H_2S and systemic inflammation

Andreas Papapetropoulos BPharm, PhD, FAHA, FBPhS

Faculty of Pharmacy

National and Kapodistrian University of Athens, Greece

BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

Outline

1. Introduction
2. Production and degradation
3. Signaling, target and physiological functions
4. Agents regulating H_2S levels
5. H_2S, bacteria and antibiotics
6. H_2S and infection

Gasotransmitter Definition

Gasotransmitter is a gaseous messanger molecule involved in signaling processes

To be a gasotransmmiter, a molecule should:

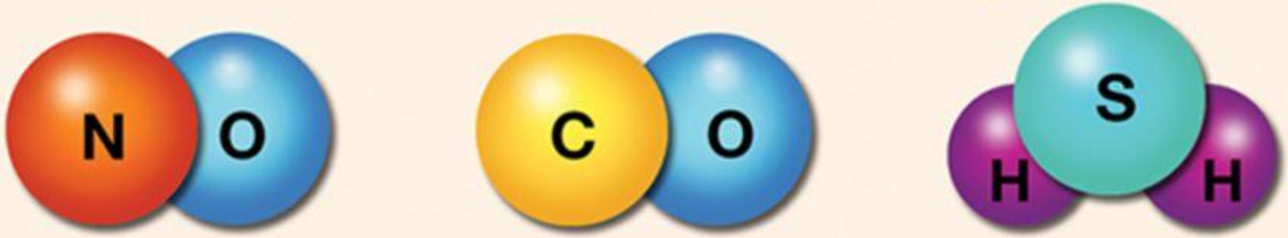
- Be a small molecules of gas
- Be freely permeable to membranes. Its effects do not rely on cognate membrane receptors
- Be endogenously and enzymatically generated; the generation is regulated
- Have well-defined specific functions at physiologically relevant concentrations; functions of this endogenous gas can be mimicked by its exogenously applied counterpart
- Its cellular effects may or may not be mediated by second messengers, but the molecule should have specific cellular and molecular targets

Wang R, FASEB J, 16:1792-1798, 2002

BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

Gasotransmitters

ENDOGENOUS GASOTRANSMITTERS



	Nitric Oxide	Carbon Monoxide	Hydrogen Sulfide
Enzymatic Production	nNOS iNOS eNOS	HO-1	CBS CSE (CGL) 3MST
Blood Concentration	low nM	nM-μM	high nM – low μM
Half-life (*in vivo*)	seconds	minutes	seconds – minutes
Year of Discovery as a Physiological Modulator	1987	1991	1996

Polhemus & Lefer, Circ Res 114:730–737, 2014

BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

Comparison of gasotransmitter properties

	NO	CO	H_2S
Biological sources	• NO synthases • Non-enzymatic processes (for example, via conversion from nitrite) • Conversion from nitrite by several bacteria (for instance, in the oral cavity)	• Haem oxygenases	• Produced in mammalian cells from L-cysteine by at least three distinct enzymes • Produced from D-cysteine in certain tissues (for example, the kidneys) • Non-enzymatic processes • Produced by enteral bacterial flora (for example, in the oral cavity and intestines)
Chemical properties	• A diffusible and labile free-radical gas	• A diffusible and labile gas	• A diffusible and labile gas
Biological half-life	• Short (a few seconds)	• Long (minutes)	• Medium (seconds to minutes)
Elimination	• Mainly via the urine as nitrite and nitrate • A small amount is exhaled	• Mainly unaltered, in the exhaled air	• Via the urine as sulfite, sulfate and thiosulfate • A small amount is exhaled
Key biological reactions	• Reacts with haem iron centres in various proteins • Reacts with protein cysteines to initiate S-nitrosylation. • Has multiple reactions with oxygen free radicals (for example, with superoxide, to yield peroxynitrite) • Reacts with haemoglobin to yield nitrosyl-haemoglobin and met-haemoglobin	• Binds to haem iron centres • Reacts with haemoglobin to yield carboxyhaemoglobin	• Binds to protein cysteines to initiate sulfhydration • Reacts with oxygen free radicals • Can form persulfides and polysulfides • Reacts with haemoglobin to yield sulfhaemoglobin
Selected signalling pathways	• Activates guanylyl cyclase to increase cGMP levels • Post-transcriptional protein modification via nitrosylation and reactions with haem groups • Activates (opens) K_{ATP} channels	• Reactions with haem groups • Activates guanylyl cyclase (less potently than NO), which then forms cGMP • Activates (opens) K_{Ca} channels	• Post-transcriptional protein modification via sulfhydration • Activates (opens) K_{ATP} channels • Inhibits cGMP and cAMP phosphodiesterases

Szabo, Nat Rev Drug Discov. 15:185-203, 2016

BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

H_2S toxicity

BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

H_2S toxicity

0.00047 ppm recognition threshold

< 10 ppm, exposure limit 8hr/d

50–100 ppm eye damage

100–150 ppm olfactory nerve paralysis

320–530 ppm leads to pulmonary edema

530–1000 ppm CNS stimulation, loss of breathing

800 ppm LC_{50} for 5min exposure

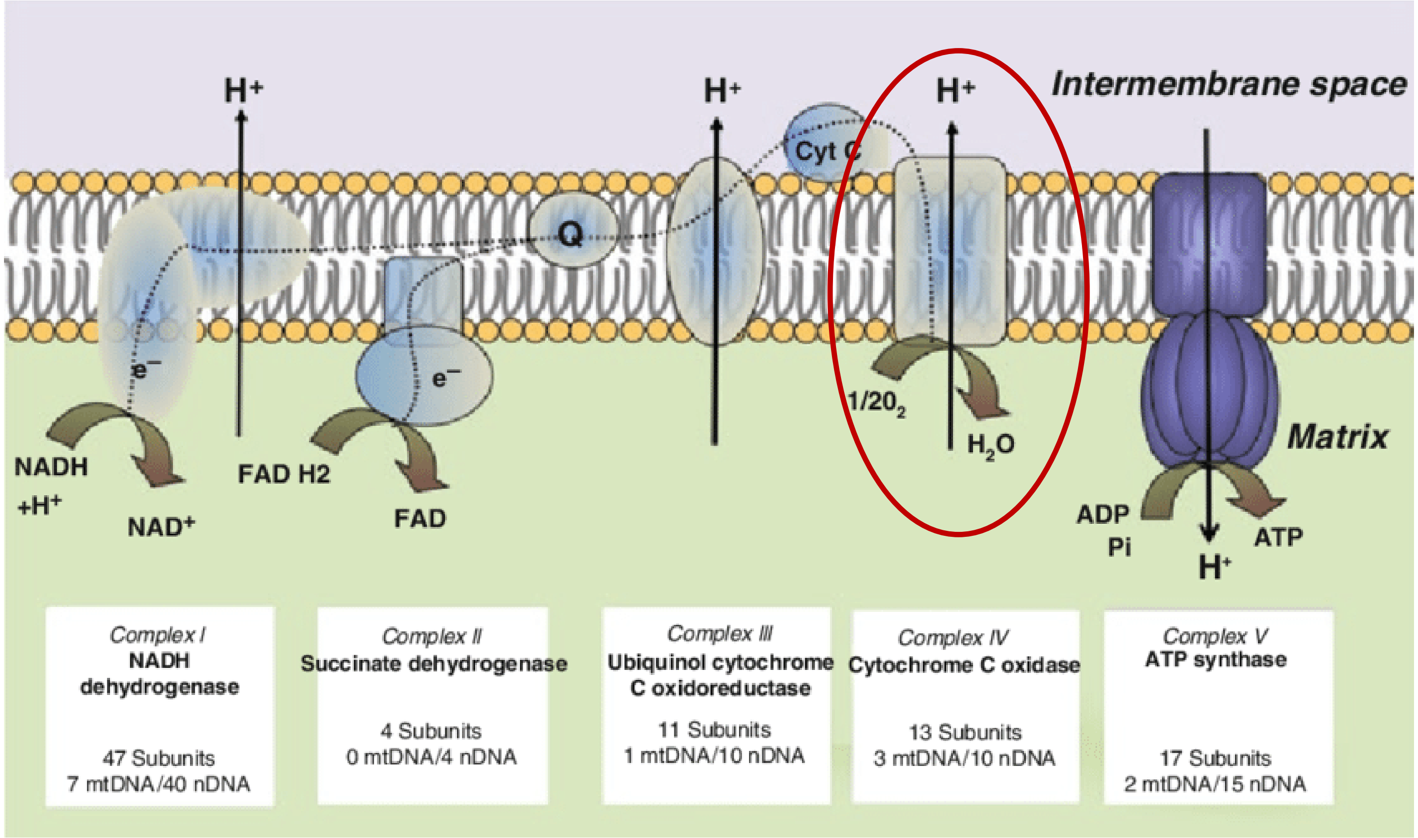
> 1000 ppm immediate collapse with loss of breathing

Dangerous Japanese 'Detergent Suicide' Technique Creeps Into U.S.

By Kevin Poulsen March 13, 2009 | 1:55 pm | Categories: Threats

BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

Gasotransmitters target cytochrome C oxidase

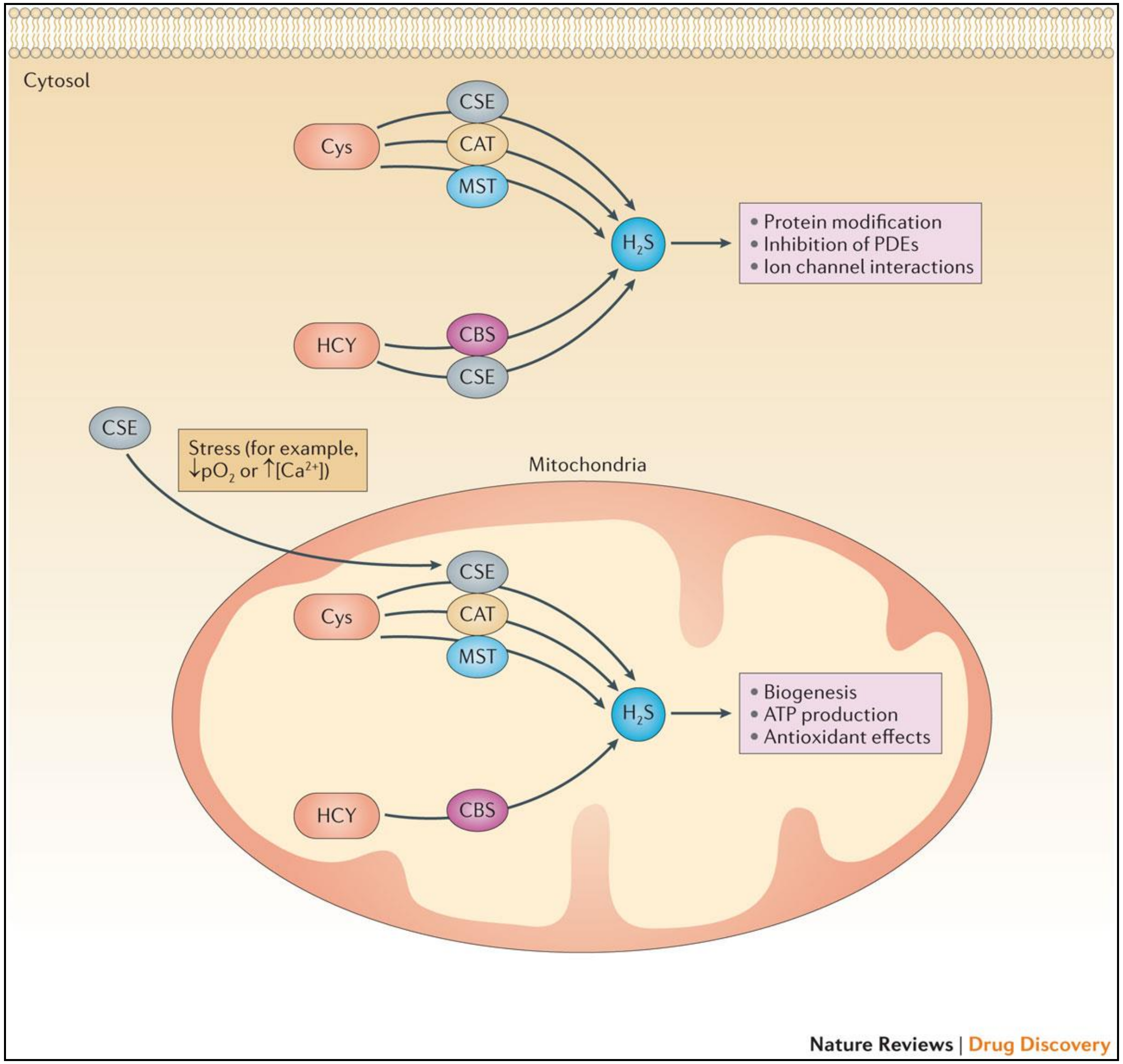


BIOMEDICAL RESEARCH FOUNDATION ACADEMY OF ATHENS

Outline

1. Introduction
2. **Production and degradation**
3. Signaling, target and physiological functions
4. Agents regulating H_2S levels
5. H_2S, bacteria and antibiotics
6. H_2S and infection

