

## Review

# Hydrogen Sulfide-Regulated NF- $\kappa$ B Signaling via Persulfidation: A Review

Liang Xu <sup>1,2,†</sup>, Keke Liang <sup>1,2,†</sup> , Renjie Wang <sup>2,3</sup>, Yanling Ta <sup>1,2</sup>, Yongrun Yang <sup>1,2</sup>, Jiaying Wang <sup>1,2</sup>, Xianxie Zhang <sup>1,2</sup> , Yuguang Wang <sup>2,3</sup>, Chengrong Xiao <sup>2</sup>, Yihao Wang <sup>2,\*</sup> and Maoxing Li <sup>1,2,3,\*</sup>

- <sup>1</sup> College of Pharmacy, Gansu University of Chinese Medicine, Lanzhou 730000, China; xuliang021026@163.com (L.X.); 18394248206@163.com (K.L.); tyl20250510@163.com (Y.T.); yangyongrun0207@163.com (Y.Y.); wx010890@163.com (J.W.); zhangxianxie@163.com (X.Z.)  
<sup>2</sup> Academy of Military Medical Sciences, Beijing 100850, China; wangrenjiegy@163.com (R.W.); wyg79@139.com (Y.W.); xiaocr2026@163.com (C.X.)  
<sup>3</sup> College of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China  
\* Correspondence: hao\_1216@126.com (Y.W.); limaox2020@aliyun.com (M.L.)  
† These authors contributed equally to this work.

## Abstract

Hydrogen sulfide (H<sub>2</sub>S) is an important endogenous gaseous signaling molecule that regulates diverse physiological and pathological processes through protein persulfidation. As a central regulator of inflammation and immune responses, NF- $\kappa$ B signaling is precisely controlled by post-translational modifications, and its dysregulation contributes to various inflammatory diseases. Recent studies reveal that H<sub>2</sub>S-mediated persulfidation is a key mechanism for fine-tuning NF- $\kappa$ B activity by targeting critical components, including p65, IKK $\beta$ , I $\kappa$ B $\alpha$ , and upstream regulators. Through site-specific S-sulfhydration, H<sub>2</sub>S modulates IKK activation, I $\kappa$ B $\alpha$  degradation, and p65 nuclear translocation, thereby limiting excessive NF- $\kappa$ B activation and inflammatory cytokine production. This review provides an integrated view of how endogenous H<sub>2</sub>S production and persulfidation-dependent signaling regulate inflammatory responses. Rather than simply summarizing individual pathways, we focus on the molecular mechanisms underlying H<sub>2</sub>S-mediated regulation of NF- $\kappa$ B-associated networks, including TLR4/NF- $\kappa$ B, PI3K/Akt/NF- $\kappa$ B, and MAPK/NF- $\kappa$ B pathways, and highlight its roles in oxidative stress, apoptosis, pyroptosis, and tissue repair. We further discuss current challenges in identifying persulfidation sites, understanding endogenous H<sub>2</sub>S regulation, and improving detection technologies. By proposing H<sub>2</sub>S as a precision modulator of inflammatory signaling, this review provides new insights into H<sub>2</sub>S biology and highlights future opportunities for developing targeted H<sub>2</sub>S-based therapeutic strategies.

**Keywords:** hydrogen sulfide (H<sub>2</sub>S); persulfidation (or persulfidation modification); nuclear factor kappa B (NF- $\kappa$ B) signaling pathway; inflammation; oxidative stress



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## 1. Introduction

Hydrogen sulfide (H<sub>2</sub>S) is a colorless, irritating gas with a rotten egg odor. Traditionally, it has been regarded as a toxic and harmful gas [1]. However, recent molecular biology studies have confirmed that the body endogenously produces small amounts of H<sub>2</sub>S. Alongside nitric oxide (NO) and carbon monoxide (CO), H<sub>2</sub>S is now recognized as an important gaseous signaling molecule in the body, playing key regulatory roles

in various physiological and pathological processes, including antioxidative stress [2], anti-inflammatory responses [3], inhibition of neuronal apoptosis [4], and protection of blood–brain barrier integrity [5]. Persulfidation is a special post-translational protein modification discovered after phosphorylation and acetylation. Its core mechanism involves the introduction of a sulfhydryl group (-SH) onto cysteine residues of proteins, forming a persulfide group (-SSH), which in turn regulates protein activity and function. This modification participates in intracellular signal transduction and the regulation of diverse biological processes, making it a hotspot in the field of protein modification [6].

Nuclear factor  $\kappa$ B (NF- $\kappa$ B) is an important class of nuclear transcription factors. Its activation depends on the dissociation of the inhibitor of NF- $\kappa$ B (I $\kappa$ B), after which NF- $\kappa$ B translocates from the cytoplasm to the nucleus, binds to specific sites on target genes, and initiates transcription of downstream genes, thereby exerting its biological effects [7]. The NF- $\kappa$ B signaling pathway is central to the regulation of inflammatory responses and apoptosis. Previous studies have shown that endogenous H<sub>2</sub>S can participate in regulating key biological processes such as inflammation and apoptosis by S-sulfhydrating the RelA (p65) protein within the NF- $\kappa$ B pathway [8]. This review summarizes recent research progress on the molecular mechanisms and biological significance of H<sub>2</sub>S-mediated regulation of the NF- $\kappa$ B signaling pathway through persulfidation.

## 2. NF- $\kappa$ B Signaling Pathway

### 2.1. Overview of the NF- $\kappa$ B Signaling Pathway

Nuclear factor  $\kappa$ B (NF- $\kappa$ B) is a ubiquitous and important nuclear transcription factor present in various cell types across almost all animal species. Its family members include five proteins: RelA (p65), RelB, c-Rel, p50/p105 (NF- $\kappa$ B1), and p52/p100 (NF- $\kappa$ B2). These typically exist as homo- or heterodimers, such as p50/p65 and p65/c-Rel, among which the p50/p65 heterodimer is the most common [9,10]. There are eight known inhibitors of NF- $\kappa$ B (I $\kappa$ Bs): the classical I $\kappa$ B proteins (I $\kappa$ B $\alpha$ , I $\kappa$ B $\beta$ , I $\kappa$ B $\epsilon$ ), the NF- $\kappa$ B precursor proteins (p100, p105), and the nuclear I $\kappa$ Bs (I $\kappa$ B $\zeta$ , Bcl-3, and I $\kappa$ BNS). In most cells, these I $\kappa$ Bs exist as inactive complexes in the cytoplasm and bind to NF- $\kappa$ B through specific ankyrin-repeat sequences at their C-termini [11,12]. Upstream of I $\kappa$ B, the I $\kappa$ B kinase (IKK) complex is a crucial kinase that activates the NF- $\kappa$ B system. IKK is a serine/threonine kinase complex composed of three subunits: IKK $\alpha$  (CHUK), IKK $\beta$  (IKKBK), and NEMO (IKBK $\gamma$ ). It primarily functions by phosphorylating the I $\kappa$ B $\alpha$  protein bound to NF- $\kappa$ B in the cytoplasmic matrix, thereby releasing the dimeric protein and enhancing gene transcription [13,14].

### 2.2. Classical Activation Pathway

The aforementioned NF- $\kappa$ B dimers, I $\kappa$ B proteins, and IKKs constitute the NF- $\kappa$ B signaling pathway. Under resting conditions, NF- $\kappa$ B directly binds to I $\kappa$ B family inhibitory proteins and remains in an inactive state in the cytoplasm [15]. However, upon stimulation by signals such as tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin 1 (IL-1), bacterial lipopolysaccharide (LPS), or ultraviolet irradiation, RIP1 kinase is activated, facilitating the binding of these signals to their corresponding receptors to form complexes, which subsequently activate IKK. Activated IKK $\beta$  then phosphorylates I $\kappa$ B $\alpha$  at two amino acid residues, Ser32 and Ser36, at its N-terminus. Subsequently, phosphorylated I $\kappa$ B $\alpha$  undergoes ubiquitination and is hydrolyzed by the 26S proteasome, leading to its dissociation from NF- $\kappa$ B. This releases the NF- $\kappa$ B dimer, exposing its nuclear localization signal, which promotes the translocation (nuclear shift) of the NF- $\kappa$ B dimer from the cytoplasm to the nucleus. Once in the nucleus, the NF- $\kappa$ B dimer binds to specific sites on the promoters or enhancers of target genes, inducing their transcription [16–19]. There are hundreds of such target genes, including those encoding major proinflammatory cytokines, chemokines,

I $\kappa$ B $\alpha$ , and RelB, thereby broadly participating in various biological processes such as immune responses, inflammation, cell proliferation, and apoptosis [20]. This is the classical pathway of NF- $\kappa$ B signaling activation.

### 2.3. Non-Canonical Activation Pathway

The NF- $\kappa$ B signaling pathway can also be activated through the non-canonical pathway. In the unstimulated state, NF- $\kappa$ B-inducing kinase (NIK) is recruited by TRAF2 and TRAF3 to cIAP1/2 (cellular inhibitor of apoptosis protein 1/2, cIAP1 and cIAP2), leading to K48-linked ubiquitination and subsequent degradation of NIK [21]. Activation of the non-canonical pathway primarily depends on stimulation by factors such as lymphotoxin-beta (LT- $\beta$ ), B-cell activating factor of the TNF family (BAFF), and cluster of differentiation 40 (CD40). Upon binding of these signaling factors to their cognate receptors, TRAF2 and TRAF3 are directly recruited to the receptors and degraded via the proteasome. Concurrently, the targets of cIAP1/2 are shifted, promoting NIK accumulation and subsequent phosphorylation, thereby activating IKK $\alpha$ . Activated IKK $\alpha$  then phosphorylates p100, which is partially ubiquitinated by the recruited SCF $\beta$ TrCP $\beta$ TrCP ubiquitin ligase complex, followed by proteolytic digestion of its C-terminal portion to release the N-terminal portion, i.e., p52. Subsequently, p52 forms RelB–p52 heterodimer with RelB and translocates into the nucleus to induce transcription of specific genes [22,23].

Under normal physiological conditions, the activation of the NF- $\kappa$ B signaling pathway is tightly regulated to maintain internal homeostasis. However, excessive activation or dysregulation of this pathway can lead to uncontrolled inflammatory responses and imbalance in cell proliferation and apoptosis. This is associated with the occurrence and progression of many diseases, including cardiovascular diseases, cancer, neurodegenerative diseases, and autoimmune diseases.

