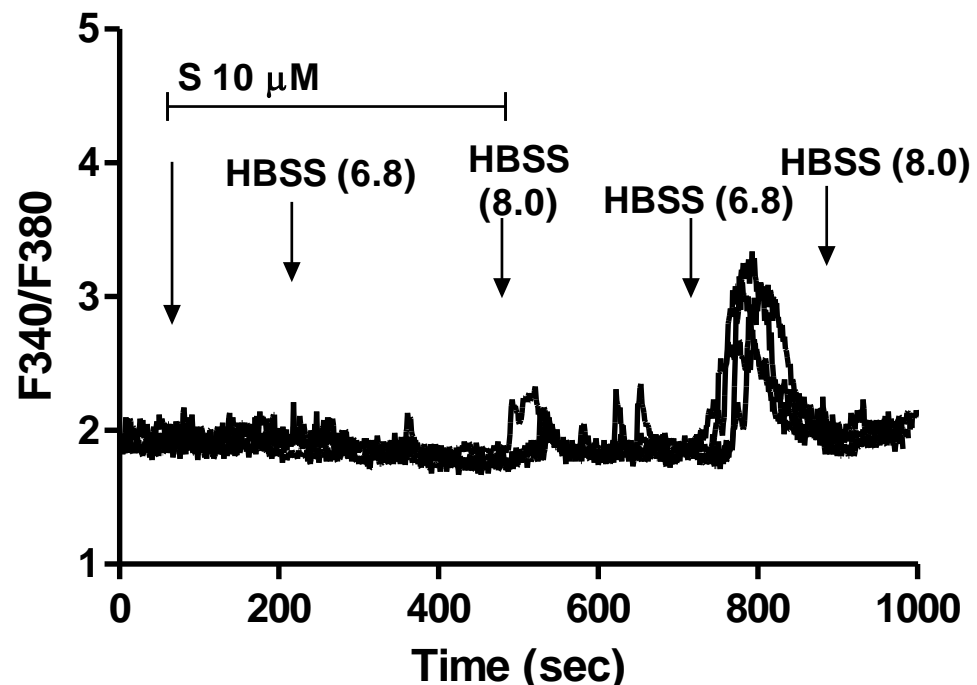
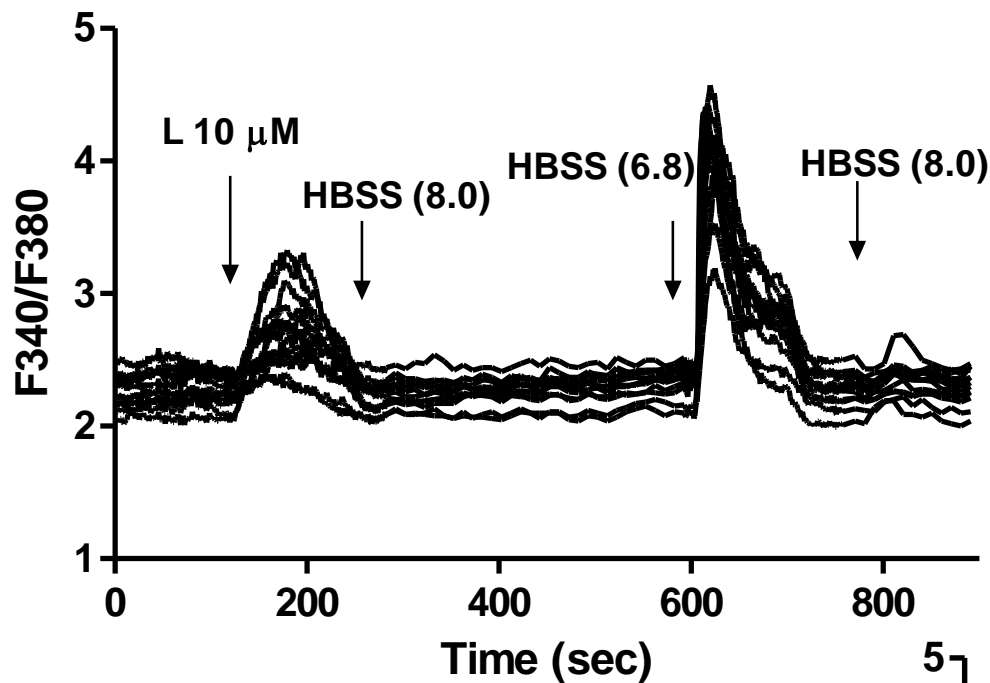
- Control pHo or pHi
- Block, activate, or bias GPCR signaling:
 - a. Downstream signaling- usual suspect pathway inhibitors, COX inhibitors, antagonists of induced GPCR ligands, tyrphostins.