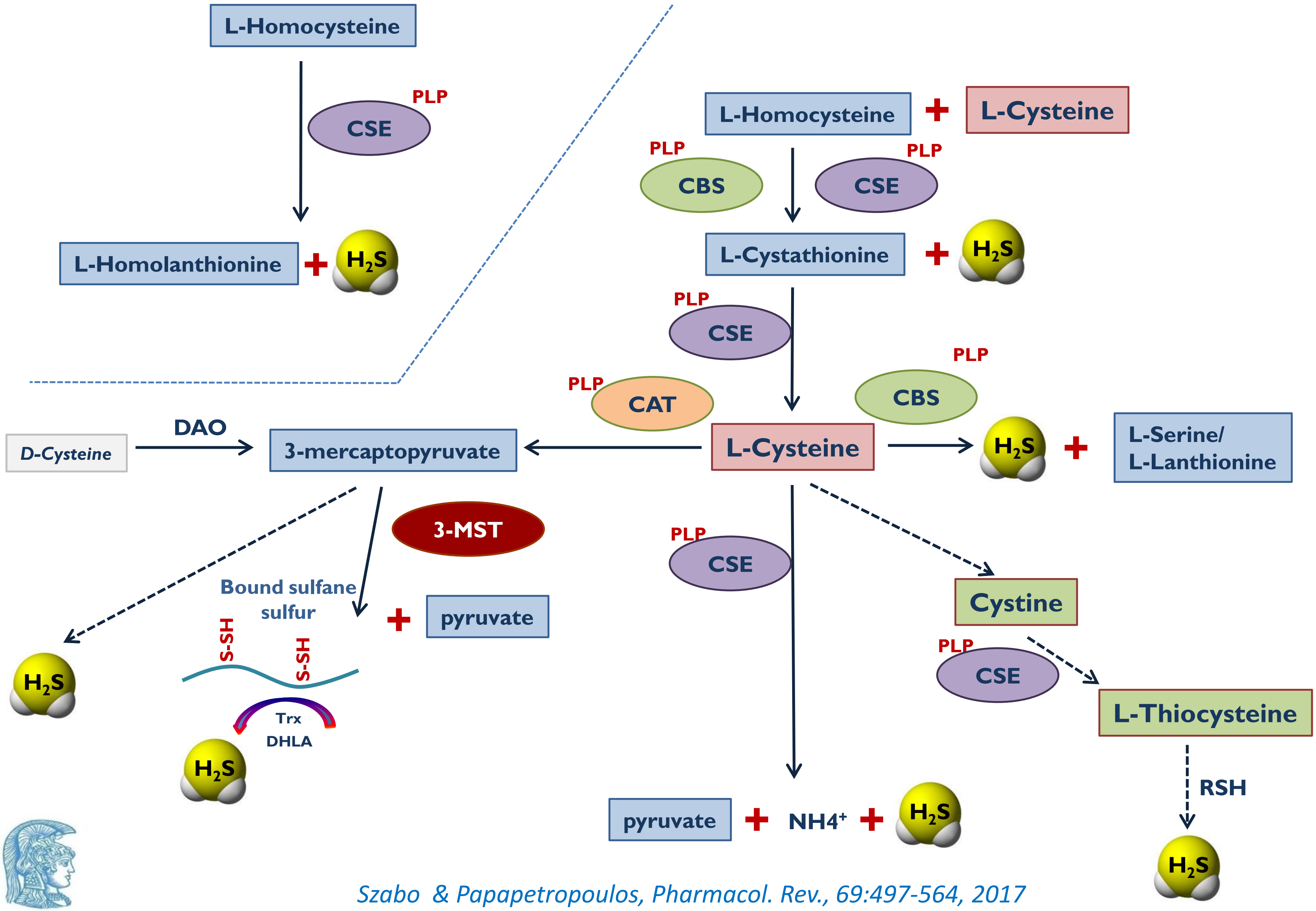
H_2S biosynthesis



Wang & Wallace, Nat. Rev. Drug Discov, 14:329-45, 2015

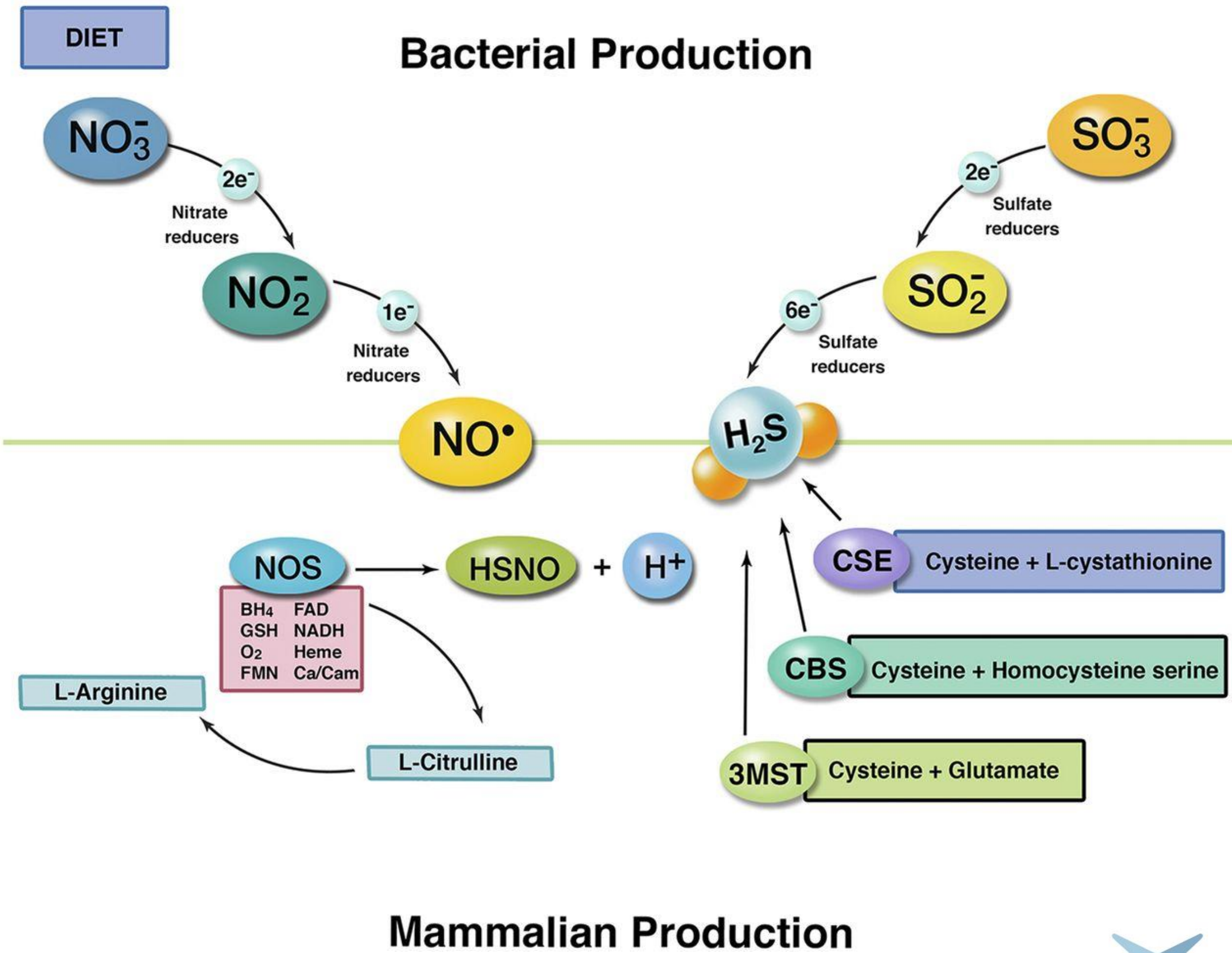
BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

H_2S synthesis

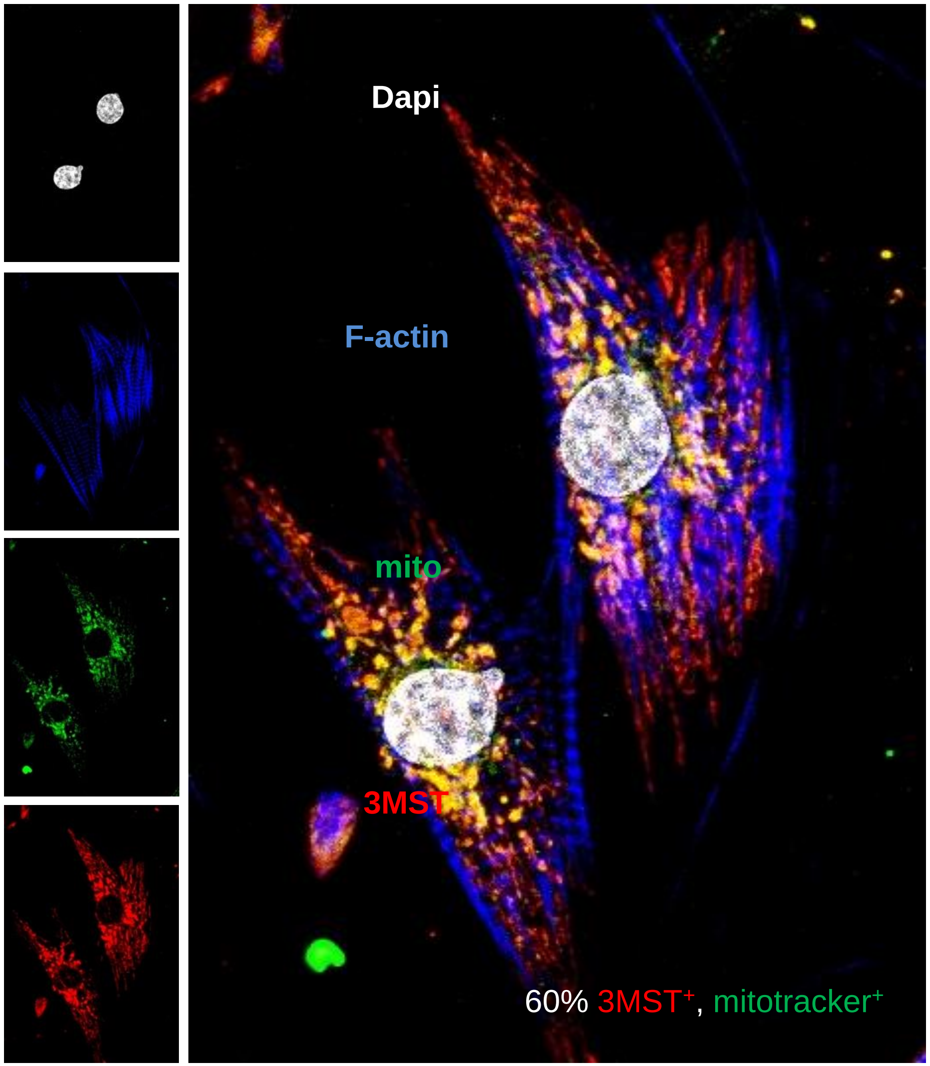


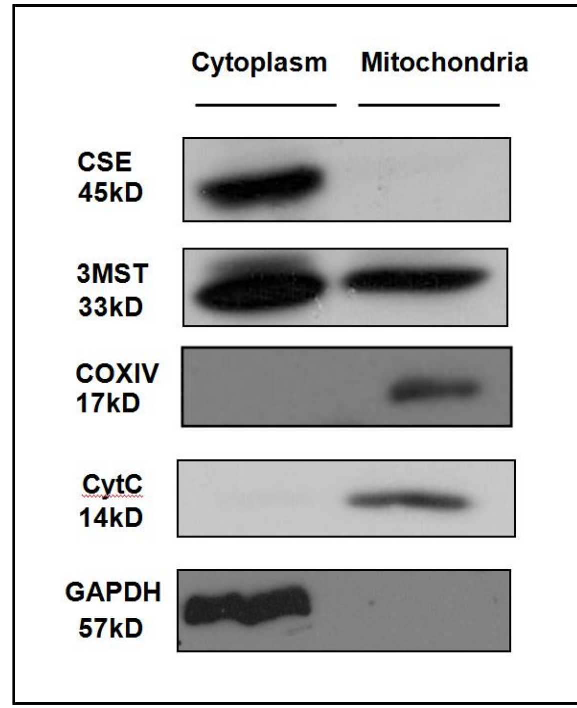
Szabo & Papapetropoulos, Pharmacol. Rev., 69:497-564, 2017

H_2S synthesis in bacteria



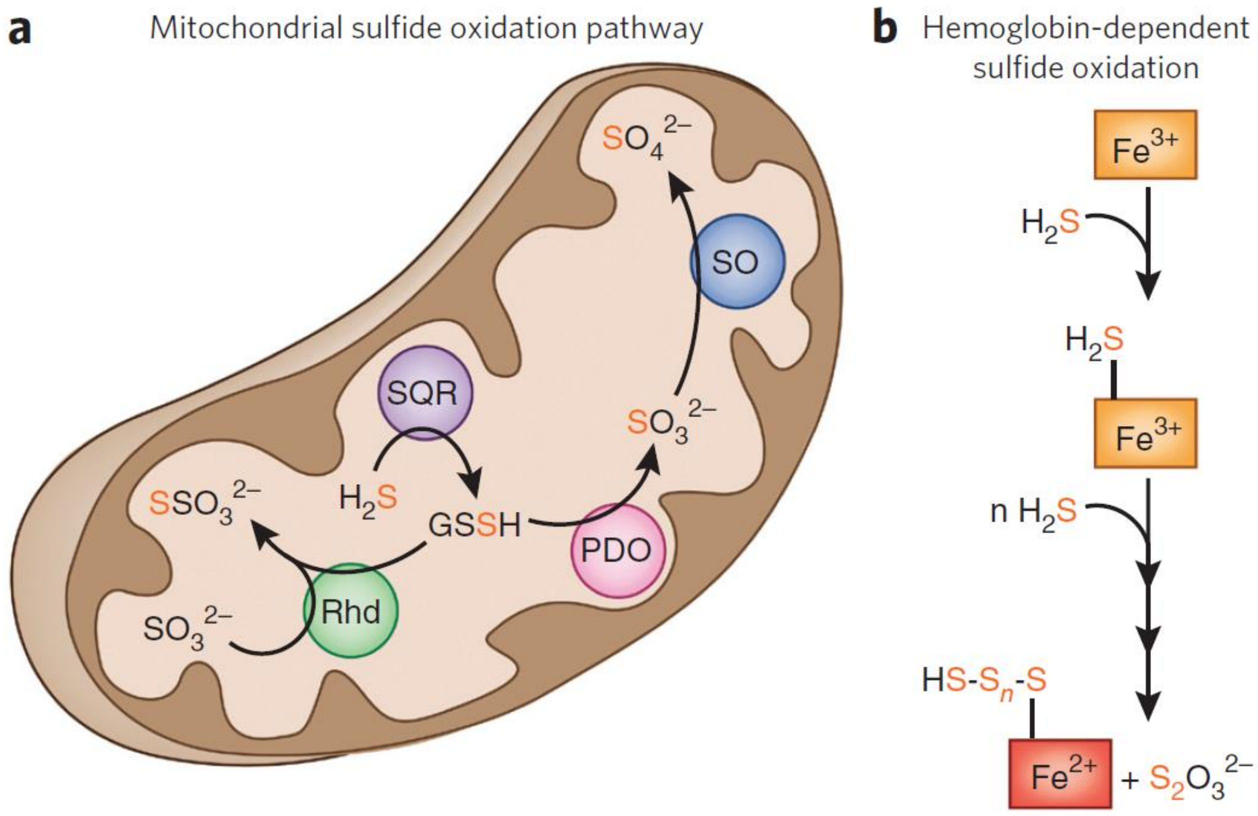
H_2S-synthesizing enzyme localization





Peleli et al, Biochem Pharm, 176:113833, 2020

H_2S degradation

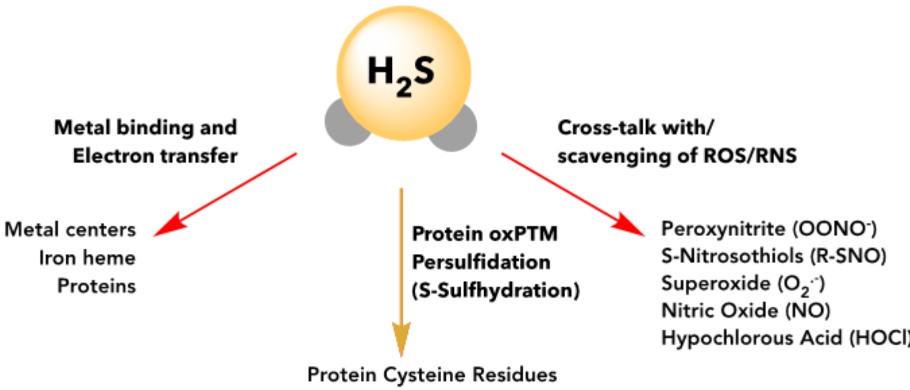


Mishanina et al., Nature Chem Biol, 11:457-464, 2015

Outline

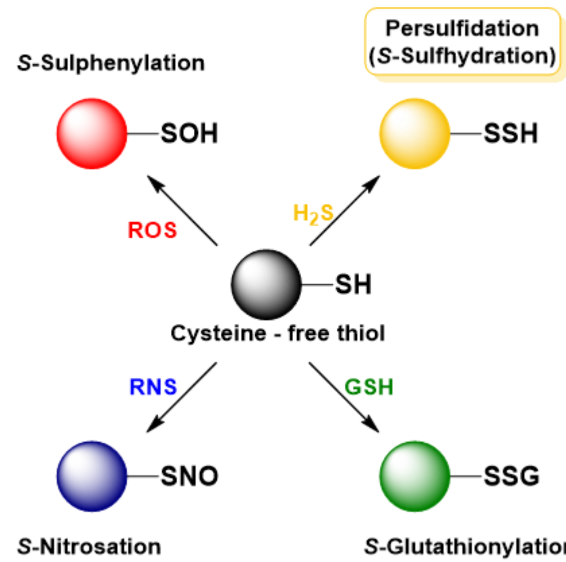
1. Introduction
2. Production and degradation
3. **Signaling, target and physiological functions**
4. Agents regulating H_2S levels
5. H_2S and bacteria
6. H_2S and infection