## 3. Endogenous Generation of Hydrogen Sulfide and Persulfidation Modification

### 3.1. Endogenous Hydrogen Sulfide Generation Pathways: Enzymatic and Non-Enzymatic Pathways

H<sub>2</sub>S has recently been recognized as a novel endogenous gaseous signaling molecule and has garnered widespread attention due to its anti-inflammatory, antioxidant, and cardiovascular protective effects. In mammals, the majority of H<sub>2</sub>S is generated via enzymatic pathways. The primary enzymes involved in H<sub>2</sub>S production are cystathionine- $\beta$ -synthase (CBS), cystathionine- $\gamma$ -lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST) [24]. These three enzymes have distinct subcellular localizations and exhibit different expression levels across tissues. Among them, CBS is the major H<sub>2</sub>S synthase in the nervous system, with high expression in the brain, CSE is expressed in the cardiovascular system, as well as in the liver and kidneys, and 3-MST is mainly localized in mitochondria, with highest expression in the kidneys, followed by the liver and heart. Moreover, these three enzymes employ different catalytic mechanisms. CBS predominantly catalyzes the condensation of two cysteine molecules via  $\beta$ -replacement or the reaction of homocysteine with cysteine via  $\gamma$ -replacement to produce H<sub>2</sub>S. CSE can generate H<sub>2</sub>S directly through  $\alpha$ -elimination, as well as via  $\alpha,\gamma$ -elimination or  $\gamma$ -replacement. 3-MST, in coordination with cysteine aminotransferase (CAT), produces 3-mercaptopyruvate, which then forms a persulfide intermediate and subsequently reacts with endogenous reducing agents such as thioredoxin to generate H<sub>2</sub>S [25–27]. Notably, a small amount of H<sub>2</sub>S is also produced through non-enzymatic reactions, for example, by sulfate- and sulfite-reducing bacteria [28]. This reaction occurs predominantly in red blood cells. Beyond H<sub>2</sub>S biosynthesis itself, the gas also engages in sophisticated cross talk with other sulfur-containing metabolic networks.

Substantial evidence indicates that H<sub>2</sub>S does not regulate GSH metabolism through a single linear pathway, but rather acts at multiple interconnected levels. H<sub>2</sub>S intersects with GSH biosynthesis through the transsulfuration pathway. H<sub>2</sub>S is produced from L-cysteine by cystathionine  $\beta$ -synthase (CBS) and cystathionine  $\gamma$ -lyase (CSE), both of which also generate cysteine. Thus, H<sub>2</sub>S production and cysteine availability are metabolically coupled. Moreover, cysteine derived from N-acetylcysteine (NAC) serves as a substrate for both GSH synthesis and H<sub>2</sub>S production, suggesting a dynamic interaction between these two sulfur-containing metabolites. At the redox cycling level, H<sub>2</sub>S maintains the GSH/GSSG ratio by enhancing the activity of glutathione reductase (GR), promoting the regeneration of GSSG to GSH. This effect is particularly important under oxidative stress conditions, where maintaining the GSH–GSSG balance is critical for cellular redox homeostasis [29,30]. Furthermore, H<sub>2</sub>S plays important roles in antioxidative stress, apoptosis, inflammatory responses, and cardiovascular diseases, with persulfidation mediated by H<sub>2</sub>S being the primary mechanism underlying its biological effects [31].

In addition, CSE is not constitutively highly expressed in humans. Rather, its expression is upregulated upon stimulation, when transcription factor SP1 binds to the promoter region of CSE, leading to a significant increase in CSE mRNA. Elevated CSE protein levels subsequently result in increased intracellular H<sub>2</sub>S production. In the NF- $\kappa$ B signaling pathway, H<sub>2</sub>S acts as a nucleophile to attach an additional sulfur atom to the thiol group (–SH) of p65-Cys38, generating a persulfide group (–SSH). This modification induces a conformational change in the N-terminal region of p65, exposing the binding site for coactivators. The persulfidated p65 then recruits ribosomal protein S3 (RPS3), ultimately protecting the organism from injury. In CSE-knockdown models, however, this cascade of events fails to occur [8,32].

Notably, studies have shown that 3-mercaptopyruvate sulfurtransferase (3-MST) itself functions as a protein persulfidase. It specifically recognizes 3-mercaptopyruvate (3-MPA) and attacks the sulfur atom in 3-MPA, initiating a  $\beta$ -elimination reaction to generate the 3-MST-SSH intermediate. In this intermediate, the outer sulfur atom forms an unstable persulfide bond (–S–S–) with the sulfur atom at the enzyme's active site. Subsequently, the thiol group of a cysteine residue on the target protein acts as a nucleophile, directly attacking the outer sulfur atom of 3-MST-SSH, resulting in the transfer of the outer sulfur atom to the target protein and formation of the target protein persulfide. The active-site cysteine of 3-MST is restored to its free thiol state, enabling the next round of catalysis. However, due to steric constraints, the inner sulfur atom of 3-MST-SSH is not readily attacked by nucleophiles, and thus this reaction rarely occurs [33].

In addition, quantification methods for H<sub>2</sub>S include UV-vis spectrophotometry, chromatography, and fluorescence probe methods, etc. Enzyme activity detection methods include LC-MS/MS and fluorescence probe methods, etc. [29], as shown in Tables 1 and 2.

**Table 1.** Methods for the quantitative detection of H<sub>2</sub>S.

| Method Category                                           | Detailed Method                                           | Operating Steps                                                                                                                                           | Applicable Samples                          | Reference |
|-----------------------------------------------------------|-----------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|-----------|
| Direct ultraviolet-visible spectrophotometric measurement | Direct ultraviolet-visible spectrophotometric measurement | Observe the absorbance of sulfide species in the ultraviolet region (214–300 nm) of the spectrum.                                                         | Dissolved sulfides in natural water samples | [34]      |
|                                                           | Zinc capture—methylene blue method                        | Zinc-trapped sulfides are acidified and reacted with DMPD/Fe <sup>3+</sup> to yield methylene blue (670 nm); LC-MS can be coupled for higher sensitivity. | Water samples, biological samples           | [29]      |

Table 1. Cont.

| Method Category                                 | Detailed Method                                                              | Operating Steps                                                                                                                                                                                                                                  | Applicable Samples                                    | Reference |
|-------------------------------------------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|-----------|
| Chromatography                                  | Gas chromatography coupled with a flame photometric detector (GC-FPD)        | Acid-labile sulfur (acid) and sulfane sulfur (cyanide) are detected by GC-FPD; alternatively, oxidation to sulfate/thiosulfate permits IC-electrochemical detection.                                                                             | Rat liver and cardiac tissue samples                  | [35]      |
|                                                 | Mobile gas dialysis chromatography                                           | Samples are treated with HCl to release H <sub>2</sub> S, which is trapped in NaOH, concentrated, and detected by IC using a silver electrode for electrochemical detection.                                                                     | Human and rat brain tissues                           | [36]      |
|                                                 | Gas chromatography coupled with a sulfur chemiluminescence detector (GC-SCD) | Samples are injected onto a Teflon column (Chromosil 330) with nitrogen carrier, detected by chemiluminescence, and quantified by peak area.                                                                                                     | Mouse tissues, human blood                            | [37]      |
|                                                 | Gas chromatography with silver particle trapping                             | Ag particles adsorb H <sub>2</sub> S to Ag <sub>2</sub> S; release with thiourea/H <sub>2</sub> SO <sub>4</sub> and GC; differentiates acid-labile from bound sulfur.                                                                            | Mouse blood, rat brain and tissue homogenate          | [38]      |
|                                                 | High-performance liquid chromatography (HPLC)                                | Zinc-trapped analyte derivatized to methylene blue, analyzed by RP-HPLC-visible; or pre-column fluorescent derivatization with fluorescence detection.                                                                                           | Cow brain; Human red blood cells                      | [39]      |
|                                                 | Liquid chromatography–tandem mass spectrometry (LC-MS/MS)                    | CBS/CSE-generated H <sub>2</sub> S byproducts cystathionine, lanthionine, and homolanthionine (thioethers) were measured by MRM.                                                                                                                 | Human plasma and serum                                | [40]      |
|                                                 | HPLC-DAD method based on MMB or MBB                                          | MBB alkylates H <sub>2</sub> S/HS <sup>−</sup> to UV/fluorescent SPB/SDB for HPLC detection.                                                                                                                                                     | Plasma, rat tissues                                   | [41]      |
| Fluorescence probe method                       | Benzodisulfide cyclopentanone probe                                          | Bis-electrophilic center reacts with H <sub>2</sub> S to fluorescence; product also MS-analyzable.                                                                                                                                               | Bovine plasma                                         | [42]      |
|                                                 | Azide-reduced probe                                                          | H <sub>2</sub> S reduces azide to amine with fluorescence; rapid, no pretreatment.                                                                                                                                                               | Aqueous solution, bovine serum, mouse blood           | [43]      |
|                                                 | Chemiluminescence based on luminol                                           | Samples mixed with CLSS-1/2, light recorded, H <sub>2</sub> S quantified by standard curve.                                                                                                                                                      | Cell lysis solution or culture medium                 | [44]      |
|                                                 | Nitro-reduced fluorescent probe                                              | NMO probe incubated with sample in PBS (pH 7.4), measure fluorescence at 550 nm (ex 430 nm); for cell imaging, co-incubate with HEK293T and view under fluorescence microscope.                                                                  | HEK293T cells                                         | [45]      |
|                                                 | Acetyl benzocoumarin fluorophore                                             | H <sub>2</sub> S reacts with probe's $\alpha,\beta$ -unsaturated carbonyl via tandem Michael/aldol to turn weak to strong red fluorescence ( $\lambda_{em}$ 628 nm). 900 nm two-photon excitation gives red emission, avoiding autofluorescence. | Saliva, phosphate-buffered solution                   | [46]      |
|                                                 | H <sub>2</sub> S-responsive near-infrared fluorescent probe                  | HT probe in DMSO/H <sub>2</sub> O with sample: monitor A ↓ 535, new peak 700 nm; record F ↑ 785 (ex700). For cell imaging, co-incubate and view by fluorescence microscopy.                                                                      | DMSO/H <sub>2</sub> O solution, A549 cells, nude mice | [47]      |
|                                                 | Chlorinated coumarin malononitrile fluorescence probe                        | H <sub>2</sub> S replaces Cl, then intramolecular addition forms cyclic imine coumarin (1b), blocking ICT/PET, enhancing fluorescence ( $\lambda_{ex}/em$ 515/564 nm).                                                                           | MCF-7 cells                                           | [48]      |
| Sulfide-specific ion-selective electrodes (ISE) | Direct potential method                                                      | Alkaline conversion of sulfides to S <sup>2−</sup> , measure electrode potential; protein interference may occur, so use with caution.                                                                                                           | Bovine serum albumin (BSA) and buffer solution        | [49]      |

Table 1. Cont.

| Method Category          | Detailed Method                       | Operating Steps                                                                                                                                        | Applicable Samples                                             | Reference |
|--------------------------|---------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------|-----------|
| Polarographic electrodes | Polarographic hydrogen sulfide sensor | H <sub>2</sub> S concentration was recorded real-time in a respirometer chamber using an H <sub>2</sub> S-permeable membrane and anode/cathode system. | Rat aortic smooth muscle cells and intact thoracic aorta       | [50]      |
|                          | Amperometric microsensor method       | H <sub>2</sub> S-selective sensors (e.g., TBR4100) directly measure H <sub>2</sub> S in samples electrochemically.                                     | Serum, tissue homogenate, kidney sections, culture supernatant | [51]      |