 - b. At the receptor level for proton-sensing GPCRs; this quite hard given no ligands!

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

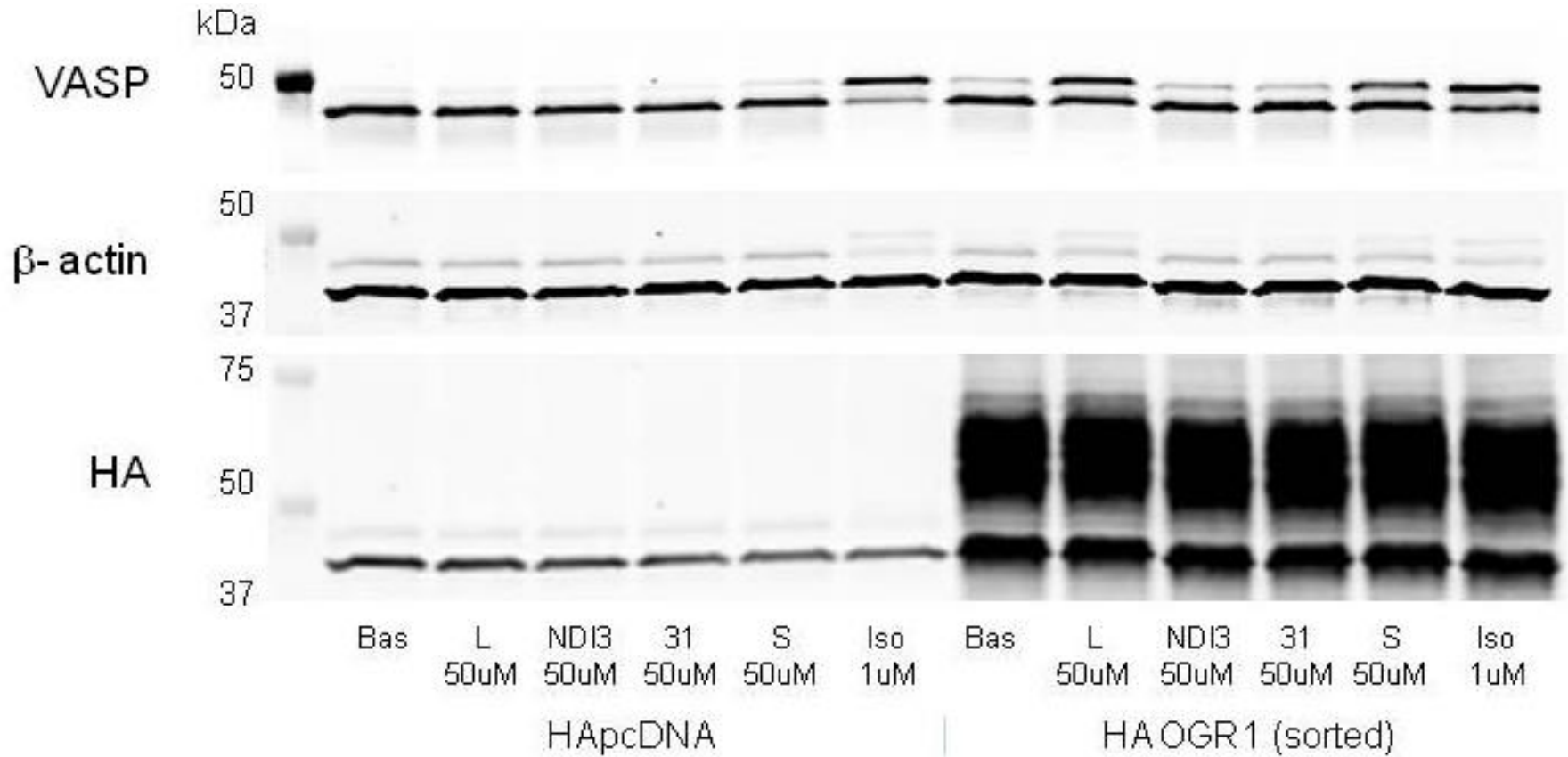
**A subclass of (can't tell you which because confidential!)