H_2S signaling



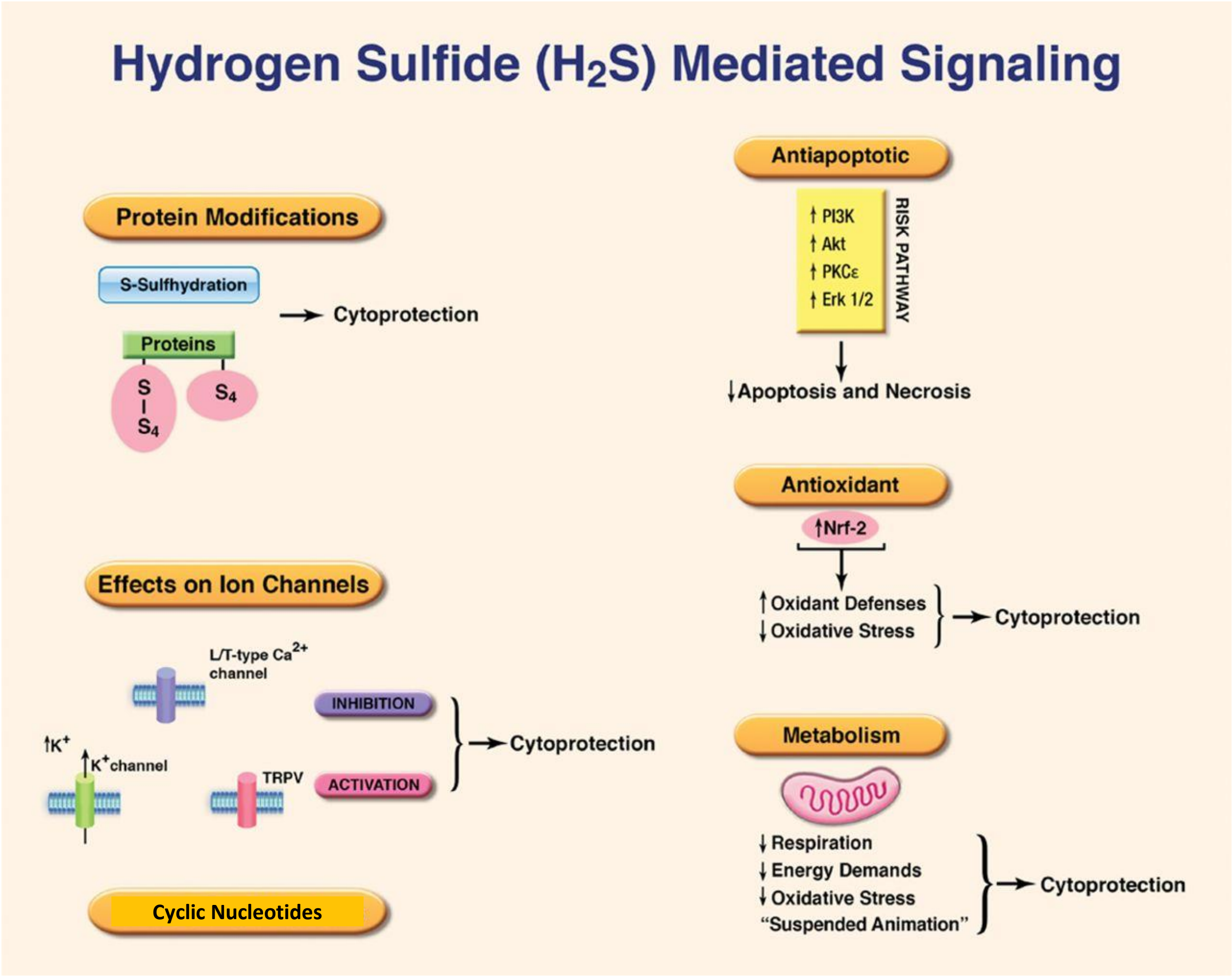
Persulfidation

A means of explaining biological effects of H_2S through the modulation of cysteine residues?



Oxidative Posttranslational modifications (oxPTM) of Cysteines

Examples of biological targets of H_2S

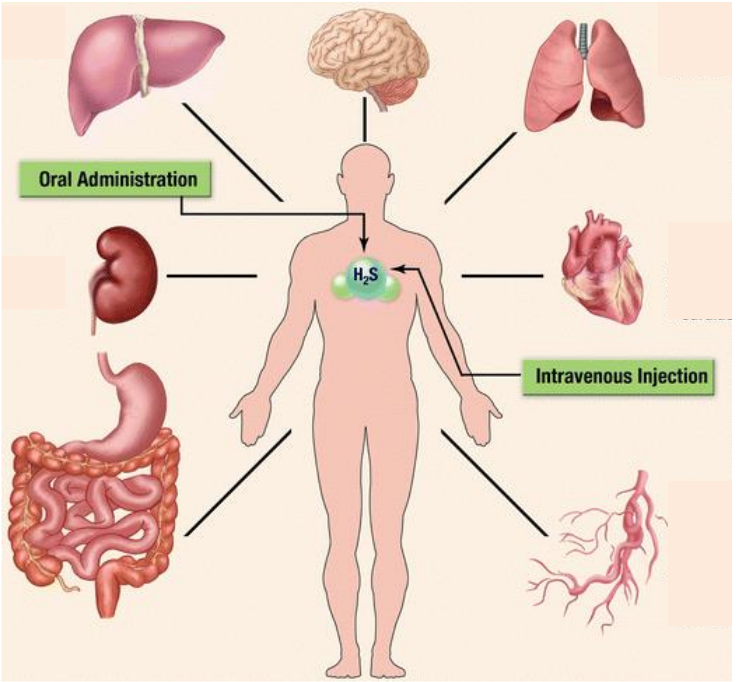


Adapted from Polhemus D J , and Lefer D J Circ Res 114:730-737, 2014

BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

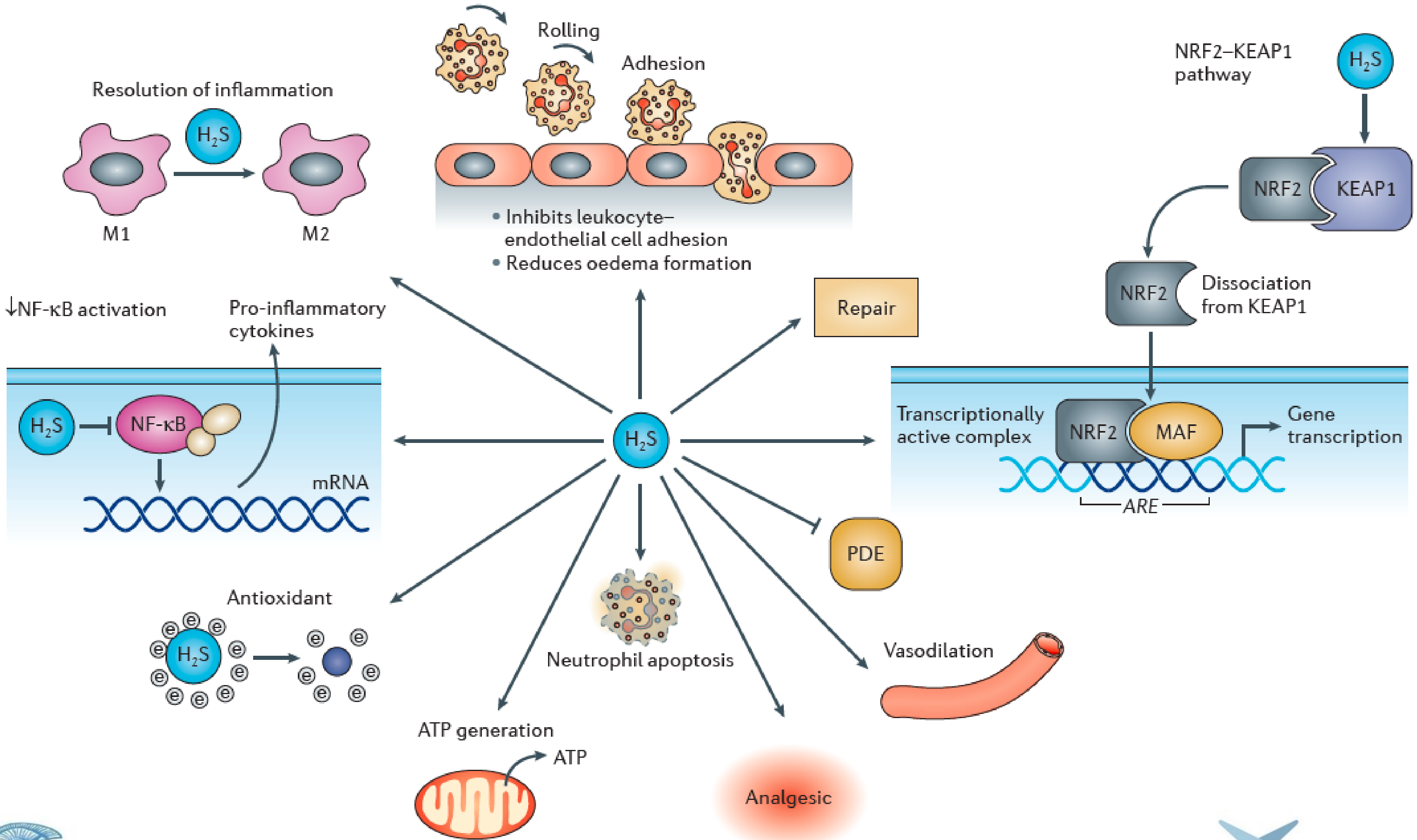
Physiological effects of H_2S

- Antioxidant
- Anti-apoptotic
- Angiogenesis stimulator
- Bronchodilation
- Cardioprotective
- Glucose and lipid homeostasis
- Inhibits atherosclerosis
- Inhibits fibrosis
- Inhibits inflammation
- Promotes physiological calcification (bone)
- Smooth muscle relaxation
- Vasorelaxation



BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

Hydrogen Sulfide is an Anti-Inflammatory Molecule

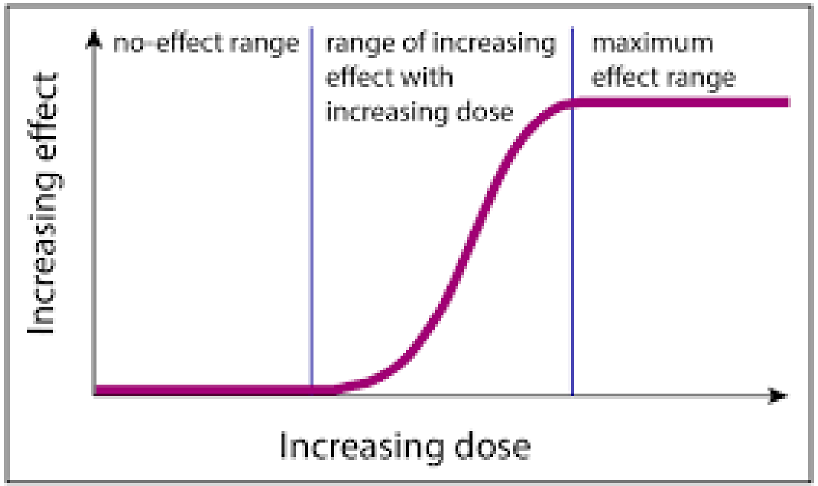


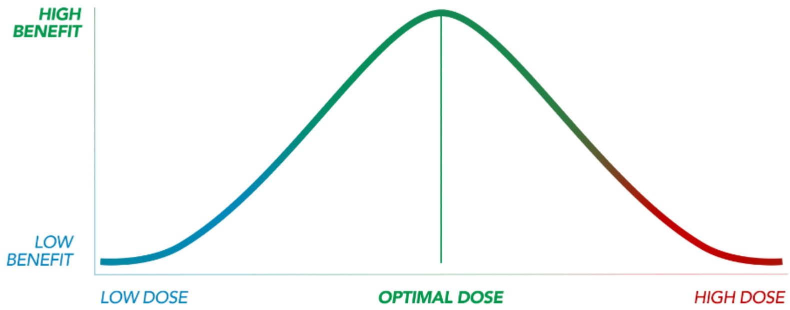
Wang & Wallace, Nat Rev Drug Discov, 14:329-45, 2105

Outline

1. Introduction
2. Production and degradation
3. Signaling, target and physiological functions
4. **Agents regulating H_2S levels**
5. H_2S, bacteria and antibiotics
6. H_2S and infection

Concentration-response curves of gasotransmitters





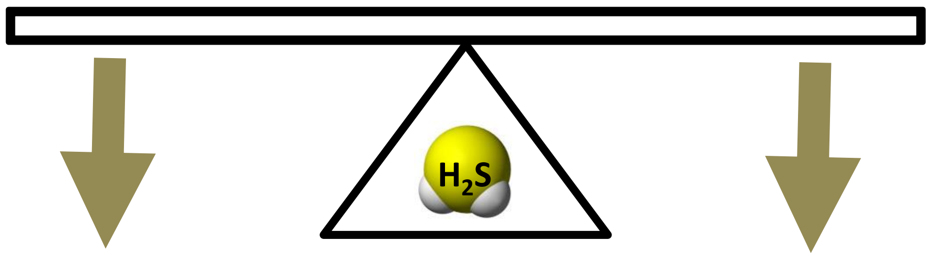
BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

"H_2S-poor" state

Aging
Ischemia
Heart failure
Liver disease
Hypertension
Atherosclerosis
Obesity
Endothelial dysfunction
Diabetic complications
(cardiovascular system)
Preeclampsia
Alzheimer's disease
Huntington's disease

Circulatory shock
Burns
Cancer
Sleep apnea
Down Syndrome
Diabetes onset
(beta cells)
Stroke
Schizophrenia

"H_2S-rich" state



Increased H_2S consumption
and/or
Downregulation of H_2S producing enzymes

<u>H_2S donors</u>

Upregulation of H_2S producing enzymes

<u>CBS/CSE inhibitors</u>

Szabo & Papapetropoulos, Pharmacol Rev. 69:497-564, 2017

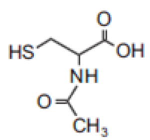
BIOMEDICAL RESEARCH FOUNDATION ACADEMY OF ATHENS

Properties & Differences of H_2S donors

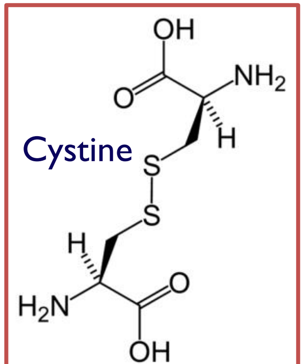
- Source (naturally occurring, synthetic)
- Sulfide salts (Na_2S, NaHS)-not really donors
- Mode of H_2S release: spontaneous vs controlled (Cys-activated, ROS-activated, pH-activated, esterase-activated)
- Rate of release
- Targeted delivery (AP39)
- Hybrid or bi-functional donors (ATB-346, adenine-H_2S, many others)
- Clinically used (NAC, cystine, zofenopril)

BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

H_2S donors/precursors approved for human use

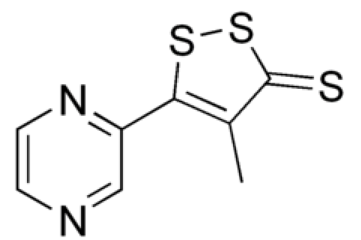


N-acetylcysteine



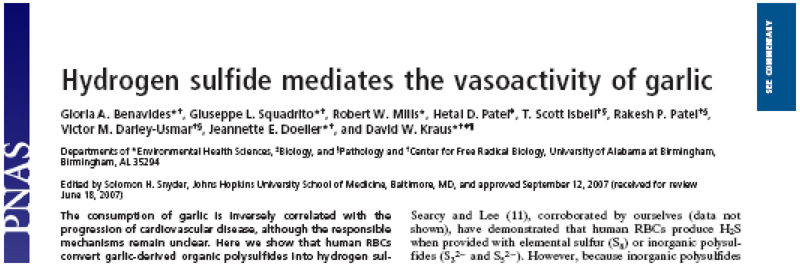
Cystine

thiosulfate
$S_2O_3^{2-}$



oltipraz

Beneficial effects of H_2S in traditional medicine



PNAS

SEE COMMENTARY

Hydrogen sulfide mediates the vasoactivity of garlic

Gloria A. Benavides*†, Giuseppe L. Squadrito*†, Robert W. Mills*, Hetal D. Patel‡, T. Scott Isbell†§, Rakesh P. Patel†§, Victor M. Darley-Usmar†§, Jeannette E. Doeller*†, and David W. Kraus*†‡¶

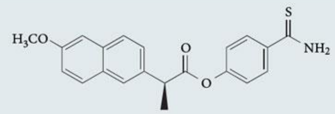
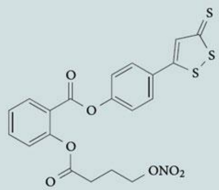
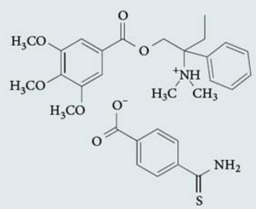
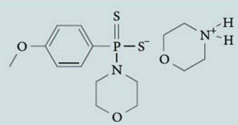
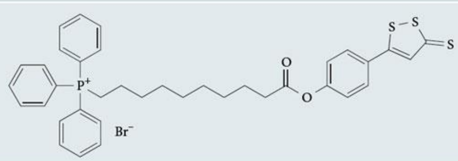
Departments of *Environmental Health Sciences, ‡Biology, and §Pathology and †Center for Free Radical Biology, University of Alabama at Birmingham, Birmingham, AL 35294