Table 2. Enzyme activity detection methods.

| Enzyme Name | Main Organizational Expression                                                   | Activity Detection Method                                                                                                                                                                                                 | Reference |
|-------------|----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| CSE         | Liver, kidneys, thoracic aorta, ileum, portal vein, islets of pancreas, placenta | CSE produces H <sub>2</sub> S from L-cysteine; H <sub>2</sub> S reacts with sulfite to thiosulfate, then with DTNB to yellow product (412 nm). Absorbance increase reflects H <sub>2</sub> S production and CSE activity. | [52]      |
| CBS         | Brain, liver, kidney, pancreas                                                   | Isotope serine -LC-MS/MS; H <sub>2</sub> S selective electrode/lead-acetic acid method                                                                                                                                    | [40]      |
| 3-MST       | Liver, kidney, heart, lung, thymus, brain, red blood cells, vascular endothelium | Coupled CAT-MST reaction system; real-time monitoring by fluorescent probe                                                                                                                                                | [53]      |

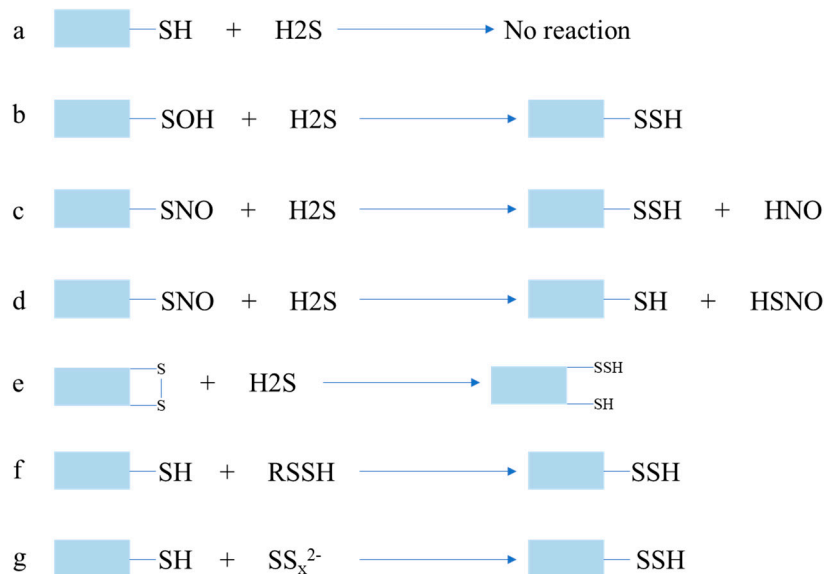
However, for exogenous H<sub>2</sub>S, each donor exhibits distinct release profiles. In the studies by Yanxi Liu [54], Tae Jun Kim [55], and colleagues, it was found that SFN and GYY4137 show similar H<sub>2</sub>S release kinetics at their respective IC<sub>50</sub> concentrations—namely a rapid release during the first 30 min, followed by a plateau. In addition, Na<sub>2</sub>S and NaHS release H<sub>2</sub>S rapidly and in large amounts in PBS; however, their release is significantly attenuated in serum-containing medium due to protein binding. In contrast, DATS and DADS show minimal H<sub>2</sub>S release in PBS, but their release increases rapidly upon the addition of glutathione (GSH).

### 3.2. Mechanisms of Persulfidation Modification

Hydrogen sulfide-mediated persulfidation is a novel post-translational protein modification that plays a critical role in maintaining cellular homeostasis and signal transduction [56]. The mechanisms by which H<sub>2</sub>S induces persulfidation include the following: (1) due to thermodynamic constraints, H<sub>2</sub>S cannot directly react with the cysteine thiol group (RSH) of proteins, but it can react with the sulfenic acid form of cysteine (Cys-SOH) to produce persulfidation; (2) under different environmental conditions, H<sub>2</sub>S can react with S-nitrosated cysteine to generate nitroxyl (HNO) or thionitrous acid (HSNO); (3) H<sub>2</sub>S reacts with cysteine disulfides (–S–S–) to form S-sulfhydrated products; (4) persulfides (RSSH) can act as carriers and participate in transpersulfidation reactions through acid–base motifs spatially close to RSH groups; and (5) persulfidation can also be formed via reactions between oxidized sulfur species such as polysulfides (S<sub>x</sub>2<sup>−</sup>x2<sup>−</sup>) and RSH groups (Figure 1) [57].

### 3.3. Detection Methods for Persulfidation

Currently, the main detection methods for persulfidation include the maleimide assay, biotin-switch assay, tag-switch assay, cysteine labeling assay, mass spectrometry, and diketone method, among others.



**Figure 1.** The formation process of persulfidation: (a)  $\text{H}_2\text{S}$  cannot directly react with thiol groups (-SH); (b)  $\text{H}_2\text{S}$  can react with the sulfenic acid form of cysteine (Cys-SOH) to generate persulfidation; (c,d)  $\text{H}_2\text{S}$  can react with S-nitrosated cysteine to generate nitroxyl (HNO) or thionitrous acid (HSNO); (e)  $\text{H}_2\text{S}$  can react with cysteine disulfides ( $-\text{S}-\text{S}-$ ) to generate persulfidation; (f) persulfides (RSSH) can act as carriers to undergo transpersulfidation reactions with RSH groups; (g) oxidized sulfur species such as polysulfides ( $\text{S}_x^{2-}$ ) can react with RSH to generate persulfidation.

### 3.3.1. Maleimide Assay

This method was proposed by Professor Snyder and his team at Johns Hopkins University in 2012. It is a relatively sensitive technique for detecting protein persulfidation. This approach uses an Alexa Fluor 680-conjugated C2 maleimide fluorescent probe to label the thiol groups of cysteine residues. The sample is then treated with the reducing agent dithiothreitol (DTT), which selectively cleaves disulfide bonds and removes the -SH group from -SSH, leading to a decrease in fluorescence signal. The fluorescence signal intensity can be assessed by separating proteins via gel electrophoresis and transferring them onto a PVDF membrane using transfer or dot blotting. The red fluorescent bands on the PVDF membrane are detected using a gel imaging system, allowing observation of changes in fluorescence signal intensity. A decrease in fluorescence signal indicates persulfidation of the target protein by  $\text{H}_2\text{S}$ . However, this method cannot be used for large-scale proteomic analysis [58].

In addition, another maleimide-based labeling method is the biotin-thiol assay (BTA). First, biotin-polyethylene glycol-maleimide is used to simultaneously label both thiol and persulfide sites on proteins. Second, the proteins are enzymatically digested, and all biotinylated peptides are captured using streptavidin beads. Finally, the reducing agent DTT is used to selectively cleave persulfide bonds, thereby eluting only the sulfhydrated peptides for subsequent mass spectrometry identification. However, it should be noted that high concentrations of maleimide can disrupt polysulfide chains, and the procedure is cumbersome [59].

### 3.3.2. Biotin-Switch Assay

The biotin-switch assay, proposed by Mustafa and colleagues, was the original method for detecting S-sulfhydrated proteins. This method is a modification of an earlier approach used for detecting S-nitrosylation of cysteine thiols. Briefly, the thiol-blocking agent methyl methanethiosulfonate (MMTS) is first reacted with -SH groups. The MMTS is then removed with acetone, and -SSH groups are labeled with biotin-HPDP (N-(6-(biotinamido)hexyl)-

3'-(2'-pyridyldithio)-propionamide) in dimethyl sulfoxide (biotin-HPDP does not react with MMTS). Biotinylated proteins are then captured and pulled down using streptavidin-agarose beads, followed by washing with HENS buffer. Finally, the biotinylated proteins are eluted with SDS-PAGE sample buffer and analyzed by Western blotting [60]. A limitation of this method is that MMTS cannot distinguish between -SH and -SSH and blocks both, preventing biotin-HPDP from labeling -SSH. Consequently, this method suffers from low specificity and a high false-positive rate.

### 3.3.3. Tag-Switch Assay

Zhang and colleagues developed a method termed the “tag-switch assay” for detecting protein persulfidation. This group used methylsulfonyl benzothiazole (MSBT) and its more water-soluble analog MSBT-A to react specifically with sulfhydrated sites (-SSH), generating mixed aromatic disulfides that selectively block both free thiols and sulfhydrated sites. Subsequently, a nucleophilic biotinylated cyanoacetate derivative reacts with the disulfide bonds, and the labeled proteins are specifically captured by streptavidin-agarose beads via biotin. Finally, the proteins are analyzed by Western blotting to quantitatively assess persulfidation levels.

In addition, this group developed cyanoacetate derivatives bearing a fluorescent BODIPY moiety (CN-BOT) or a Cy3 dye (CN-Cy3) to detect sulfhydrated proteins by fluorescence microscopy or gel staining, thereby enhancing detection sensitivity. However, CN-Cy3 is difficult to wash out from fixed cells; therefore, CN-BOT is used for in situ detection of protein sulfhydration, while CN-Cy3 is used for detecting protein sulfhydration in cell lysates [61]. A limitation of this method is that the MSBT reagent cannot penetrate cell membranes and thus cannot be used for real-time detection in live, intact cells: it is only applicable to cell lysates or fixed cells.

### 3.3.4. Cysteine Labeling Assay

This method was proposed by Krishnan and colleagues in 2011. First, iodoacetic acid (IAA) is used to label all -SH and -SSH groups. Then, DTT is used to reduce -SSH to -SH. Finally, an iodoacetamide-linked biotin probe (IAP) is used for labeling [62]. A limitation of this method is that it cannot distinguish -SSH from other reducible modifications such as S-nitrosylation.

### 3.3.5. Mass Spectrometry

Longen and colleagues developed a mass spectrometry-based method for detecting protein persulfidation in 2016. First, the team treated cells with trichloroacetic acid (TCA) to achieve complete cell lysis and protein denaturation. Next, a biotinylation reagent called iodoacetyl-PEG2-biotin (IAMBio) was used to simultaneously label both -SH and -SSH groups on all proteins. The labeled proteins were then digested with trypsin, enriched using streptavidin-agarose beads, and eluted using tris(2-carboxyethyl)phosphine (TCEP) to release -SSH-derived peptides. Finally, the samples were analyzed by LC-MS/MS [63].

### 3.3.6. Diketone Method

The diketone method is a novel approach for detecting protein persulfidation based on dimedone developed by Zivanovic and colleagues in 2019. First, they used a new labeling agent, 4-chloro-7-nitrobenzofurazan (NBF-Cl), which reacts with thiols (-SH), persulfides (-SSH), sulfenic acids, and amines. Then, a biotinylated dimedone probe (DCP-Bio1) was employed for “tag switching,” allowing -SSH to be readily captured by streptavidin magnetic beads and subsequently detected [64]. The limitations of this method include its relatively complex procedure and the need to consider the membrane permeability of the probes.