regulate OGR1-mediated Ca^{2+} mobilization.**



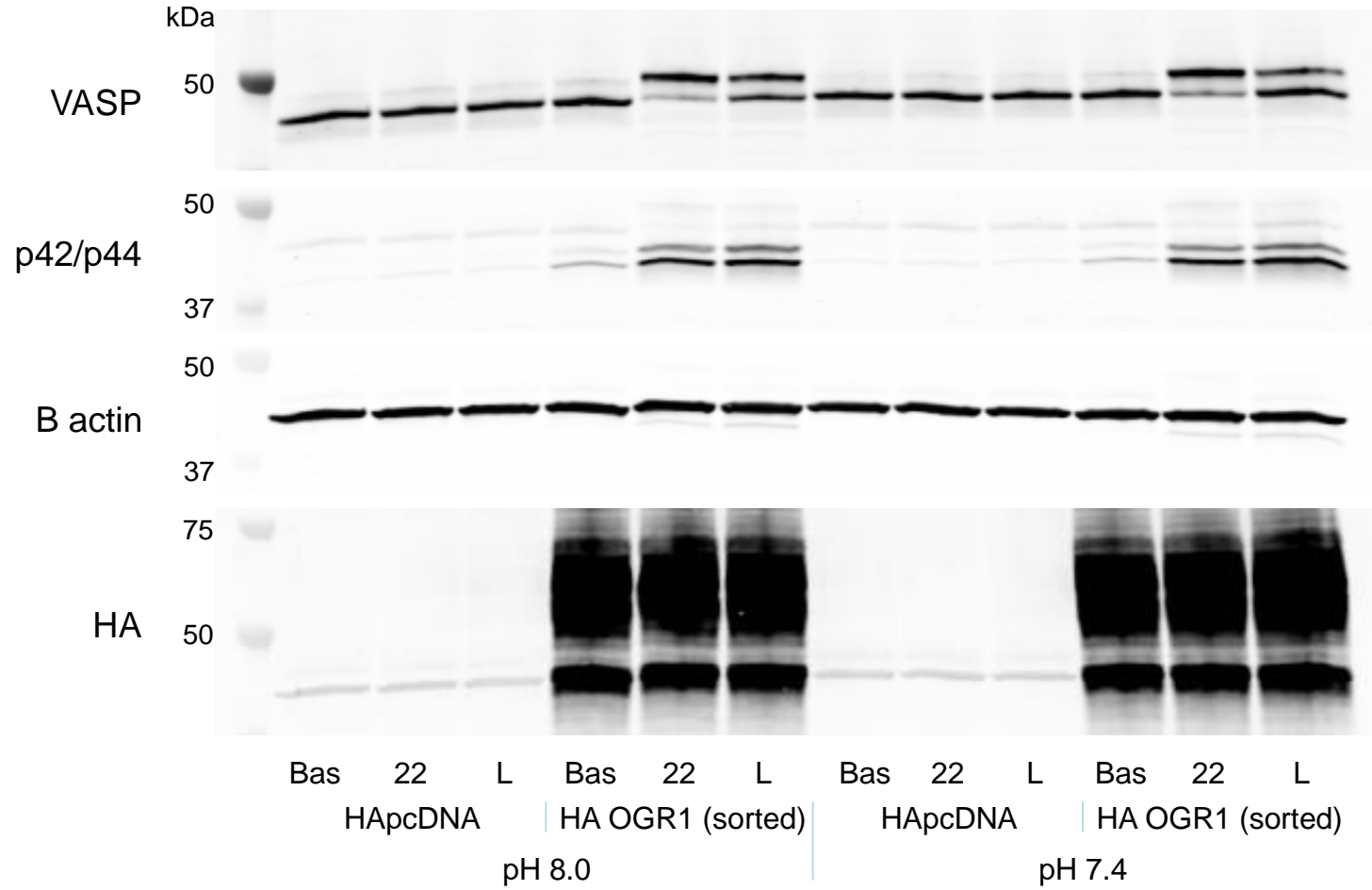
Some ligands appear balanced (L), some biased (S)



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)