Edited by Solomon H. Snyder, Johns Hopkins University School of Medicine, Baltimore, MD, and approved September 12, 2007 (received for review June 18, 2007)

The consumption of garlic is inversely correlated with the progression of cardiovascular disease, although the responsible mechanisms remain unclear. Here we show that human RBCs convert garlic-derived organic polysulfides into hydrogen sul-

Searcy and Lee (11), corroborated by ourselves (data not shown), have demonstrated that human RBCs produce H_2S when provided with elemental sulfur (S_8) or inorganic polysulfides (S_3^{2-} and S_5^{2-}). However, because inorganic polysulfides

Translation of H_2S donors

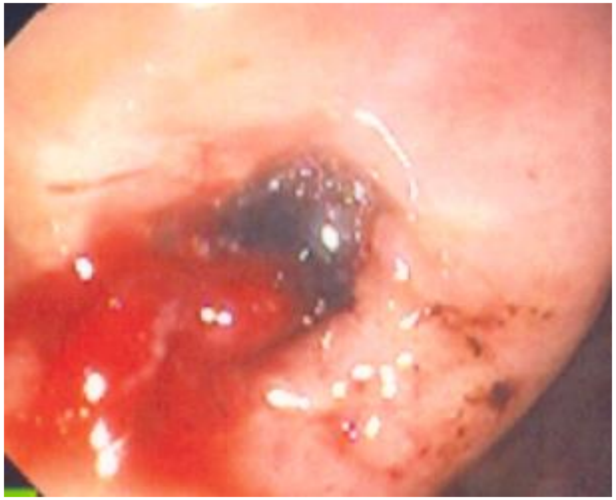
Institution (location)	Structure	Clinical indications	Lead drug	Comment	Stage of development
Antibe Therapeutics (Toronto, Ontario, Canada)	 **ATB-346**	Osteoarthritis	ATB-346	Naproxen derivative	Phase I
		Acute pain	ATB-352	Ketoprofen derivative	Preclinical
		Veterinary (pain)	ATB-338	Diclofenac derivative	Preclinical
		Thrombosis	ATB-350	Aspirin derivative	Preclinical
City University of New York (New York, USA)		Cancer	NBS-1120	Aspirin derivative	Preclinical
GIcare Pharma (Montreal, Quebec, Canada)		Colonic pain	GIC-1001	Trimebutine salt; licensed from Antibe Therapeutics	Phase II for analgesia during colonoscopy*
National University of Singapore (Singapore)		Hypertension, inflammation, cancer	GYY4137	Slow-releasing H_2S donor	Unknown
Sova Pharmaceuticals (La Jolla, California, USA	No structure available	Pain, metabolic disorders	Unknown	Inhibitor of CSE activity	Unknown
SulfaGENIX (New Orleans, Louisiana, USA)		Oxidative stress	SG-1002	Polyvalent sulfur	Phase II for heart failure‡
University of Exeter (Exeter, UK)		Inflammation, oxidative stress	AP39	Mitochondrion-targeted H_2S release	Preclinical

CSE, cystathionine γ-lyase; H_2S, hydrogen sulfide. *ClinicalTrials.gov identifiers: NCT01926444 and NCT02276768. ‡ClinicalTrials.gov identifier: NCT01989208.

Wang & Wallace, Nat Rev Drug Discov, 14:329-45, 2105

BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

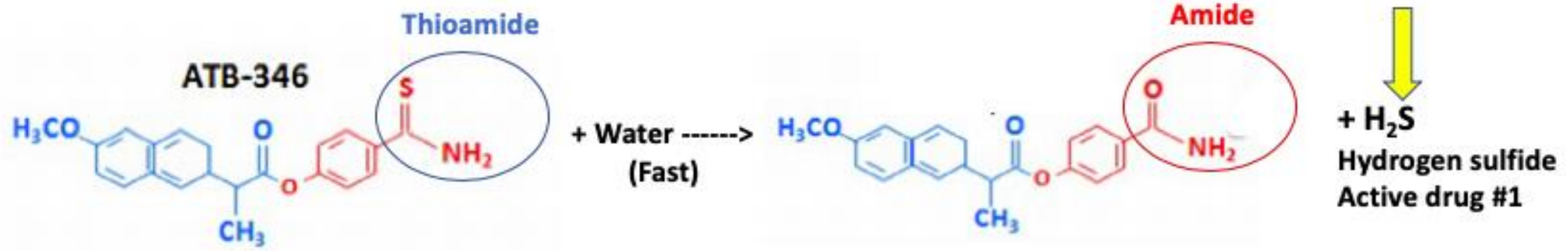
H_2S-Releasing Anti-Inflammatory Drugs



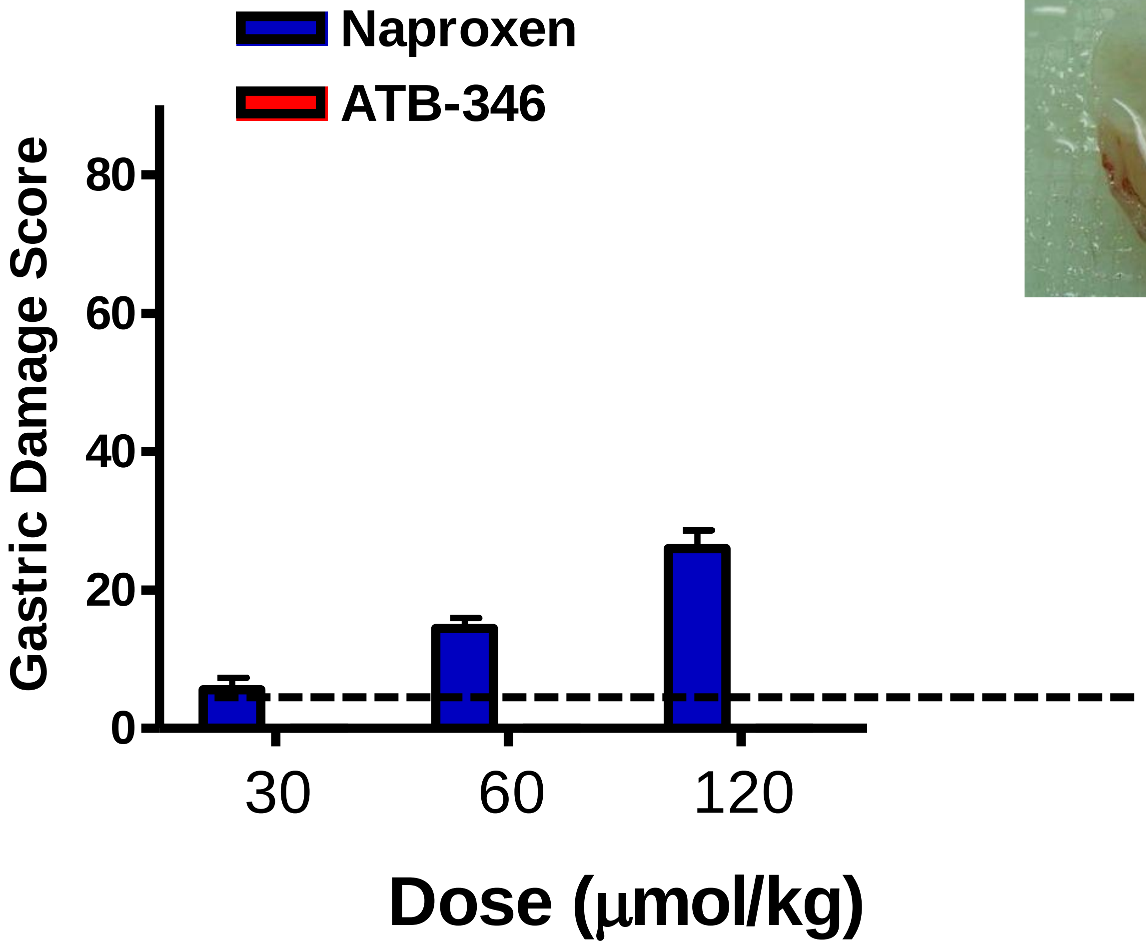
- 30 million people take NSAIDs on a daily basis
- 15.3 deaths per 100,000 users

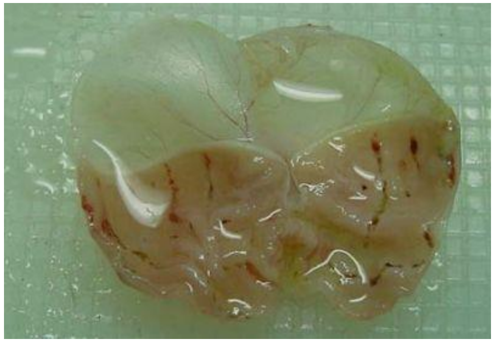
BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

Otenaproxesul (ATB-346)



ATB-346 Causes Negligible Gastric Damage

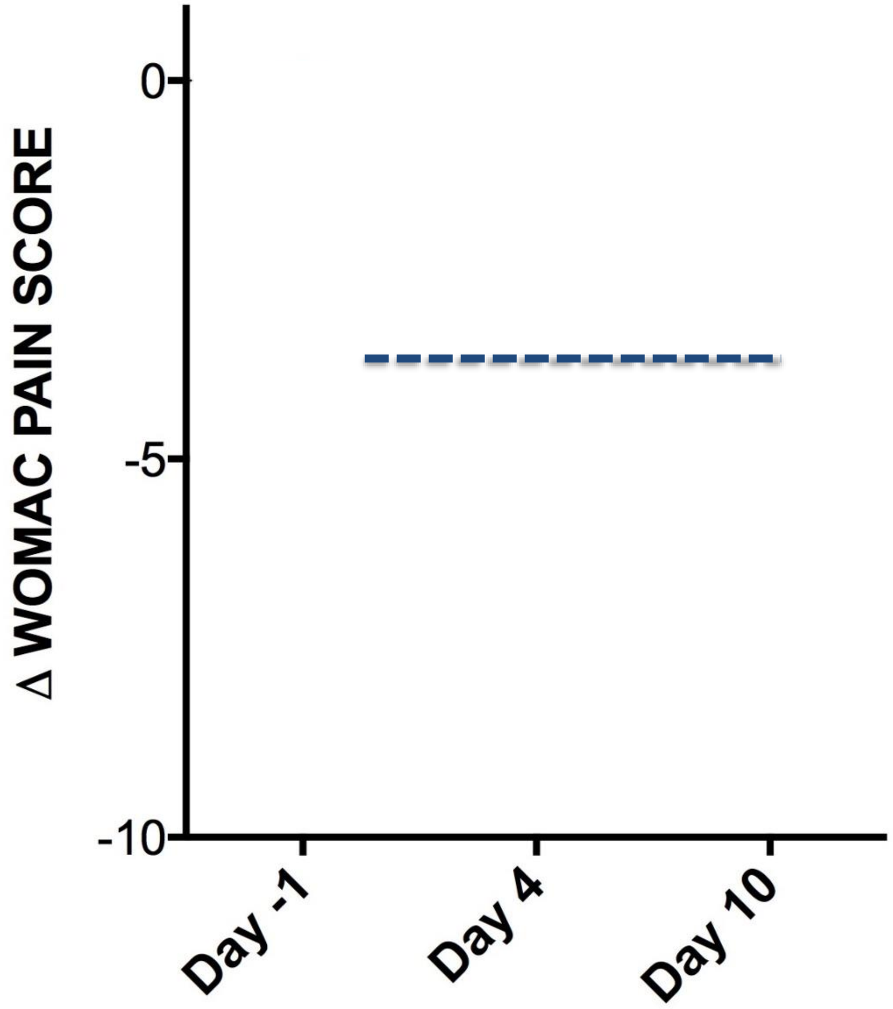




Wallace et al., Br J Pharmacol. 159: 1236–1246, 2010

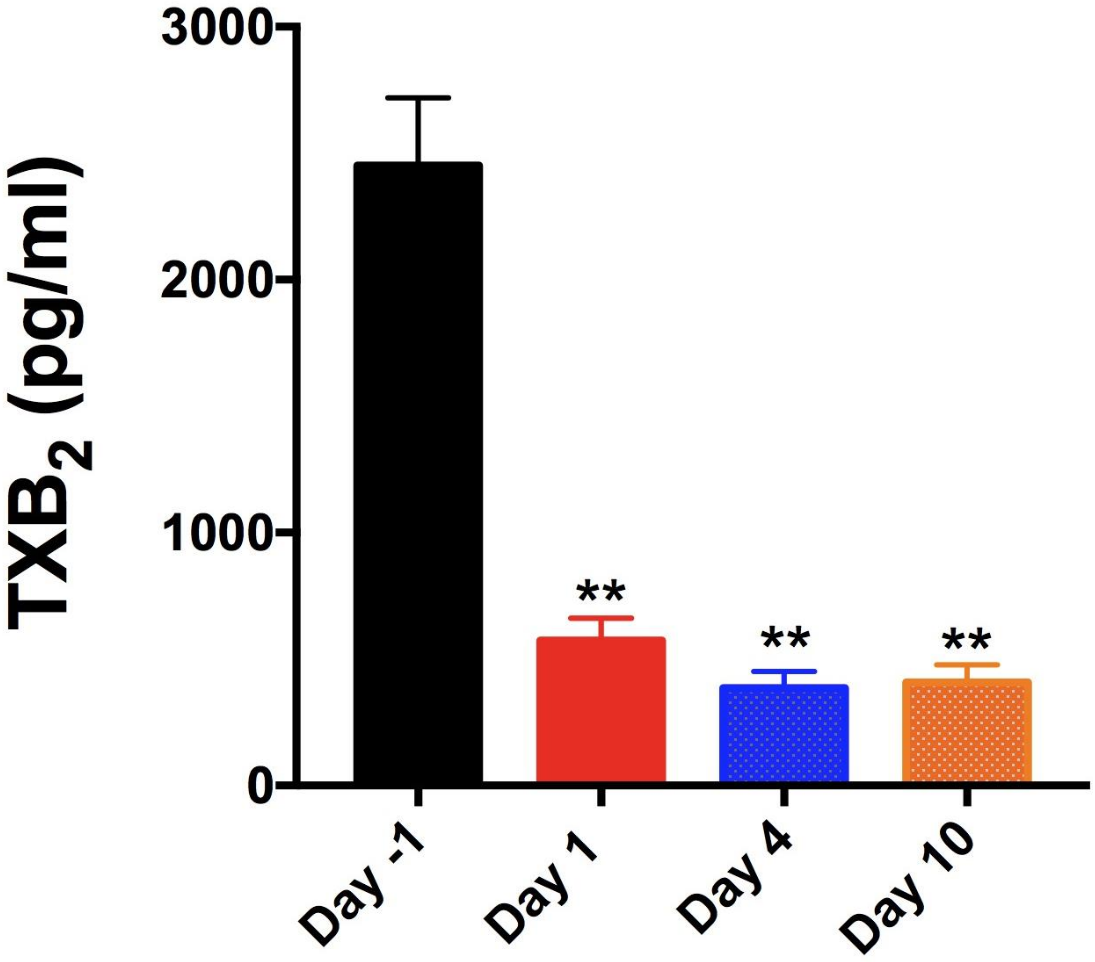
BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

Otenaproxesul reduces pain in patients with OA



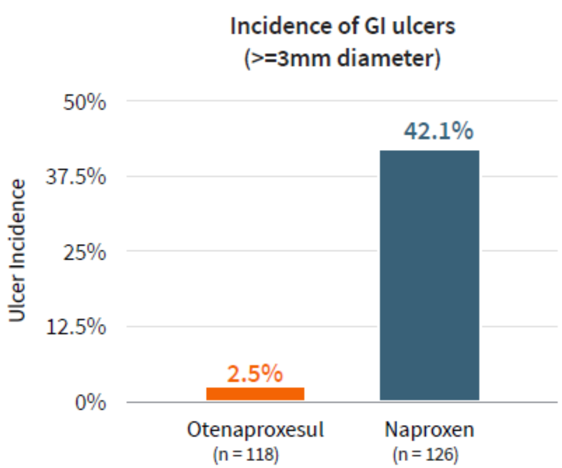
Antibe Therapeutics

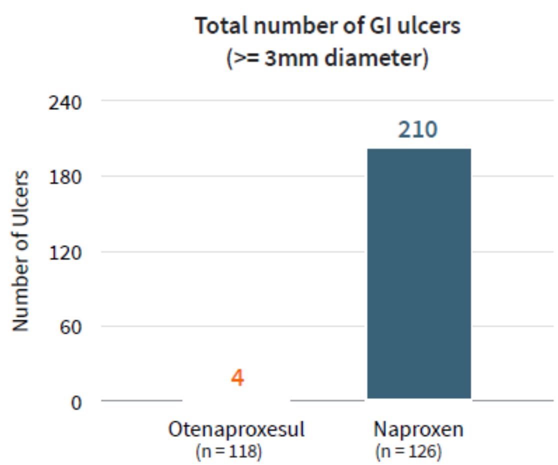
Otenaproxesul reduces prostaglandin synthesis in patients with OA