## 4. Biological Effects of H<sub>2</sub>S-Mediated Persulfidation of NF-κB

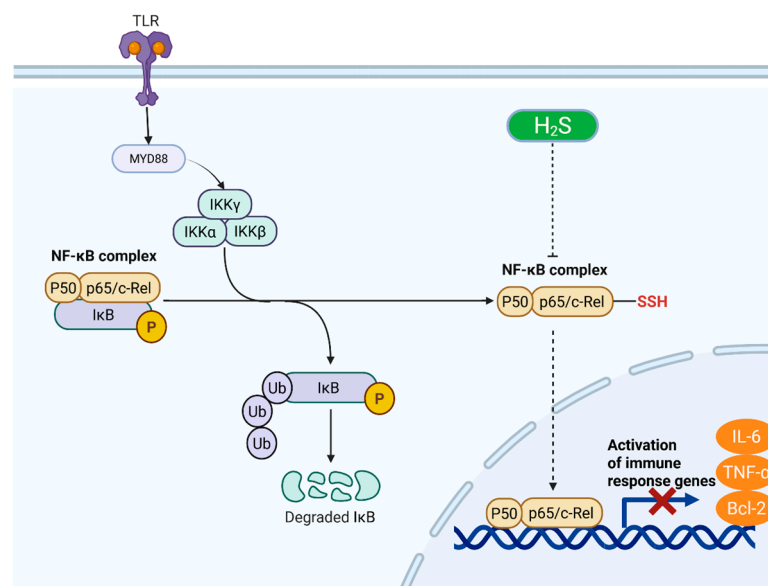
### 4.1. Biological Effects of H<sub>2</sub>S-Mediated Persulfidation of TLR4/NF-κB

Toll-like receptor 4 (TLR4) is a transmembrane protein and a member of the pattern recognition receptor family, which is indispensable for the activation of innate and adaptive immune responses [65]. It consists of an extracellular domain, a transmembrane domain, and a cytoplasmic Toll–interleukin 1 receptor (TIR) domain. The extracellular domain contains multiple leucine-rich repeat (LRR) motifs, each composed of 20–29 residues, providing specific sites for ligand interactions [66]. The transmembrane domain primarily maintains the topology of TLR4. The TIR domain plays a critical role in transducing extracellular signals into the intracellular space. Upon exposure to external stimuli, TLR4 recognizes and binds to these stimuli, subsequently recruiting the downstream adaptor protein MyD88, which further activates the IKK complex, promotes IκB phosphorylation and ubiquitination-dependent degradation, and ultimately enables NF-κB dimers to translocate into the nucleus to initiate gene transcription [67].

In recent years, numerous studies have demonstrated that H<sub>2</sub>S-mediated persulfidation of TLR4/NF-κB plays a significant role in regulating immune and inflammatory responses. In a study by Sun et al. [68], a lipopolysaccharide (LPS)-induced inflammatory model in bovine mammary epithelial cells (MAC-T) showed decreased cell viability and upregulated expression of TLR4 and NF-κB, effects that were reversed by NaHS treatment. In a Parkinson's disease model, Cao et al. [69] found decreased cell viability, increased apoptosis rate, elevated levels of proinflammatory cytokines, and increased protein expression of TLR4, p-IκBα, p-NF-κB p65, and iNOS. Exogenous H<sub>2</sub>S treatment suppressed these increases, reduced proinflammatory cytokine release, and improved cell viability. Furthermore, in an LPS-induced infectious preterm birth model [70], NaHS administration reduced ASC and caspase 1 activity and downregulated TLR4 and NF-κB expression, suggesting that exogenous H<sub>2</sub>S alleviates LPS-induced inflammatory responses, potentially involving over-vulcanization modification. Liang et al. [71] established a salt-sensitive hypertensive rat model and found that protein levels of TLR4, MyD88, p-NF-κB p65, and p-IKKβ were significantly higher in the model group compared with the control group. Exogenous H<sub>2</sub>S inhibited the activation of the TLR4/MyD88/NF-κB signaling pathway, alleviated central neuroinflammation, subsequently reduced peripheral sympathetic nerve activity, and effectively lowered blood pressure. In a chronic obstructive pulmonary disease (COPD) model established by cigarette smoke exposure, Wang et al. [72] found that the expression levels of GSDMD-N, cleaved caspase 1, LDH, TLR4, and NF-κB were significantly increased compared with controls, and NaHS treatment reversed these changes. Additionally, Abdel-Latif [73] used CP to induce a rat hepatotoxicity model and observed decreased GSH levels, increased TBARS and NOx levels, and significantly increased expression of TLR2, TLR4, p-JNK, and NF-κB. NaHS treatment inhibited these changes. Similarly, in an alcohol-induced fatty liver model established by ethanol gavage [74], the NaHS-treated group showed significantly improved liver function indices and downregulated protein expression of TLR4 and NF-κB.

Taken together, current studies both domestically and internationally have confirmed the regulatory role of H<sub>2</sub>S in the TLR4/NF-κB signaling pathway across various disease models and at different cellular levels. The core mechanism consistently points to persulfidation modification: H<sub>2</sub>S can S-sulfhydrate key proteins in the TLR4/NF-κB pathway (such as TLR4, RelA/p65, and IKK), thereby inhibiting excessive activation of the pathway, alleviating overt inflammatory responses, suppressing aberrant apoptosis, and exerting tissue-protective effects. However, existing studies still have certain limitations. Most research has focused on the intervention effects of exogenous H<sub>2</sub>S, whereas the regulatory mechanisms of endogenous H<sub>2</sub>S-mediated persulfidation remain insufficiently explored.

Moreover, the precise modification sites on key proteins within the TLR4/NF- $\kappa$ B pathway, modification efficiency, and cross talk with other post-translational modifications require further in-depth investigation. In the future, with continued advances in research technologies, a deeper understanding of the molecular mechanisms of H<sub>2</sub>S-mediated persulfidation of the TLR4/NF- $\kappa$ B pathway will provide new insights and potential therapeutic targets for the prevention and treatment of various diseases, including inflammation-related diseases, neurodegenerative disorders, and cardiovascular diseases (Figure 2).



**Figure 2.** H<sub>2</sub>S regulates the TLR4/NF- $\kappa$ B signaling pathway through persulfidation modification. Abbreviations: NF- $\kappa$ B: nuclear factor  $\kappa$ B; I $\kappa$ B: inhibitor of NF- $\kappa$ B; TLR: Toll-like receptor. Created in BioRender. Liang, K. (2026) <https://BioRender.com/0kc3rdt> (accessed on 22 July 2026).

#### 4.2. Biological Effects of H<sub>2</sub>S-Mediated Persulfidation of PI3K/Akt/NF- $\kappa$ B

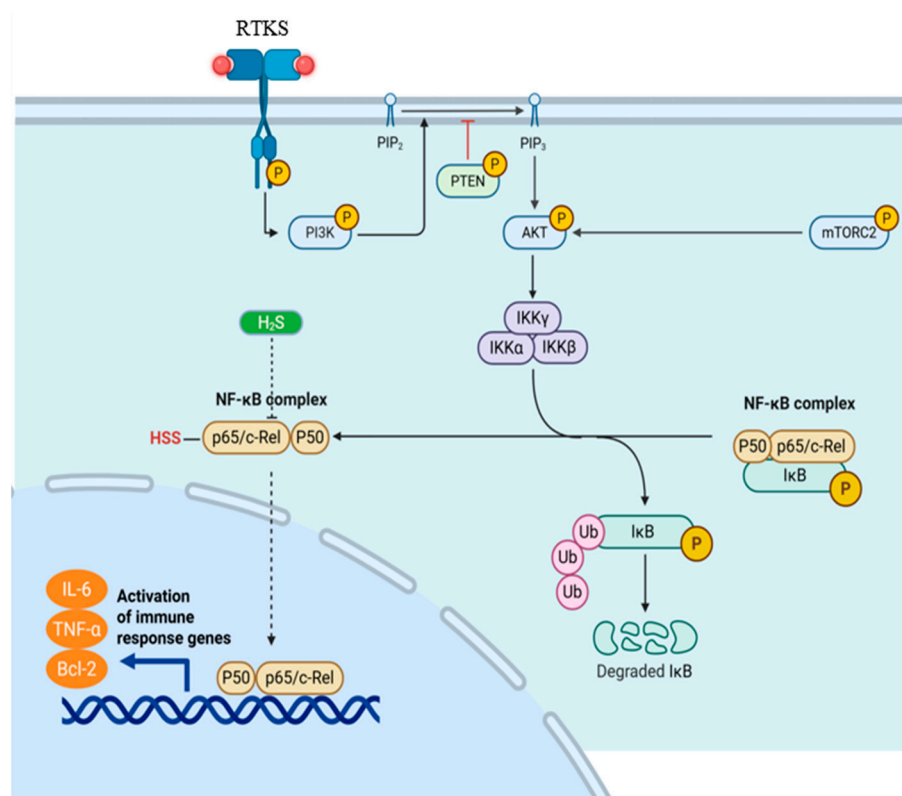
Phosphoinositide 3-kinase (PI3K) is a member of the lipid kinase family. PI3K is classified into three classes (I, II, and III) based on its structure and substrate specificity. Class I PI3K is the most extensively studied and is further divided into the IA and IB subclasses. Class IA PI3K is a heterodimer composed of a catalytic subunit p110 (p110 $\alpha$ , p110 $\beta$ , and p110 $\gamma$ ) and a regulatory subunit p85 (p85 $\alpha$ , p85 $\beta$ , and p85 $\gamma$ ). Class IB PI3K consists of a regulatory subunit p101 and a catalytic subunit p110 $\gamma$  [75,76]. Protein kinase B (Akt) is a member of the serine/threonine kinase family and is a key downstream target of PI3K. It consists of three conserved domains: an N-terminal pleckstrin homology (PH) domain, a central kinase catalytic domain, and a C-terminal hydrophobic motif (HM). Akt has three isoforms: PKB $\alpha$  (Akt1), PKB $\beta$  (Akt2), and PKB $\gamma$  (Akt3). Each isoform exhibits different tissue expression levels. Akt1 is widely expressed across various tissues, whereas Akt2 is highly expressed in insulin-sensitive tissues, and Akt3 is specifically found in the brain, lung, heart, kidney, testis, and skeletal muscle [77,78].

PI3K can be activated via receptor tyrosine kinases (RTKs), G protein-coupled receptors (GPCRs), and Ras-related small G proteins. Activated PI3K converts phosphatidylinositol 4,5-bisphosphate (PIP2) on the cell membrane into the second messenger phosphatidylinositol 3,4,5-trisphosphate (PIP3). The generated PIP3 specifically binds to the PH domain of downstream Akt, promoting the translocation of Akt and 3-phosphoinositide-dependent protein kinase 1 (PDK1) to the cell membrane. Subsequently, PDK1 and mammalian target of rapamycin complex 2 (mTORC2) catalyze the phosphorylation of Akt at Thr-308 and Ser-473, respectively, thereby activating Akt. The activated PI3K/Akt pathway stimulates

the IKK complex, leading to I $\kappa$ B phosphorylation and degradation, promoting NF- $\kappa$ B nuclear translocation, and consequently initiating gene transcription [79].

It is worth noting the substantial evidence that H<sub>2</sub>S-mediated persulfidation of the PI3K–Akt–NF- $\kappa$ B axis plays an important role in maintaining body homeostasis. In a sepsis model, Liu et al. [80] found that exogenous H<sub>2</sub>S administration increased PI3K/Akt phosphorylation and decreased the expression of NF- $\kappa$ B, Bax, and caspase 3. In a septic acute lung injury model [81], exogenous H<sub>2</sub>S improved survival and reduced lung injury in septic mice. Moreover, compared with the model group, GYY4137 decreased p-Akt and p-NF- $\kappa$ B p65 protein expression levels in a dose-dependent manner, a process potentially related to persulfidation modification. Wang et al. [82] established an LPS-induced inflammatory model in bovine mammary epithelial cells and found that exogenous H<sub>2</sub>S activated the PI3K/Akt/NF- $\kappa$ B signaling pathway and regulated LPS-induced inflammation and apoptosis in a concentration-dependent manner. In a portal hypertension model [83], exogenous H<sub>2</sub>S administration led to significantly increased p-Akt and NF- $\kappa$ B levels, decreased I $\kappa$ B $\alpha$  and Bcl-2 levels, and increased apoptosis compared with the model group. In addition, research [84] found that H<sub>2</sub>S promoted LPS-induced osteoblast apoptosis and exacerbated inhibition of osteogenic differentiation by inhibiting the AKT/NF- $\kappa$ B signaling pathway and enhancing the FAS/FASL-mediated death receptor pathway. Furthermore, in an acute pancreatitis model induced by caerulein, Fu et al. [85] found that NaHS treatment inhibited the PI3K/AKT–NF- $\kappa$ B signaling pathway and downregulated proinflammatory cytokine levels, thereby alleviating inflammatory damage to pancreatic acinar cells. In a subsequent rat severe pancreatitis model, Rao et al. [86] observed increased p-Akt expression, decreased I $\kappa$ B $\alpha$  expression, enhanced NF- $\kappa$ B activity, and aggravated inflammation in the model group. Exogenous H<sub>2</sub>S (NaHS treatment) alleviated pancreatic inflammatory damage by inhibiting the PI3K/AKT/NF- $\kappa$ B signaling pathway and downregulating proinflammatory cytokine expression. In a myocardial injury model of sleep apnea syndrome induced by chronic intermittent hypoxia, Zheng et al. [87] found that chronic intermittent hypoxia activated the PI3K/AKT/NF- $\kappa$ B pathway, increased apoptosis and oxidative stress (OS) levels, and exacerbated pathological damage, whereas NaHS reversed these effects. Zheng et al. [88] used a hypoxia–reoxygenation model in mouse atrial cardiomyocytes and found that the model group exhibited reduced cell numbers, increased malondialdehyde (MDA) levels and NF- $\kappa$ B protein expression, and decreased superoxide dismutase (SOD) activity. Pretreatment with NaHS reversed these effects and reduced apoptosis.

Collectively, these studies demonstrate that H<sub>2</sub>S plays an important role in maintaining body homeostasis by mediating persulfidation modification of key proteins in the PI3K/Akt/NF- $\kappa$ B pathway, with its regulatory effects being disease model-dependent. However, current research has notable limitations. Most studies have focused on the intervention effects of exogenous H<sub>2</sub>S, whereas the regulatory mechanisms of endogenous H<sub>2</sub>S-mediated persulfidation of this pathway remain poorly understood. The specific key proteins within the PI3K/Akt/NF- $\kappa$ B pathway that are modified by H<sub>2</sub>S, the precise modification sites, and the modification efficiency have not yet been identified. Moreover, the reasons underlying the bidirectional regulatory effects of H<sub>2</sub>S in different disease models remain insufficiently explored. Additionally, the cross-regulatory relationships between H<sub>2</sub>S-mediated persulfidation and other post-translational modifications such as phosphorylation and acetylation within this pathway have not been elucidated. Furthermore, most existing studies are limited to cellular and animal models, with insufficient clinical evidence. These research gaps restrict a comprehensive understanding of the molecular mechanisms by which H<sub>2</sub>S regulates this pathway and hinder the development and translation of targeted therapeutic strategies based on this modification mechanism (Figure 3).



**Figure 3.** H<sub>2</sub>S regulates the PI3K/Akt/NF-κB signaling pathway through persulfidation modification. Abbreviations: NF-κB: nuclear factor κB; IκB: inhibitor of NF-κB; PI3K: phosphoinositide 3-kinase; AKT: protein kinase B. Created in BioRender. Liang, K. (2026) <https://BioRender.com/w2qn7za> (accessed on 22 July 2026).

#### 4.3. Biological Effects of H<sub>2</sub>S-Mediated Persulfidation of MAPK/NF-κB

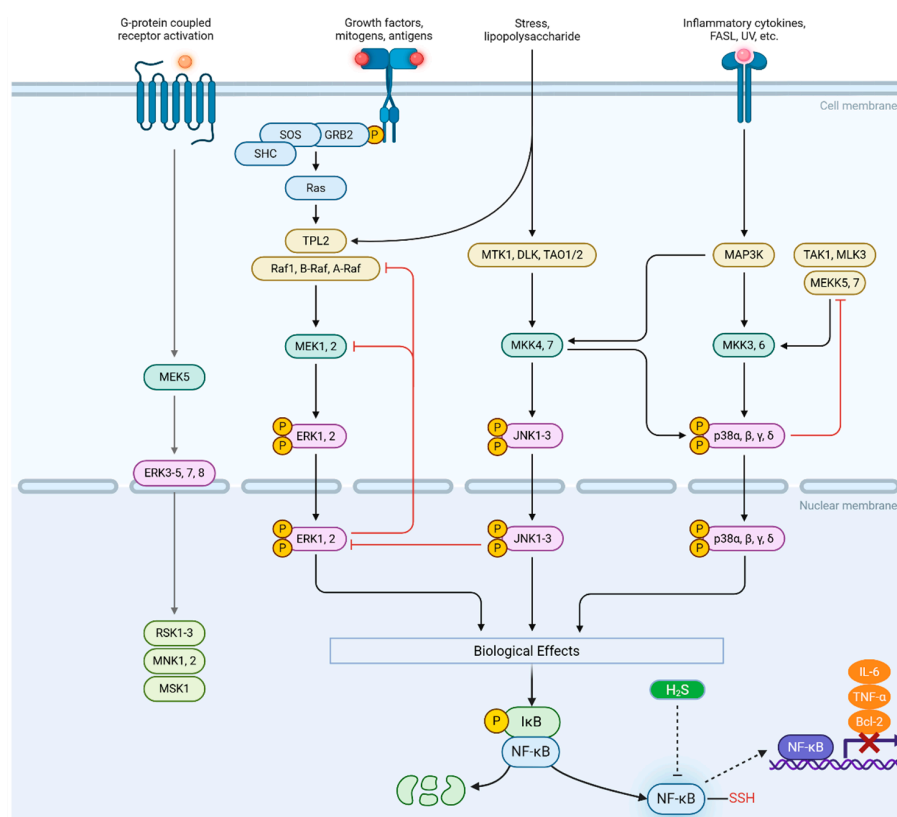
Mitogen-activated protein kinases (MAPKs) play a critical role in transducing signals from the cell surface to the nucleus. To date, 17 MAPK members have been identified in mammals, which are classified into seven subgroups. Among these, the four conventional subgroups are extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), p38 MAPK, and ERK5. The remaining three non-conventional subgroups are ERK3/4, ERK7/8, and Nemo-like kinase (NLK) [89].

The classical MAPK activation pathway strictly follows a three-tiered kinase cascade: MAPK kinase kinase (MAPKKK) → MAPK kinase (MAPKK) → MAPK [90]. Notably, the four conventional subgroups within the classical MAPK pathway can be activated by distinct stimuli, forming different signaling cascades and activating various transcription factors to exert diverse biological effects. Among them, ERK is generally associated with cell growth, whereas JNK and p38 are linked to inflammatory stress or infection [91]. In contrast, the activation pathways of atypical MAPKs do not adhere to the classical three-tiered kinase cascade, but are activated through various mechanisms, including monophosphorylation, autophosphorylation, protein-protein interactions, and ubiquitination. Upon MAPK pathway activation, extracellular signals are transduced into the cell, promoting IκB phosphorylation and ubiquitination-dependent degradation, which allows NF-κB dimers to translocate to the nucleus, activate the NF-κB pathway and promote gene transcription [92,93].

H<sub>2</sub>S plays an important role in protecting the body by S-sulfhydrating the MAPK/NF-κB signaling pathway. In chronic renal failure (CRF) [94], the protein levels of p-ERK, p-JNK, p-p38, and NF-κB were significantly elevated and NaHS intervention suppressed these increases, potentially involving over-vulcanization modification. Furthermore, in an LPS-

induced inflammatory model of rat skeletal muscle cells established by Zhang et al. [95], H<sub>2</sub>S was found to inhibit the phosphorylation of ERK, JNK, and p38 and to reduce NF- $\kappa$ B protein expression. In a study by Chi [96], male broilers exposed to H<sub>2</sub>S gas over different periods showed increased mRNA and phosphorylated protein expression levels of JNK, ERK, and p38, along with increased IKK $\alpha$ / $\beta$  and p65 expression, suggesting that H<sub>2</sub>S exposure causes cumulative damage to the avian immune system following long-term low-dose H<sub>2</sub>S exposure. In an alloxan-induced diabetic model, Song et al. [97] detected significantly higher mRNA expression of p38 MAPK and NF- $\kappa$ B p65 in the diabetic group compared with the control group. NaHS treatment reduced these expression levels, with more pronounced reduction in the high-dose group, suggesting that H<sub>2</sub>S ameliorates diabetic myocardial fibrosis. Li [98] induced NIH3T3 cells to simulate an in vitro pulmonary fibrosis microenvironment and found that exogenous H<sub>2</sub>S markedly inhibited TGF  $\beta$ 1-induced cell proliferation. In addition, NaHS suppressed the activation of the p38 MAPK/NF- $\kappa$ B signaling pathway induced by TGF  $\beta$ 1 and downregulated the transcription of the p38 MAPK and NF- $\kappa$ B genes.

A review of current related studies reveals that most remain at the level of observing pathway protein expression and pathological phenotypes, only preliminarily suggesting that the effects rely on persulfidation modification. The specific modification targets and cysteine modification sites within the MAPK/NF- $\kappa$ B pathway have not yet been identified. The intrinsic molecular mechanisms underlying the opposite biological effects of H<sub>2</sub>S at different concentrations and durations of exposure remain insufficiently explained. Moreover, there is a lack of systematic mechanistic validation and in vivo targeted intervention evidence. The relevant pathway networks and upstream/downstream cross-regulatory relationships still require further in-depth investigation and clarification (Figure 4).