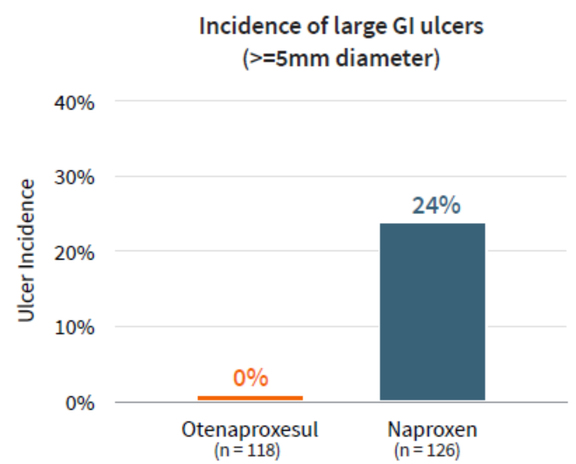


Antibe Therapeutics

GI safety of otenaproxesul







Antibe Therapeutics

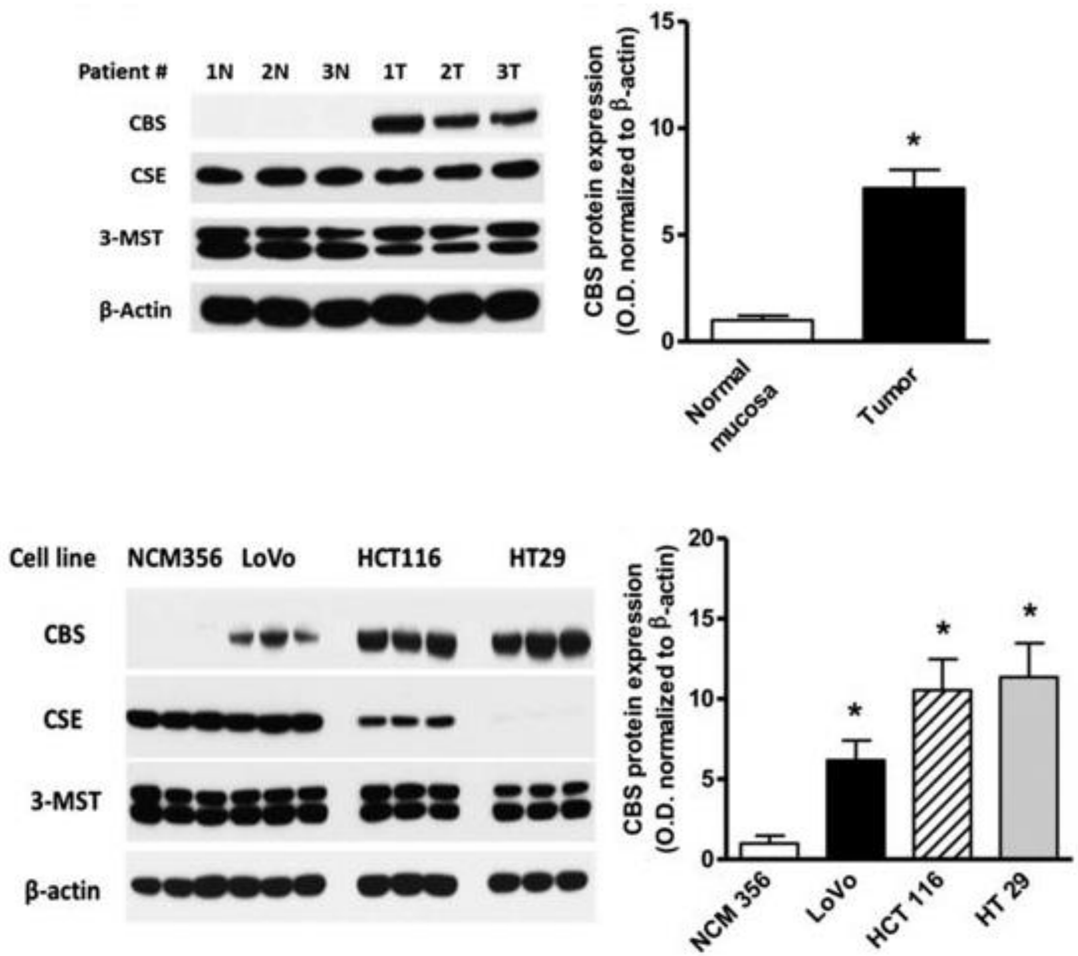
H_2S synthesis inhibitors

Compounds	CSE, IC_{50} (μM)	CBS, IC_{50} (μM)
PAG	40.0 ± 8.0	–
BCA	14.0 ± 0.16	–
HA	4.83 ± 0.31	278.0 ± 22.0
AOAA	1.09 ± 0.12	8.52 ± 0.71
Trifluoroalanine	289.0 ± 7.0	66.0 ± 9.0
AVG	1.0 ± 0.1	–

Asimakopoulou et al, Br. J. Pharmacol, 169: 922–932, 2013

BIOMEDICAL RESEARCH FOUNDATION ACADEMY OF ATHENS

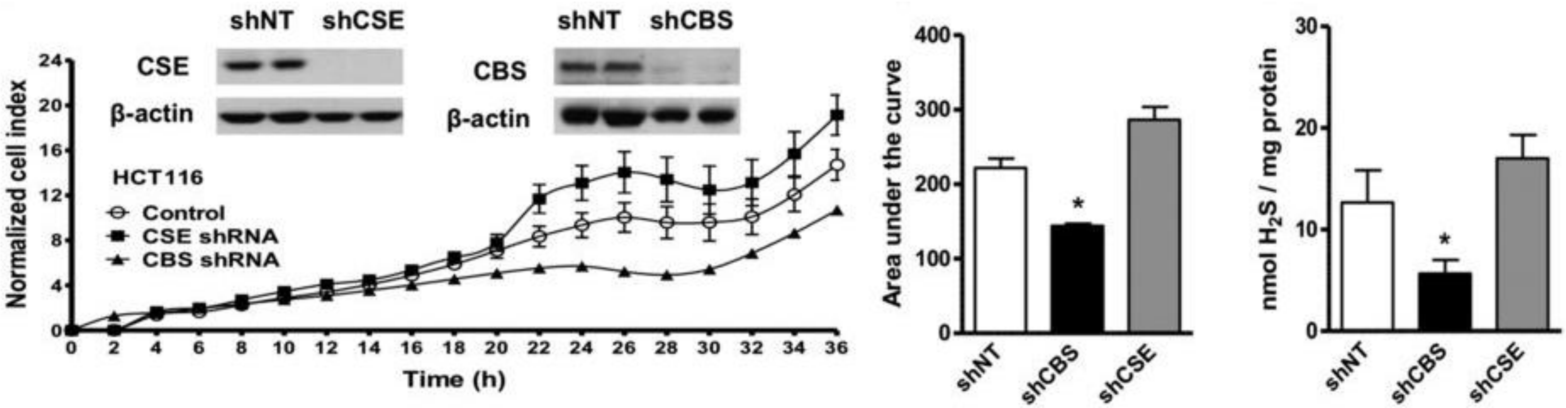
CBS is over expressed in human colorectal cancer

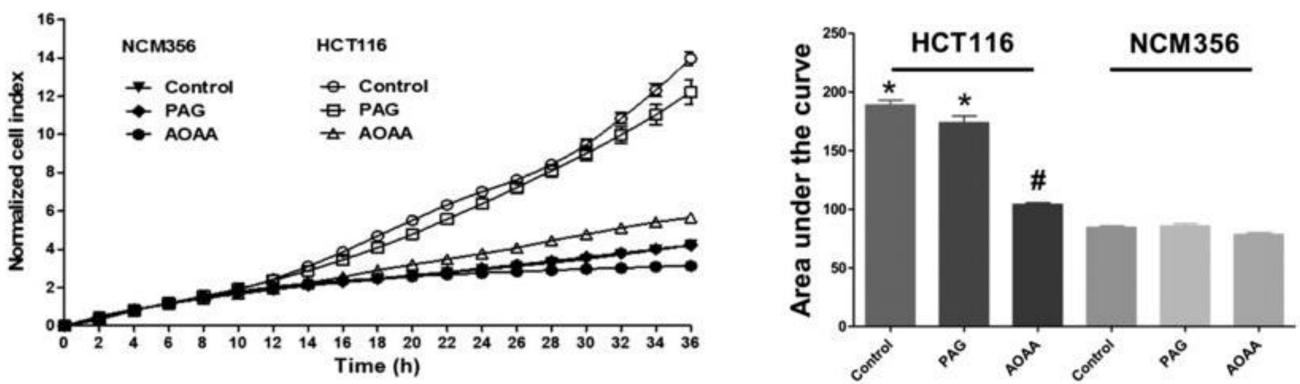


Szabo C et al. PNAS, 110:12474-12479, 2013

BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

Down-regulation or pharmacological inhibition of CBS inhibits proliferation of HCT116 cells

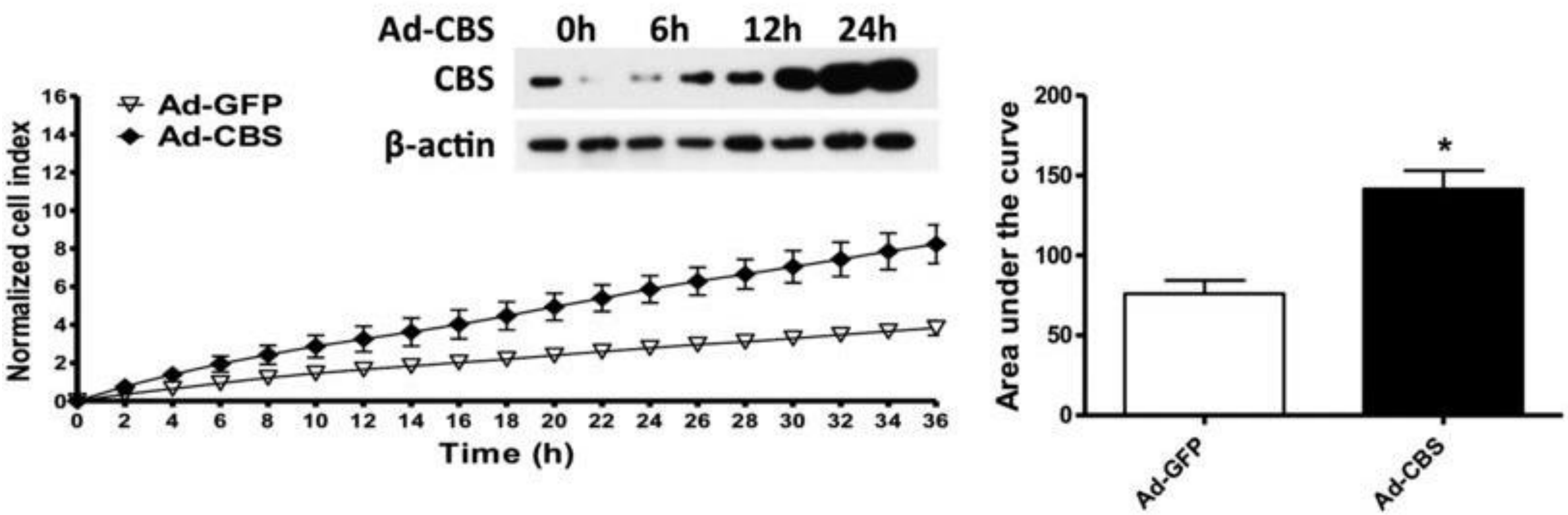




Szabo C et al. PNAS, 110:12474-12479, 2013

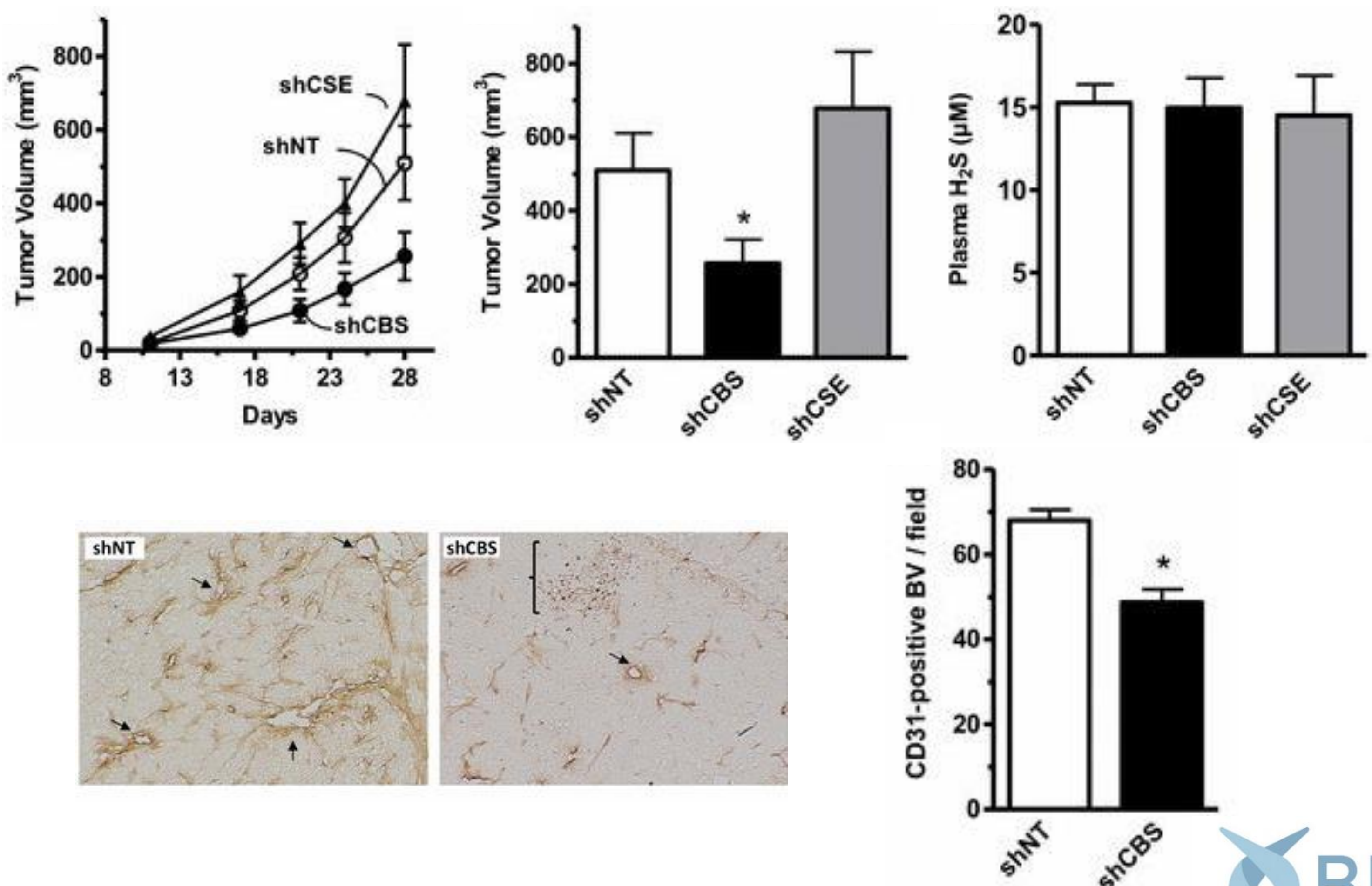
BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

Adenoviral-mediated CBS overexpression enhances the proliferation rate of NCM356 cells



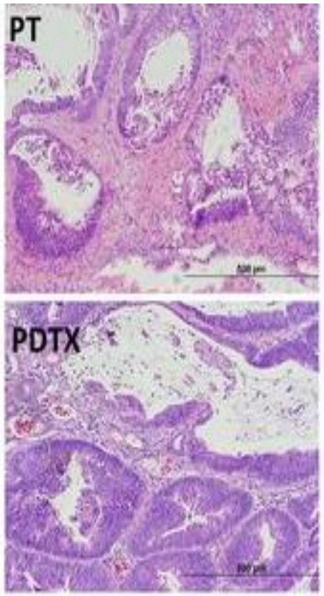
Szabo C et al. PNAS, 110:12474-12479, 2013

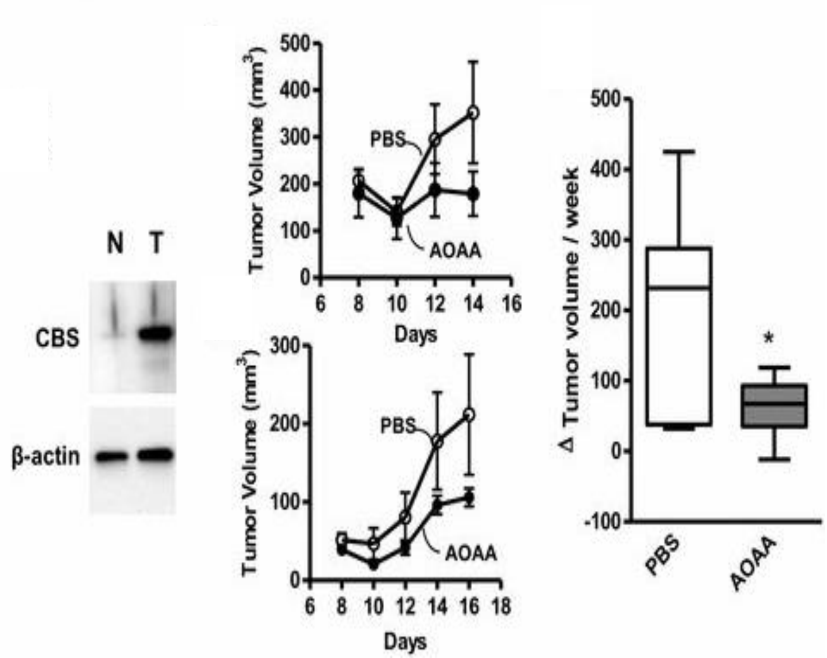
ShRNA-mediated CBS down-regulation inhibits colon cancer growth in vivo



Szabo C et al. PNAS, 110:12474-12479, 2013

AOAA inhibits colon cancer growth and tumor angiogenesis in vivo





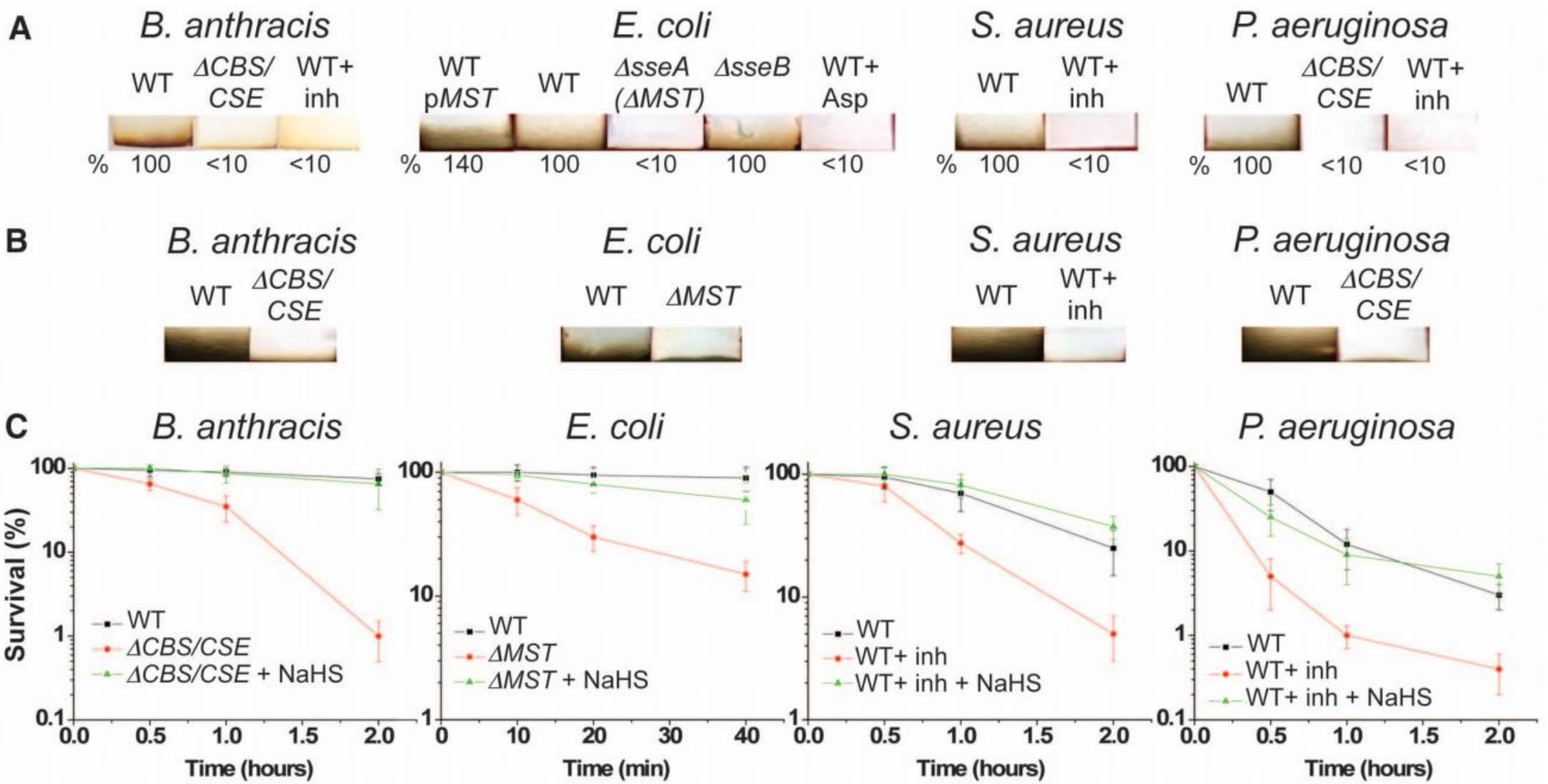
Szabo C et al. PNAS, 110:12474-12479, 2013

BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

Outline

1. Introduction
2. Production and degradation
3. Signaling, target and physiological functions
4. Agents regulating H_2S levels
5. **H_2S, bacteria and antibiotics**
6. H_2S and infection

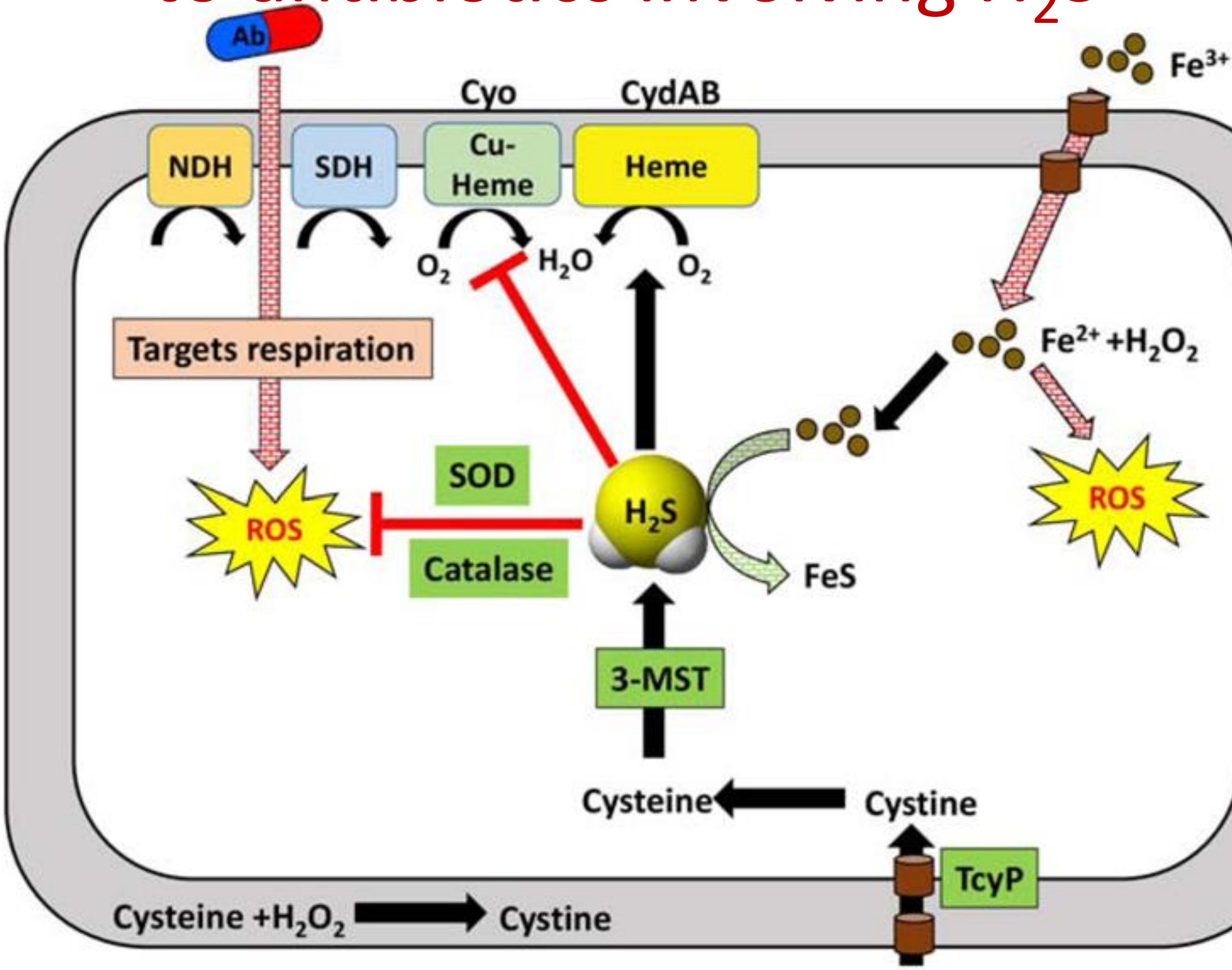
Inhibition of H_2S production renders bacteria sensitive to antibiotics



Shatalin et al., Science, 334(6058), 986–99, 2011

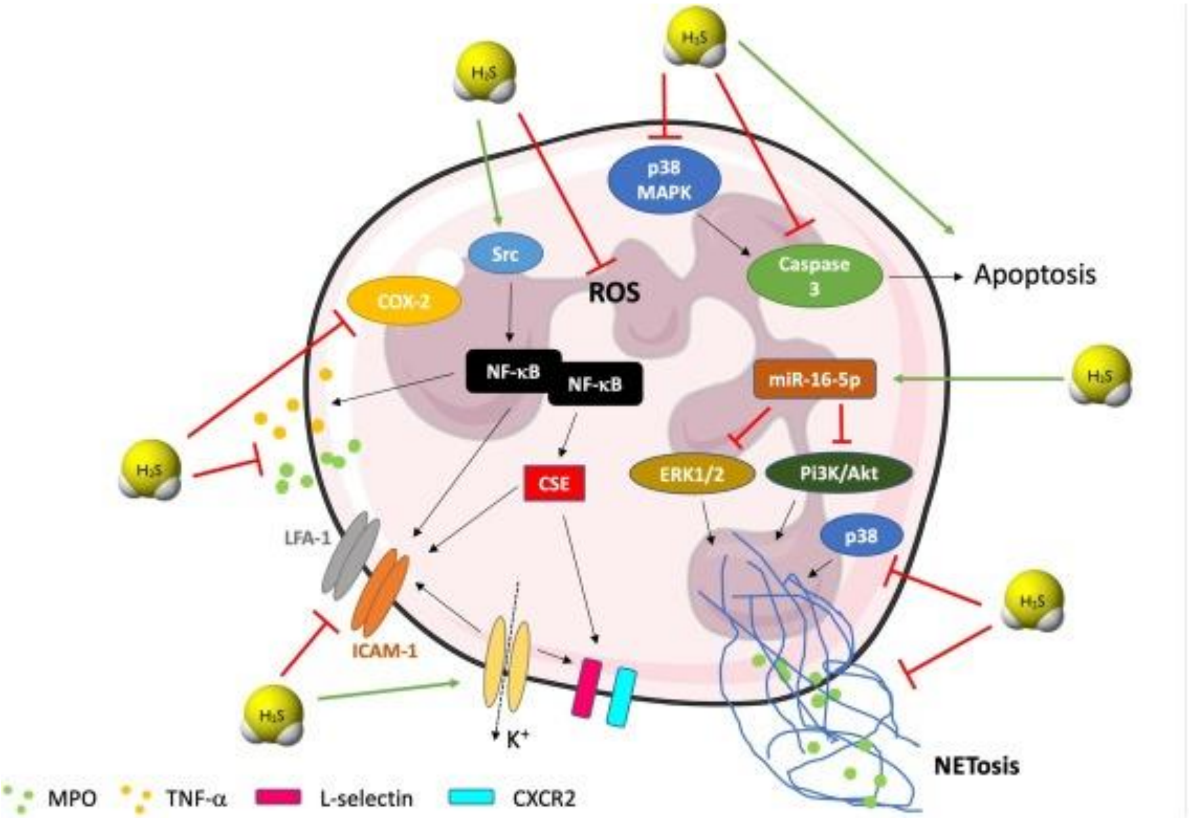
BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

Mechanisms of resistance to antibiotics involving H_2S



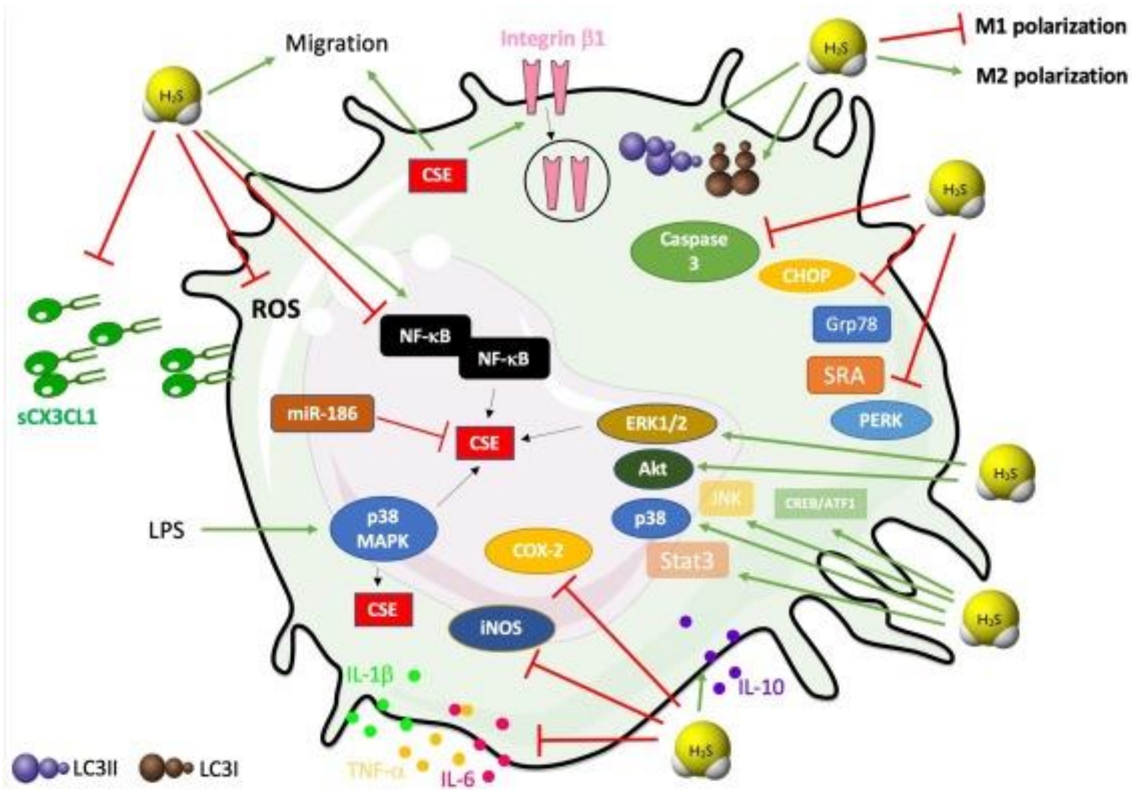
BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