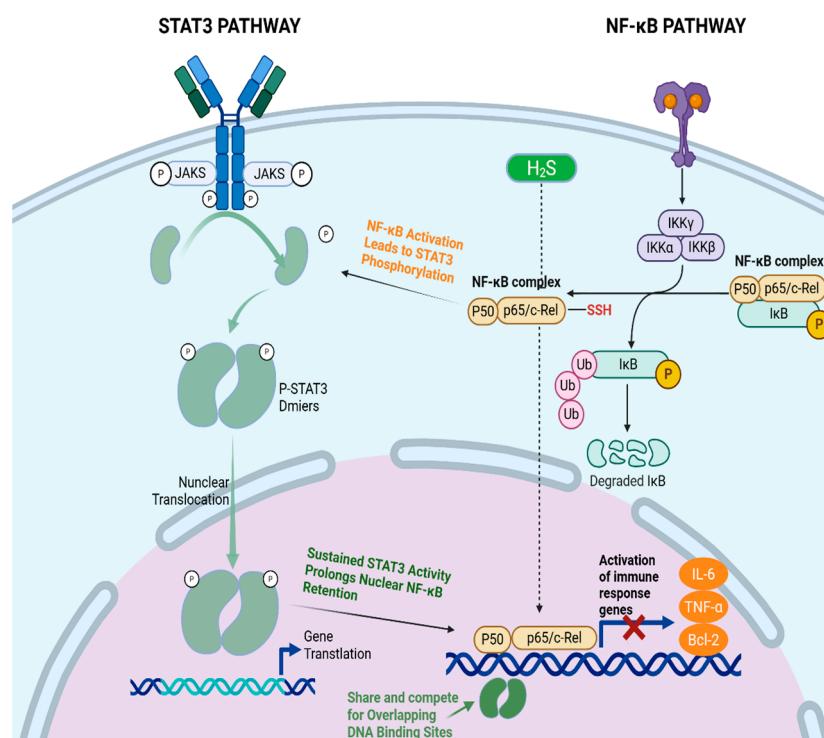
**Figure 4.** H<sub>2</sub>S regulates the MAPK/NF- $\kappa$ B signaling pathway through persulfidation modification. Abbreviations: NF- $\kappa$ B: nuclear factor  $\kappa$ B; I $\kappa$ B: inhibitor of NF- $\kappa$ B. Created in BioRender. Liang, K. (2026) <https://BioRender.com/w2qn7za> (accessed on 22 July 2026).

#### 4.4. Biological Effects of H<sub>2</sub>S-Mediated Persulfidation of STAT3/NF- $\kappa$ B

The signal transducer and activator of transcription (STAT) family consists of seven members: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. STAT family proteins respond to various extracellular growth factors and cytokines and are a class of SH2-containing signaling molecules that bind to phosphorylated tyrosine residues [99]. Among them, STAT3 is the most extensively studied. In the resting state, STAT3 exists in an inactive form in the cytoplasm. Upon stimulation by extracellular cytokines or growth factors, these factors bind to their cognate receptors, inducing conformational changes in the receptors, which in turn activate the non-receptor tyrosine kinases Janus kinases (JAK1, JAK2, JAK3, TYK2). The activated JAKs phosphorylate the receptors, creating binding sites for the SH2 domain of STAT3, promoting STAT3 binding, followed by its phosphorylation at Tyr-705 by JAKs. Subsequently, phosphorylated STAT3 dimerizes and translocates into the nucleus to initiate gene transcription [100]. STAT3 and NF- $\kappa$ B are closely interconnected. Activation of NF- $\kappa$ B can lead to STAT3 phosphorylation and nuclear translocation. Conversely, persistently activated STAT3 can prolong the nuclear retention of NF- $\kappa$ B, thereby maintaining its activity. Furthermore, they can even share or compete for overlapping DNA binding sites on the promoters of certain genes [101].

Studies have shown that H<sub>2</sub>S can alleviate inflammatory damage in the body through persulfidation of the STAT3/NF- $\kappa$ B signaling pathway. In a study by Dan et al. [102], exogenous H<sub>2</sub>S administration to mouse hepatocellular carcinoma cells decreased STAT3/NF- $\kappa$ B phosphorylation, thereby downregulating IDO1 expression and inhibiting tumor growth. In a cisplatin-induced kidney injury model, Sun et al. [103] found that STAT3/IKK $\beta$  phosphorylation and NF- $\kappa$ B nuclear translocation were increased compared with the control group. H<sub>2</sub>S inactivated IKK $\beta$  through sulfhydrylation at cysteine 179, thereby inhibiting NF- $\kappa$ B activation and the subsequent inflammatory response, thus alleviating cisplatin nephrotoxicity. In a caerulein-induced acute pancreatitis and secondary lung injury model, Mathan et al. [104] found that exogenous H<sub>2</sub>S reduced serum amylase, pancreatic and pulmonary myeloperoxidase (MPO) activity, and alleviated tissue edema and inflammatory cell infiltration while significantly downregulating TNF- $\alpha$  protein and mRNA levels. Furthermore, caerulein promoted I $\kappa$ B $\alpha$  degradation, enhanced NF- $\kappa$ B p65 DNA binding activity, increased STAT3 phosphorylation levels, and decreased SOCS3 protein and mRNA levels. Treatment with DAD activated PPAR $\gamma$  and upregulated SOCS3, thereby inhibiting STAT3 phosphorylation, while also reducing I $\kappa$ B $\alpha$  degradation and suppressing NF- $\kappa$ B activation. In an atherosclerosis model established by Ou et al. [105], ox-LDL treatment led to intracellular lipid droplet deposition, decreased cell viability, increased proinflammatory cytokine release, elevated OS levels, and upregulated pNF- $\kappa$ B p65, p JAK2, and p STAT3 protein levels. Exogenous H<sub>2</sub>S reversed these changes. Li et al. [106] found that exogenous H<sub>2</sub>S reduced reactive oxygen species (ROS) levels and STAT3/NF- $\kappa$ B phosphorylation in a hypoxia–reoxygenation model, suggesting that exogenous H<sub>2</sub>S protects aged cardiomyocytes during ischemic postconditioning, potentially through persulfidation modification.

In summary, existing studies have clarified the core mechanism by which H<sub>2</sub>S regulates the STAT3/NF- $\kappa$ B signaling pathway through persulfidation modification and have validated its protective effects in various disease models, including cancer, kidney injury, pancreatitis, atherosclerosis, and myocardial ischemia, providing new ideas and targets for the treatment of related diseases. However, the molecular details of the persulfidation modification remain unclear. Only one modification site on IKK $\beta$  has been identified. The modification sites, modes, and conformational changes of core proteins such as STAT3 and NF- $\kappa$ B p65, as well as the upstream and downstream regulatory factors associated with this modification, have not yet been determined (Figure 5).



**Figure 5.** H<sub>2</sub>S regulates the STAT3/NF-κB signaling pathway through persulfidation modification. Abbreviations: NF-κB: nuclear factor κB; IκB: inhibitor of NF-κB; STAT: signal transducer and activator of transcription. Created in BioRender. Liang, K. (2026) <https://BioRender.com/w2qn7za> (accessed on 22 July 2026).

#### 4.5. Biological Effects of H<sub>2</sub>S-Mediated Persulfidation of Nrf2/NF-κB

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a member of the cap-n-collar (CNC) basic leucine zipper (bZIP) transcription activator family. It contains a basic leucine zipper (bZIP) structure and six functional domains (Neh1–Neh6). The Nrf2 protein comprises multiple functional regions: the Neh1 domain binds to nuclear small Maf (sMaf) proteins to form a complex that recognizes the antioxidant response element (ARE) and initiates downstream gene transcription; the Neh2 domain is primarily responsible for binding to Keap1 protein; the Neh3 domain, located at the carboxyl terminus of Nrf2, activates downstream gene transcription; the Neh4 and Neh5 domains participate in regulating Nrf2 transcriptional activity by interacting with CREB protein; and the Neh6 domain, rich in serine residues, regulates Nrf2 degradation in a Keap1 independent manner, thereby inhibiting its function. Keap1 (Kelch-like ECH-associated protein 1) is an inhibitor of Nrf2 and plays a critical role in regulating Nrf2 activity [107]. Under resting conditions, Nrf2 forms a complex with Keap1 in the cytoplasm, and Keap1 acts as an adaptor for Cullin3-based ubiquitin ligase, promoting Nrf2 ubiquitination and degradation. However, under stress conditions, cysteine residues of Keap1 are oxidized, leading to conformational changes in Keap1, which reduce Nrf2 ubiquitination levels. Nrf2 is then released from the complex and translocates to the nucleus, where it binds to the ARE in association with cofactors (e.g., sMAF) and activates downstream target genes, upregulating the expression of nicotinamide adenine dinucleotide phosphate (NADPH), NAD(P)H: quinone oxidoreductase 1 (NQO1), glutathione S-transferase (GST), heme oxygenase-1 (HO-1), and superoxide dismutase (SOD), among others [108].

Under normal conditions, Nrf2 and NF-κB coordinate and interact with each other to maintain cellular homeostasis. However, this balance is disrupted under pathological conditions. Nrf2 upregulates antioxidant enzymes and GSH levels, neutralizing excess reactive oxygen species (ROS), thereby suppressing NF-κB activation. Conversely, when Nrf2 is

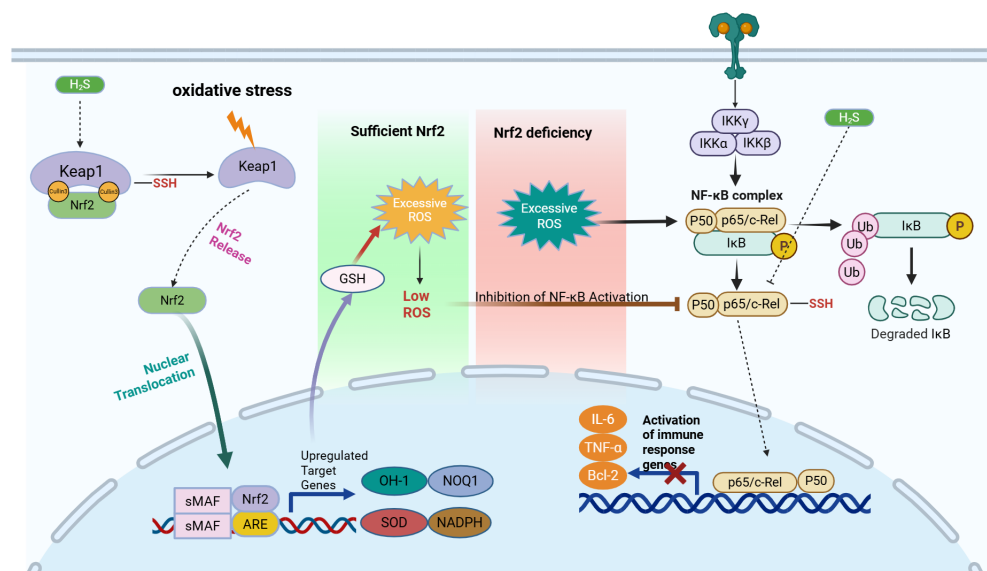
deficient, excess ROS cannot be neutralized, leading to increased I $\kappa$ B $\alpha$  phosphorylation and subsequent degradation, ultimately promoting the transcription of proinflammatory cytokines. In addition, Keap1 can directly bind to IKK $\beta$  and link to the CUL3 RBX1 ubiquitin ligase complex, regulating IKK $\beta$  ubiquitination and degradation, thereby activating a cascade of downstream responses [109]. Numerous studies have demonstrated that H<sub>2</sub>S-mediated persulfidation of the Nrf2–NF- $\kappa$ B axis plays an important role in antioxidative stress. For example, in a uranium poisoning model established by Wei et al. [110], NaHS treatment restored uranium-induced abnormalities in blood urea nitrogen and creatinine, decreased malondialdehyde (MDA) content, increased GSH levels and antioxidant enzyme activities, promoted Nrf2 nuclear translocation, upregulated the expression of downstream antioxidant enzymes HO-1, NQO1, GCLC, and TXNRD 1, reduced NF- $\kappa$ B p65 nuclear translocation, and alleviated uranium-induced renal histopathological changes. In a subsequent kidney injury model, Waz et al. [111] found that compared with the control group, the model group exhibited significantly elevated serum urea and creatinine, renal tubular degeneration and dilation, decreased p Nrf2 and HO-1 expression, and increased NF- $\kappa$ B expression. NaHS treatment reversed these changes and ameliorated pathological damage, suggesting that exogenous H<sub>2</sub>S plays a protective role against OS, with a mechanism possibly involving S-sulfhydration. Furthermore, in an alloxan-induced diabetic model [112], NaHS improved blood glucose, blood lipids, and insulin levels in rats while decreasing MDA and NO, increasing SOD, CAT, and GPx activities and Nrf2 levels, inhibiting NF- $\kappa$ B p65 activity, and alleviating diabetes-induced uterine pathological damage. In a human gastric epithelial cell ischemia–reperfusion (I–R) model, Guo et al. [113] found that NaHS intervention reduced I–R-induced elevations in ROS and MDA, increased GSH levels, inhibited NF- $\kappa$ B p65 nuclear translocation, and promoted Keap1 persulfidation, thereby facilitating Nrf2 nuclear translocation and ultimately alleviating I–R injury in gastric epithelial cells. In a study by Xie et al. [114] using streptozotocin (STZ)-induced diabetic LDLr<sup>−/−</sup> mice, GYY4137 treatment significantly reduced aortic plaque area. However, the protective effect of H<sub>2</sub>S was completely absent in Nrf2-knockout (LDLr<sup>−/−</sup> Nrf2<sup>−/−</sup>) mice or cells, indicating that H<sub>2</sub>S requires Nrf2 to exert its protective effects. Moreover, exogenous H<sub>2</sub>S S-sulfhydrated Keap1 at Cys151, thereby disrupting the Keap1–Nrf2 complex, activating the Nrf2 signaling pathway, upregulating the expression of downstream antioxidant genes (GCLC, GCLM, GR), increasing GSH levels, counteracting OS, regulating cellular senescence, and ultimately slowing the progression of diabetes-accelerated atherosclerosis.

In summary, H<sub>2</sub>S plays an important role in models of kidney injury, diabetes, atherosclerosis, and ischemia–reperfusion through persulfidation modification of the Nrf2/NF- $\kappa$ B signaling pathway, providing experimental evidence for the protection against these diseases (Figure 6).

#### 4.6. Biological Effects of H<sub>2</sub>S-Mediated Persulfidation of SIRT/NF- $\kappa$ B

Sirtuins (SIRT) are a family of histone deacetylases. To date, seven SIRT family proteins have been identified (SIRT1 to SIRT7), each with distinct subcellular localizations. SIRT1 and SIRT2 are found in the nucleus and cytoplasm, SIRT3, SIRT4, and SIRT5 are localized in mitochondria, and SIRT6 and SIRT7 are located in the nucleus. Furthermore, they participate in different physiological processes. SIRT1 is the most extensively studied and primarily functions in DNA repair, maintenance of cardiovascular homeostasis, anti-inflammation, and antioxidative stress. SIRT2 plays a role in lipid metabolism and is also closely associated with anti-inflammatory and antioxidative stress processes. SIRT3 mainly functions in mitochondria as a key regulator of mitochondrial metabolism, enhancing cellular energy metabolism efficiency and improving mitochondrial function. SIRT4 plays a critical role in suppressing tumor formation and promoting autophagy: it limits cancer cell

proliferation and reduces the probability of tumorigenesis. SIRT5 is important in fatty acid metabolism and liver protection: it improves hepatic metabolic function and reduces fat accumulation in the liver, exerting positive effects on the prevention and alleviation of fatty liver disease. SIRT6 is primarily involved in glucose metabolism and insulin regulation: it promotes insulin release, helps regulate blood glucose levels, and prevents hyperglycemia and diabetes. SIRT6 also has anti-inflammatory and antioxidative stress effects. SIRT7 mainly functions in cardioprotection and cellular homeostasis: it protects cardiomyocytes from OS and inflammatory injury and is also closely linked to DNA repair [115,116].

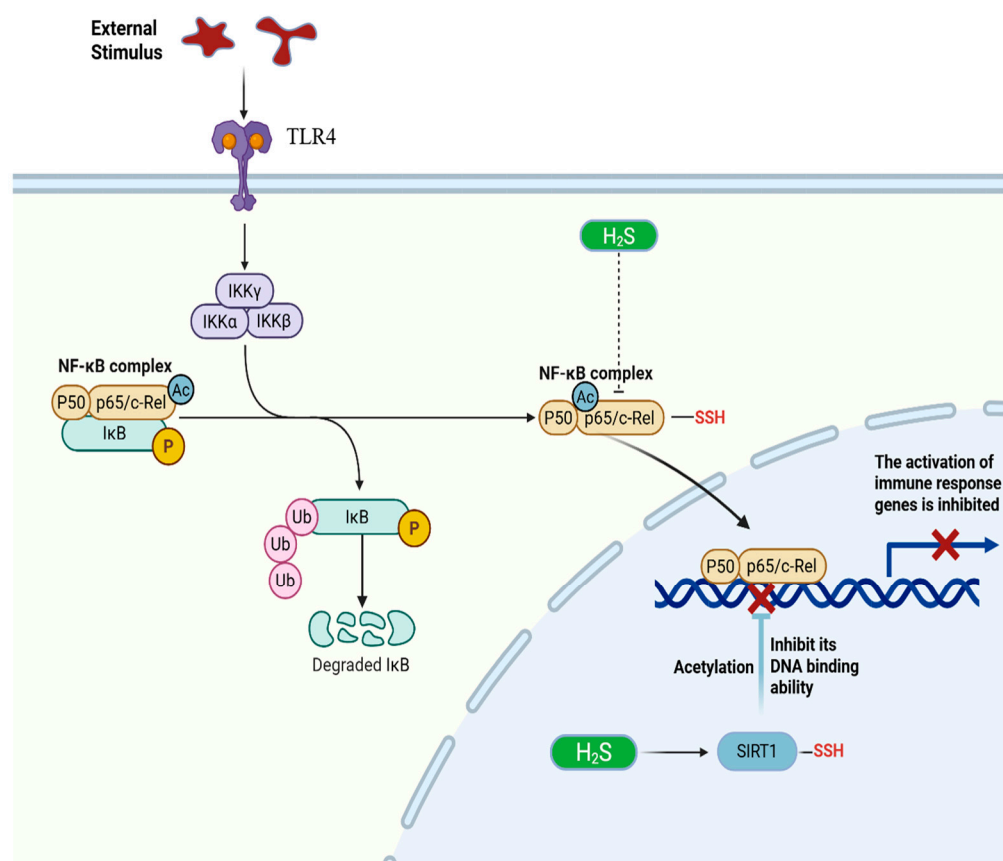


**Figure 6.** H<sub>2</sub>S regulates the Nrf2/NF-κB signaling pathway through persulfidation modification. Abbreviations: NF-κB: nuclear factor κB; IκB: inhibitor of NF-κB; Nrf2: nuclear factor erythroid 2-related factor 2; Keap1: Kelch-like ECH-associated protein 1. Created in BioRender. Liang, K. (2026) <https://BioRender.com/w2qn7za> (accessed on 22 July 2026).

SIRT1s primarily inhibit NF-κB activity by deacetylating the p65 subunit of NF-κB. When cells are stimulated by external signals, IκB kinase (IKK) phosphorylates IκB, leading to IκB ubiquitination and proteasomal degradation, which releases NF-κB and allows its translocation to the nucleus to activate proinflammatory gene transcription. SIRT1 suppresses the DNA binding ability and transcriptional activity of nuclear p65 through deacetylation, thereby downregulating proinflammatory cytokines [117]. In recent years, numerous studies have shown that H<sub>2</sub>S plays an important role in anti-inflammation and antioxidative stress by S-sulphydrating the SIRT1/NF-κB signaling pathway [118]. In a diabetic model of mouse hippocampal neurons, Li et al. [119] found that compared with the control group, the model group exhibited significantly increased levels of inflammatory cytokines such as IL-1β and IL-6 and increased phosphorylation of NF-κB p65, along with decreased SIRT1 expression. NaHS treatment reversed these effects and alleviated the inflammatory response. In a subsequent study using a trimethylamine N-oxide (TMAO)-induced mouse macrophage inflammation model, Liu et al. [120] found that exogenous H<sub>2</sub>S inhibited TMAO-induced p65 NF-κB phosphorylation and increased persulfidation levels of SIRT1. Moreover, NaHS reduced the expression of proinflammatory cytokines such as IL-1β and IL-6 and increased the levels of anti-inflammatory cytokines such as IL-4 and IL-10. In addition, El Boghdady et al. [121] used a cuprizone-induced mouse model of multiple sclerosis and found that the cuprizone group showed decreased levels of myelin basic protein (MBP), oligodendrocyte transcription factor 2 (Olig2), and ciliary neurotrophic factor (CNTF), as well as decreased expression of autophagy proteins such as ULK1 and beclin1 and SIRT1 protein. Regarding OS, the cuprizone group exhibited decreased GSH

and TAC levels and increased NF- $\kappa$ B expression. Treatment with diallyl disulfide (DADS) or S-adenosyl L-methionine (S-AMe) enhanced autophagy protein expression, improved antioxidant capacity, inhibited inflammatory factors, and restored and improved motor coordination and myelin structure. Du et al. [122] established an atherosclerosis model and found that NaHS and GYY4137 significantly reduced plaque area in ApoE<sup>−/−</sup> mice. Exogenous H<sub>2</sub>S S-sulfhydrated SIRT1 at Cys371/374 and Cys395/398, leading to deacetylation of the NF- $\kappa$ B p65 subunit, inhibition of its activity, reduced expression of inflammatory cytokines, and suppression of atherosclerosis progression. Yuan et al. [123] found that H<sub>2</sub>S S-sulfhydrated SIRT1, reducing the acetylation level of FOXO1, significantly increasing the expression of antioxidant enzymes such as MnSOD and CAT, and ultimately reducing intracellular ROS levels, thereby counteracting OS and delaying cellular senescence. Furthermore, in a cisplatin-induced acute kidney injury model, Yuan et al. [124] found that H<sub>2</sub>S S-sulfhydrated SIRT3 at Cys256, Cys259, Cys280, Cys283, and other sites, enhancing its deacetylase activity, improving mitochondrial morphology, function, and antioxidant capacity and ameliorating renal histopathological damage, thereby alleviating cisplatin-induced kidney injury.