H_2S and neutrophils



Dilek et al., Pharmacol Res, 161:105119, 2020

H_2S and macrophages

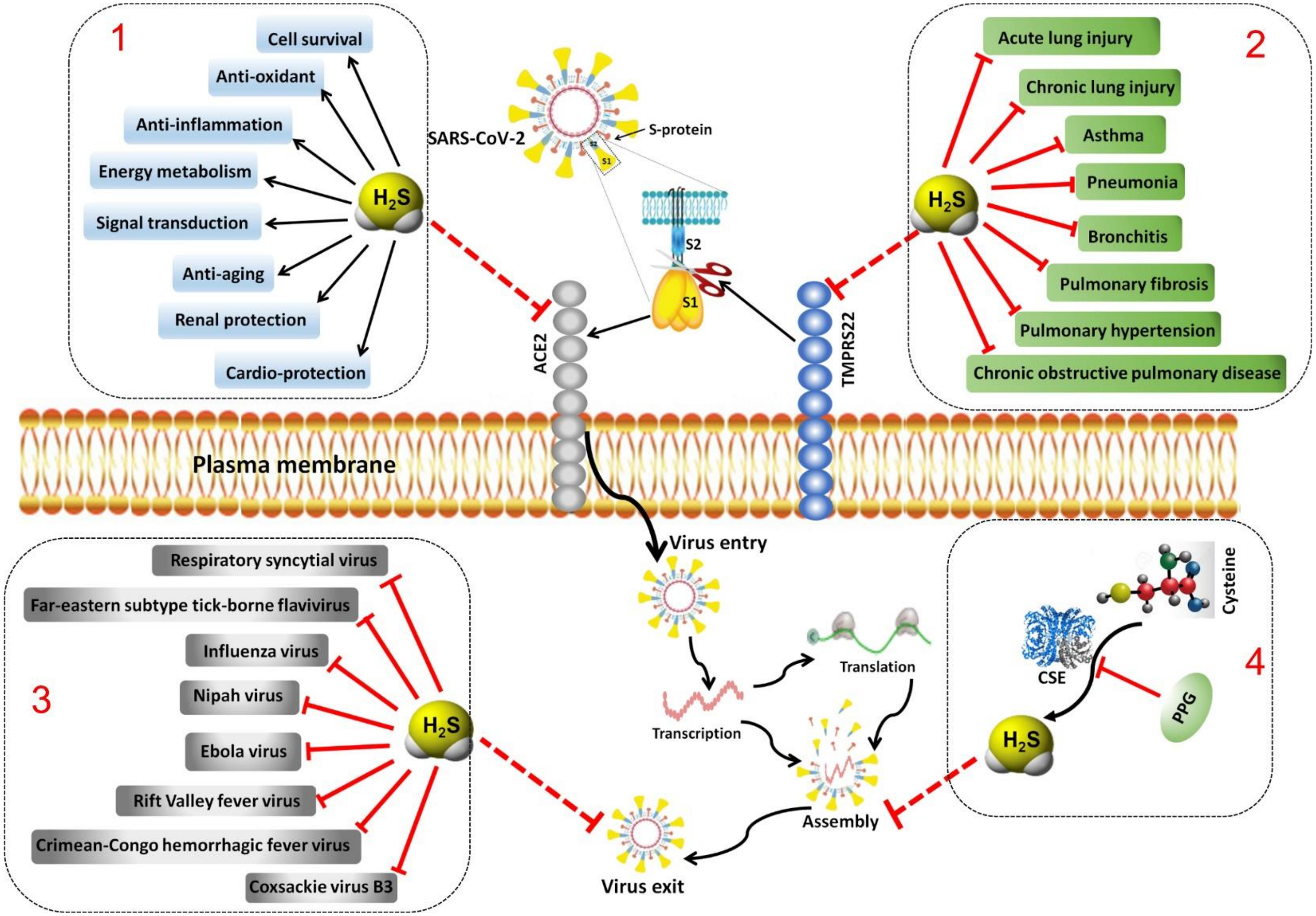


Dilek et al., Pharmacol Res, 161:105119, 2020

Outline

1. Introduction
2. Production and degradation
3. Signaling, target and physiological functions
4. Agents regulating H_2S levels
5. H_2S, bacteria and antibiotics
6. **H_2S and infection**

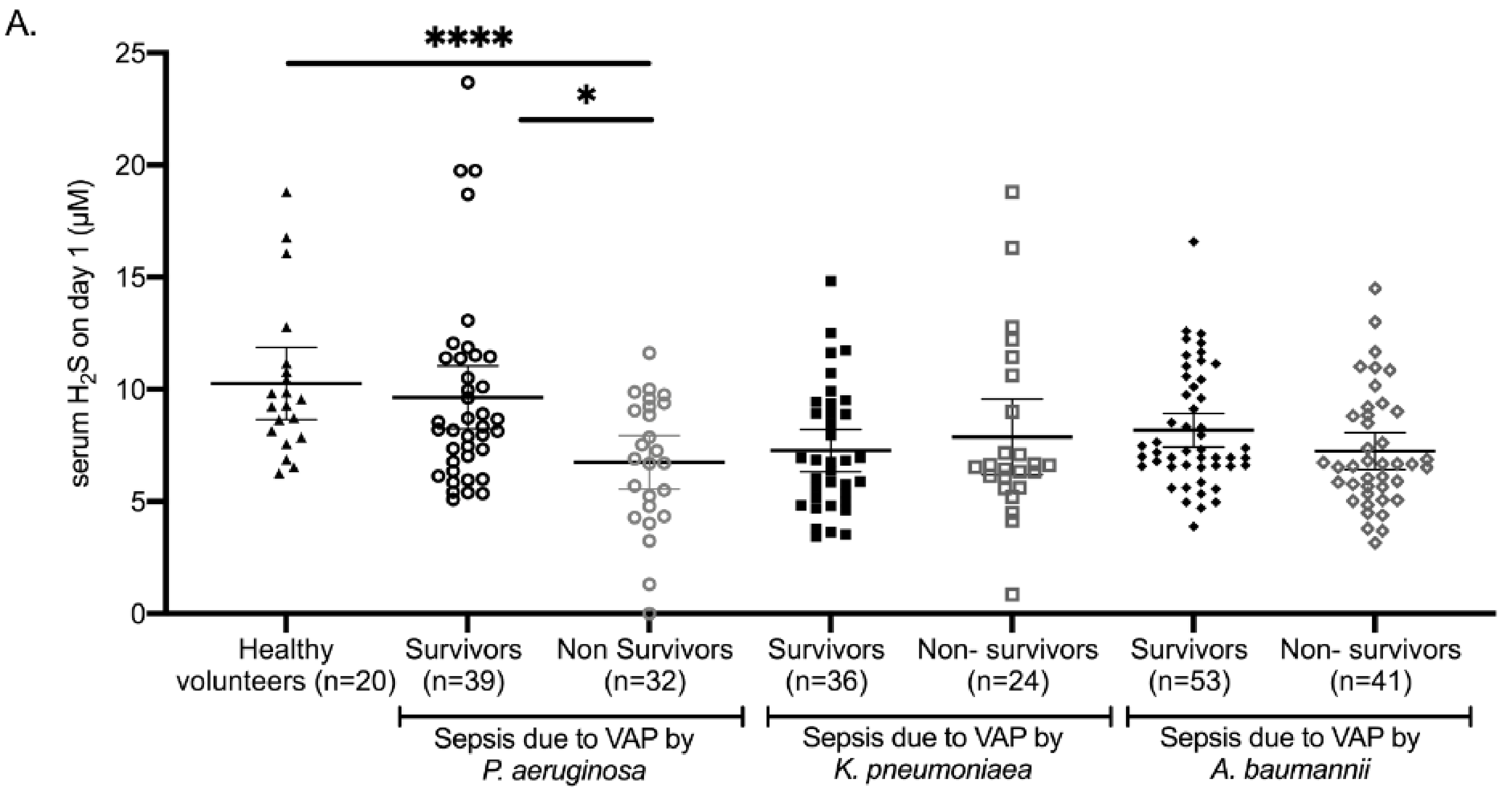
H_2S and Covid-19



Yang, Am J Physiol Heart Cic Physiol, 319: C244-C249, 2020

BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

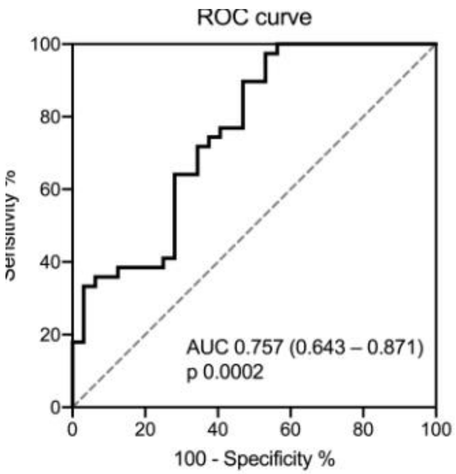
Increased survival in patients with VAP with high H_2S levels



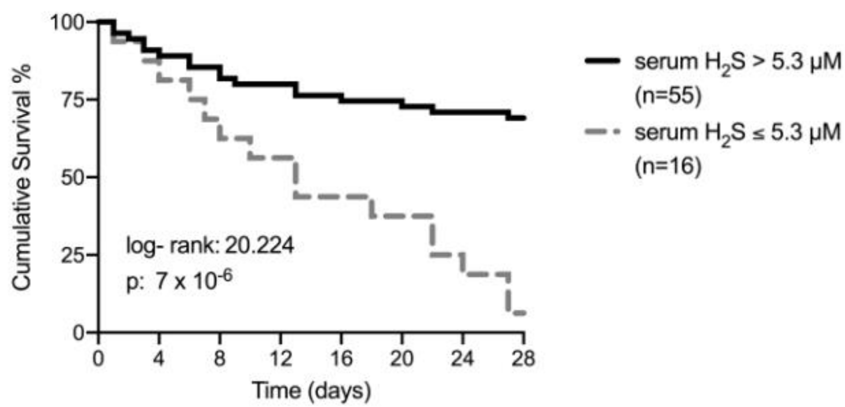
Renieris et al., PLoSPathog17(3):e1009473

BIOMEDICAL RESEARCH FOUNDATION ACADEMY OF ATHENS

Increased survival in patients with VAP with high H_2S levels



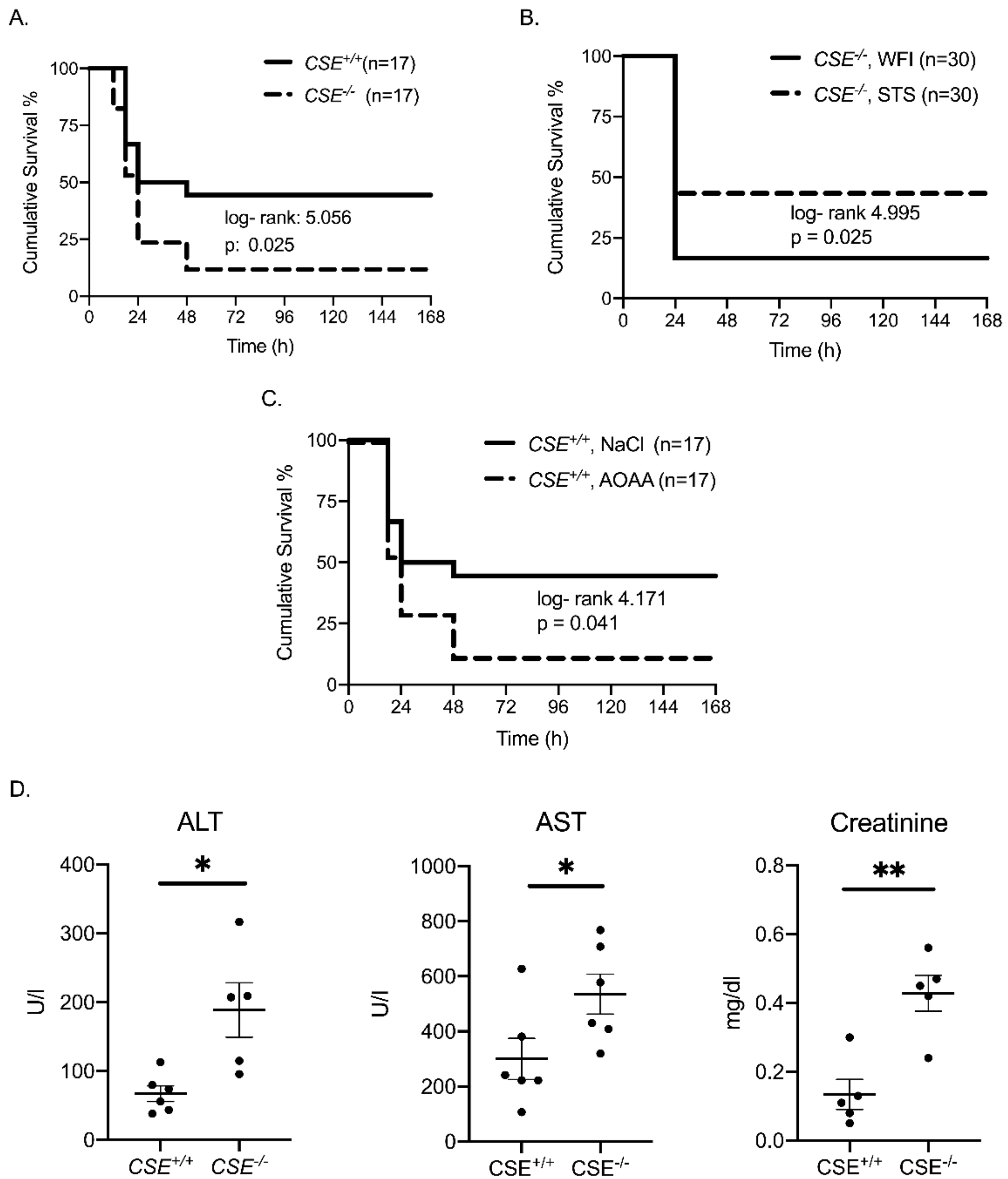
	Survivors (n patients)	Non- Survivors (n patients)	Total
$H_2S > 5.3\mu M$	36 Sensitivity: 92.3% PPV: 66.7%%	18	54
$H_2S \leq 5.3\mu M$	3	14 Specificity: 43.8% NPV: 82.4%	17
	39	32	71



Renieris et al., PLoSPathog17(3):e1009473

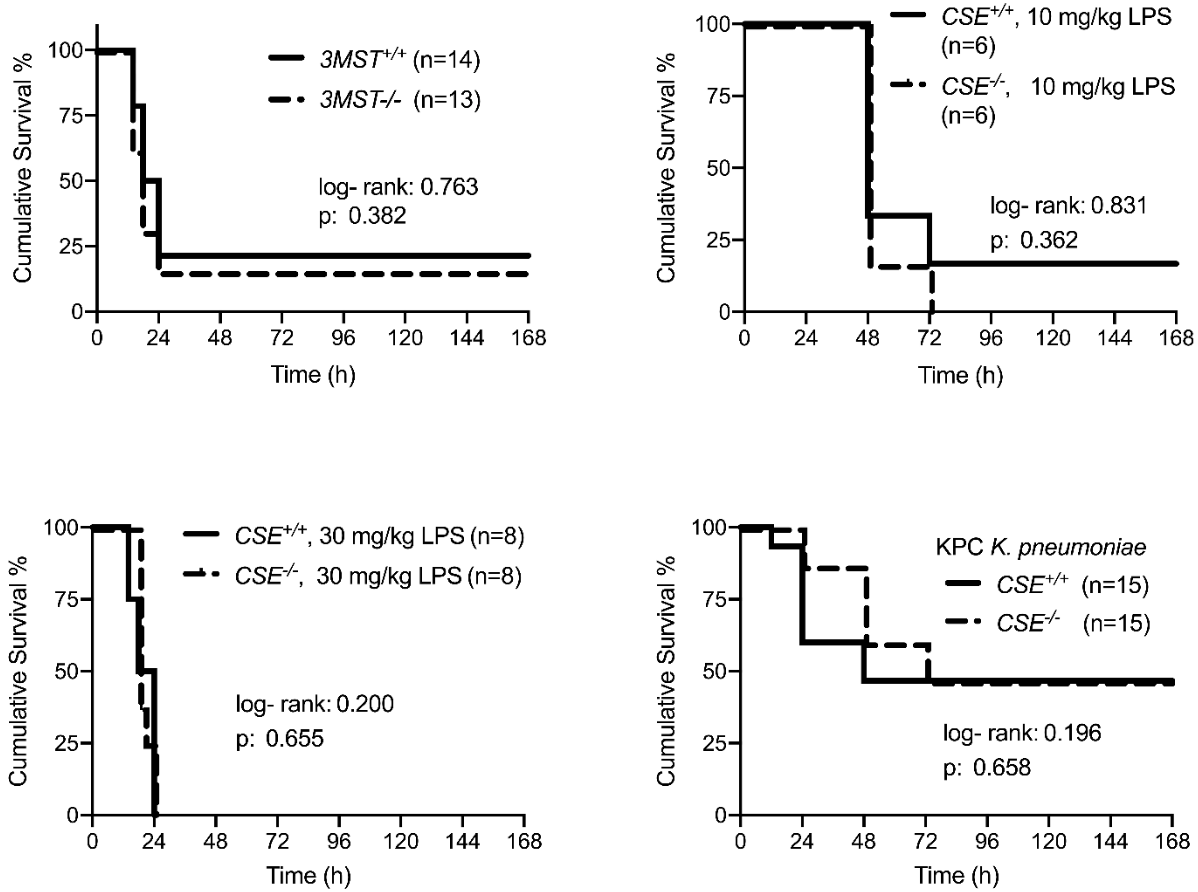
BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

Reduced survival in mice lacking CSE



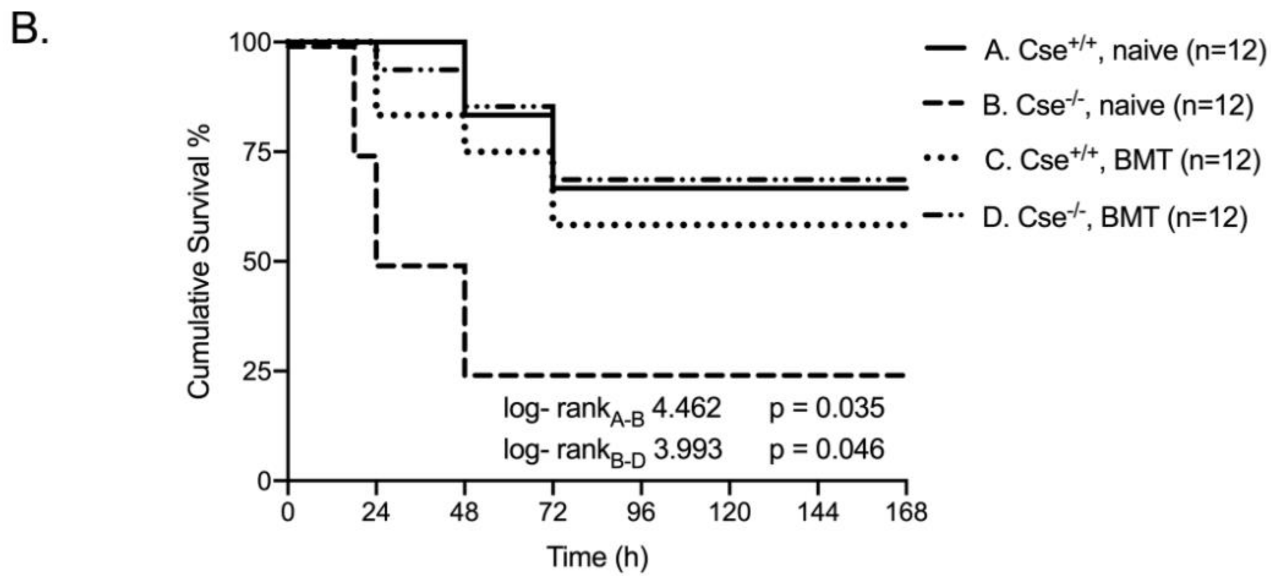
Renieris et al., PLoSPathog17(3):e1009473

CSE does not affect LPS-induced lethality



Renieris et al., PLoSPathog17(3):e1009473

BMT rescue of mice lacking CSE

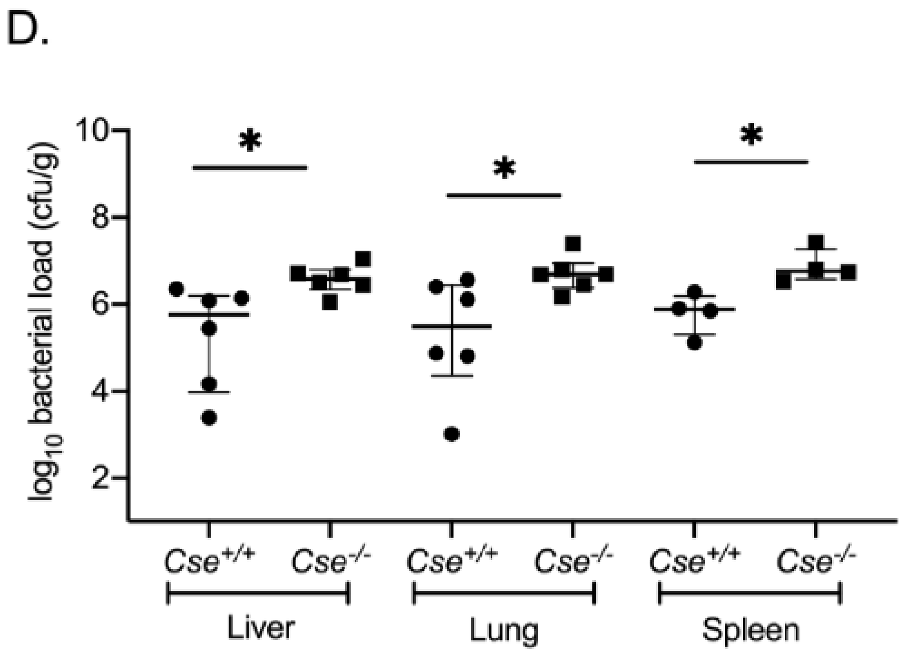


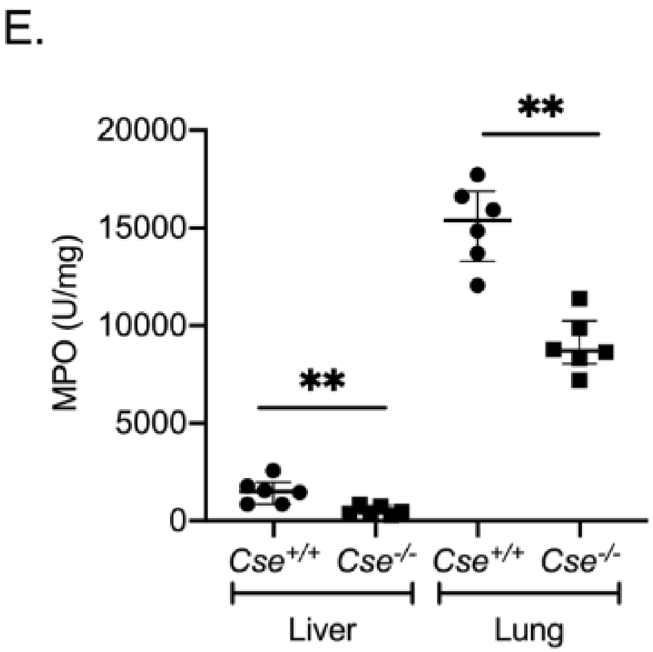
No. at risk

CSE$^{+/+}$, naive	12	12	12	10	8	8	8	8
CSE$^{-/-}$, naive	12	9	6	3	3	3	3	3
CSE$^{+/+}$, BMT	12	12	10	9	7	7	7	7
CSE$^{-/-}$, BMT	12	12	11	10	8	8	8	8

Renieris et al., PLoSPathog17(3):e1009473

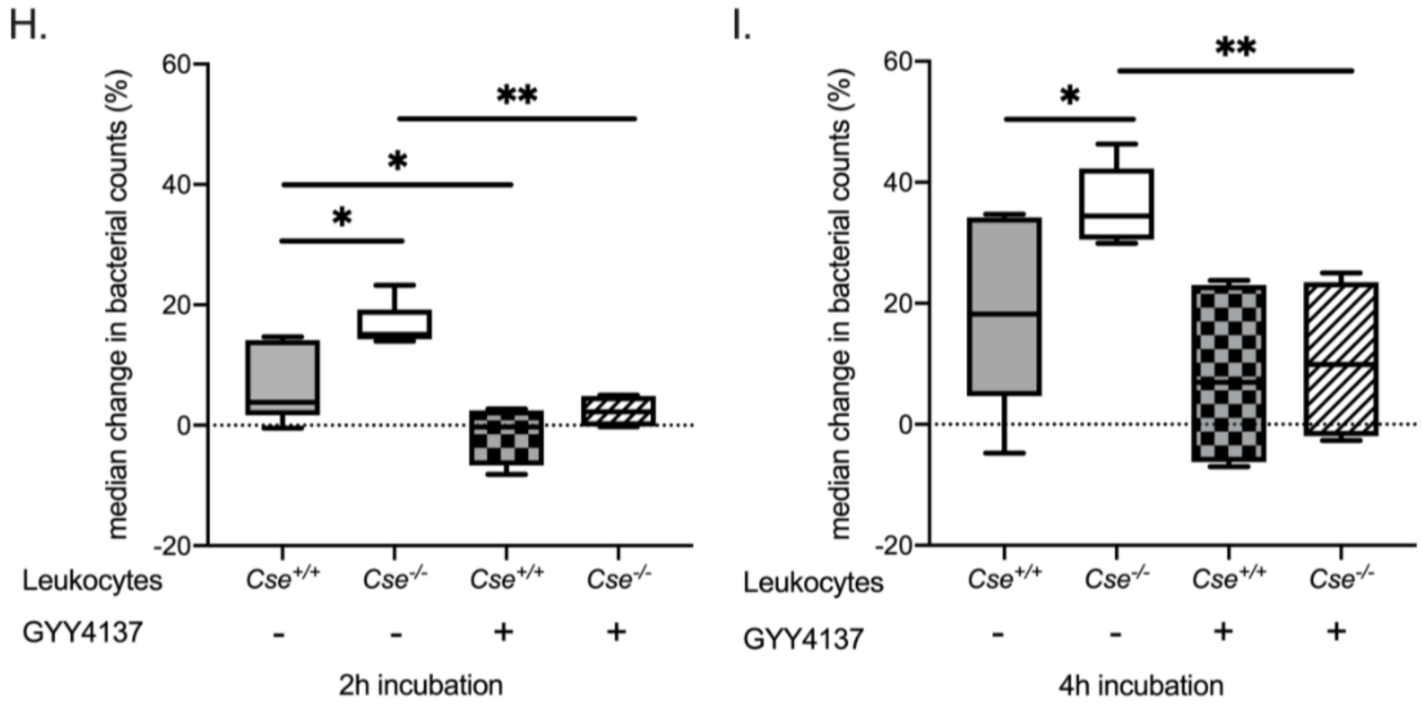
Increased bacterial load in animals lacking CSE





Renieris et al., PLoSPathog17(3):e1009473

H_2S increases bacterial killing



Renieris et al., PLoSPathog17(3):e1009473

BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

Literature

https://pubmed.ncbi.nlm.nih.gov/32781284/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6029659/

Thank you for your attention

A.

Gene	Sense primer	Antisense primers	Product size (bp)
*rpsL**	5′ - CCTCGTACATCGGTGGTGAAG - 3′	3′ - CCCTGCTTACGGTCTTTGACAC - 5′	148
lasI	5′ - CGCACATCTGGGAACTCA - 3′	3′ - CGGCACGGATCATCATCT - 5′	176
lasR	5′ - CTGTGGATGCTCAAGGACTAC - 3′	3′ - AACTGGTCTTGCCGATGG - 5′	133
rhlI	5′ - GTAGCGGGTTTGCGGATG - 3′	3′ - CGGCATCAGGTCTTCATCG - 5′	101
rhlR	5′ - GCCAGCGTCTTGTTCGG - 3′	3′ - CGGTCTGCCTGAGCCATC - 5′	160
pqsA	5′ - GACCGGCTGTATTCGATTC - 3′	3′ - GCTGAACCAGGGAAAGAAC - 5′	74

* Reference/endogenous control gene

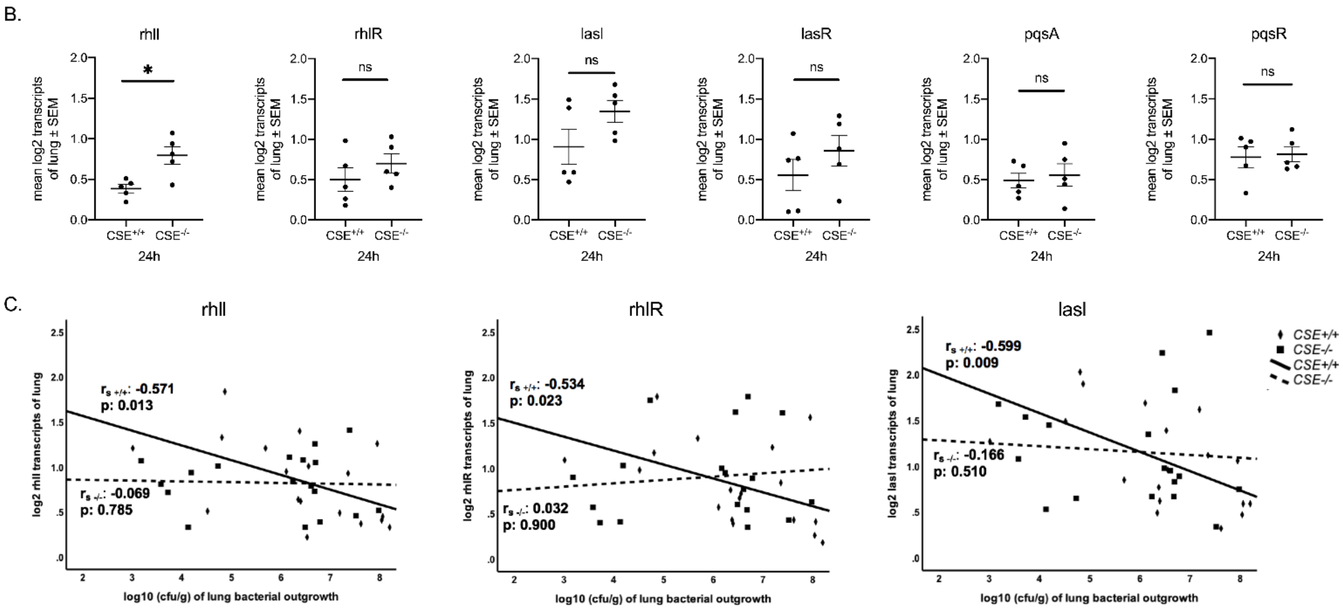


Fig. 6 Importance of endogenous H_2S in the through quorum sensing (QS) mediated multi-drug resistant (MDR) *Pseudomonas aeruginosa* growth

A) Forward and reverse primers for QS genes of *P.aeruginosa*. B) Mean expression of QS genes *rhlI*, *rhlR*, *lasI, lasR*, *pqsA*, *pqsR* in the lung. Comparison by Mann Whitney U test; * p< 0.05 and C) correlation between expression of *rhlI*, *rhlR* and *lasI* genes in the lung and bacterial outgrowth in the lung after experimental infection in $CSE^{+/+}$ and $CSE^{-/-}$ mice. Spearmann rank correlation coefficient (r_s), interpolation line for each group and relevant p- value are given.

Renieris et al., PLoSPathog17(3):e1009473