The above studies indicate that H<sub>2</sub>S plays an important role in diabetes, cellular inflammatory responses, atherosclerosis, and antioxidative stress through persulfidation modification of the SIRT/NF- $\kappa$ B signaling pathway, providing new targets and therapeutic strategies for the treatment of diseases such as diabetes, neuroinflammation, and multiple sclerosis (Figure 7).



**Figure 7.** H<sub>2</sub>S regulates the SIRT/NF- $\kappa$ B signaling pathway through persulfidation modification. Abbreviations: NF- $\kappa$ B: nuclear factor  $\kappa$ B; I $\kappa$ B: inhibitor of NF- $\kappa$ B; SIRT: sirtuin (silent information regulator). Created in BioRender. Liang, K. (2026) <https://BioRender.com/w2qn7za> (accessed on 22 July 2026).

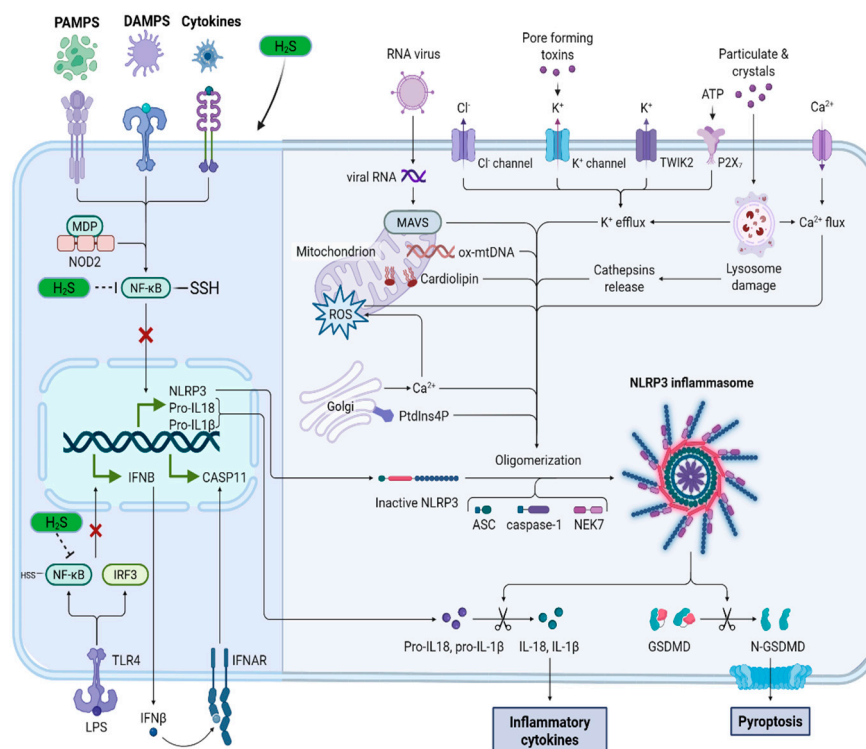
#### 4.7. Biological Effects of H<sub>2</sub>S-Mediated Persulfidation of NLRP3/NF- $\kappa$ B

NOD-like receptor protein 3 (NLRP3) is composed of a cytoplasmic multiprotein complex and resides in the cytoplasm [125]. It contains three domains: a C-terminal leucine-rich repeat (LRR) domain, an N-terminal pyrin domain (PYD), and a central nucleotide binding and oligomerization domain (NACHT) [126]. The activation of NLRP3 occurs primarily through canonical and non-canonical pathways. The canonical pathway is initiated by pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), or inflammatory factors, which activate NF- $\kappa$ B signaling by binding to cell surface receptors, upregulating NLRP3 and other proteins to complete the priming step. Subsequently, signals such as potassium efflux, mitochondrial reactive oxygen species (mtROS) released from damaged mitochondria, and cathepsin B released from ruptured lysosomes activate NLRP3, leading to the assembly of a complex with apoptosis-associated speck-like protein containing a CARD (ASC) and pro-caspase 1, ultimately activating caspase 1 and triggering pyroptosis [127]. The non-canonical pathway is primarily mediated by lipopolysaccharide (LPS). LPS enters the cytoplasm directly and binds to and activates pro-caspase 11 (or caspase 4/5 in humans), thereby inducing pyroptosis [128].

Wang et al. [129] established a palmitic acid (PA)-induced inflammatory model in human hepatocellular carcinoma cells and found that NaHS treatment significantly inhibited PA-induced inflammatory cytokine expression and release and dose-dependently suppressed NLRP3 inflammasome expression, NF- $\kappa$ B pathway activation, and p65 nuclear translocation, thereby alleviating hepatocyte inflammation. In an LPS-induced mouse depression model, Bao et al. [130] found that exogenous H<sub>2</sub>S inhibited LPS-induced NF- $\kappa$ B p65 phosphorylation in the hippocampus, as well as the protein expression of NLRP3, ASC, and caspase 1. It also reduced LPS-induced GSDMD N protein expression and the number of TUNEL positive cells in the hippocampal CA1, CA3, and DG regions. Furthermore, NaHS ameliorated LPS-induced mitochondrial swelling, blurred cristae structure, and improved membrane potential and ATP production. These findings suggest that exogenous H<sub>2</sub>S prevents LPS-induced depression-like behavior by inhibiting activation of the hippocampal NF- $\kappa$ B/NLRP3 inflammasome pathway, reducing pyroptosis, and improving mitochondrial morphology and function, with the mechanism potentially involve over-vulcanization modification. Wu [131] established an LPS+ATP model and found that NaHS pretreatment significantly reduced LPS+ATP-induced expression of NF- $\kappa$ B and NLRP3 inflammasome related proteins. In addition, exogenous H<sub>2</sub>S reversed LPS+ATP-induced autophagy inhibition, thereby alleviating the inflammatory response. Huang et al. [132] used rat embryonic cardiomyocyte cell line H9c2 to establish a high-glucose-induced cardiotoxicity model and found that the high-glucose group exhibited decreased cell viability, increased apoptosis, elevated ROS levels, and decreased mitochondrial membrane potential. Moreover, NF- $\kappa$ B, NLRP3 inflammasome, and downstream inflammatory factors were upregulated. NaHS pretreatment reversed these effects, thereby alleviating high-glucose-induced cardiomyocyte inflammation and apoptosis. Furthermore, Li et al. [81] established a sepsis model and found that exogenous H<sub>2</sub>S significantly inhibited the expression of pNF- $\kappa$ B, NLRP3, and downstream inflammatory factors while markedly improving mouse survival and alleviating histopathological conditions such as pulmonary edema and vascular leakage. In further cellular experiments, GYY4137 similarly inhibited NLRP3 inflammasome release and reduced the cellular inflammatory response. In an LPS/ATP-induced macrophage pyroptosis model, Ma et al. [133] found that GYY4137 reduced the expression of NLRP3, ASC, and GSDMD, restored LPS/ATP-induced mitochondrial structural damage, decreased membrane potential, and reduced respiratory chain complex activity. In a subsequent mouse model of osteoarthritis, GYY4137 treatment alleviated pathological damage and cartilage degradation, inhibited caspase 1 expression in the synovium, and reduced p65

phosphorylation and its DNA binding activity through persulfidation at Cys38 of p65, ultimately inhibiting synovial macrophage pyroptosis, alleviating cartilage degradation, and slowing osteoarthritis (OA) progression.

To date, it has been demonstrated in various pathological models, including liver inflammation, neuropsychiatric injury, myocardial glycolipid toxicity injury, sepsis, and osteoarthritis, that H<sub>2</sub>S, using persulfidation as the core regulatory mechanism, targets and modifies key sites of NF- $\kappa$ B, inhibits NLRP3 inflammasome assembly and activation at the transcriptional level and simultaneously modulates mitochondrial function, autophagy, and pyroptosis, thereby achieving multi-target anti-inflammatory organ protection (Figure 8).



**Figure 8.** H<sub>2</sub>S regulates the NLRP3/NF- $\kappa$ B signaling pathway through persulfidation modification. Abbreviations: NF- $\kappa$ B: nuclear factor  $\kappa$ B; I $\kappa$ B: inhibitor of NF- $\kappa$ B; NLRP3: NOD-like receptor protein 3. Created in BioRender. Liang, K. (2026) <https://BioRender.com/w2qn7za> (accessed on 22 July 2026).

#### 4.8. Biological Effects of H<sub>2</sub>S-Mediated Persulfidation of TNF- $\alpha$ /NF- $\kappa$ B

TNF- $\alpha$  is a cytokine of the TNF superfamily, composed of 157 amino acids and featuring a unique trimeric structure. TNF- $\alpha$  exerts its effects by binding to two distinct receptors: type I tumor necrosis factor receptor (TNFR1) and type II tumor necrosis factor receptor (TNFR2). TNFR1 recruits various adaptor proteins, including TNFR-associated death domain (TRADD), TNFR-associated factor 2 or 5 (TRAF2/5), receptor-interacting protein (RIP) kinase, and inhibitors of apoptosis proteins (IAPs), to form a complex. This complex subsequently activates IKK, which then activates the NF- $\kappa$ B pathway by degrading the I $\kappa$ B complex, thereby releasing the p50 subunit. p50 translocates to the nucleus and directly regulates gene expression. TNFR2, unlike TNFR1, lacks a death domain and therefore cannot regulate apoptosis. However, activated TNFR2 recruits the adaptor proteins TRAF2 and IAPs and can also activate NF- $\kappa$ B signaling via the canonical pathway. In addition, TNFR2, TRAF2, and IAPs can form a complex with NIK. Upon stimulation, NIK is released from the complex and activated. Active NIK induces the non-canonical NF- $\kappa$ B pathway through IKK $\alpha$ , ultimately generating the transcriptionally active p52 subunit and inducing gene transcription [134,135]. It is noteworthy that NF- $\kappa$ B activation requires stimulation by TNF- $\alpha$ , and activated NF- $\kappa$ B promotes the transcription of proinflammatory cytokines

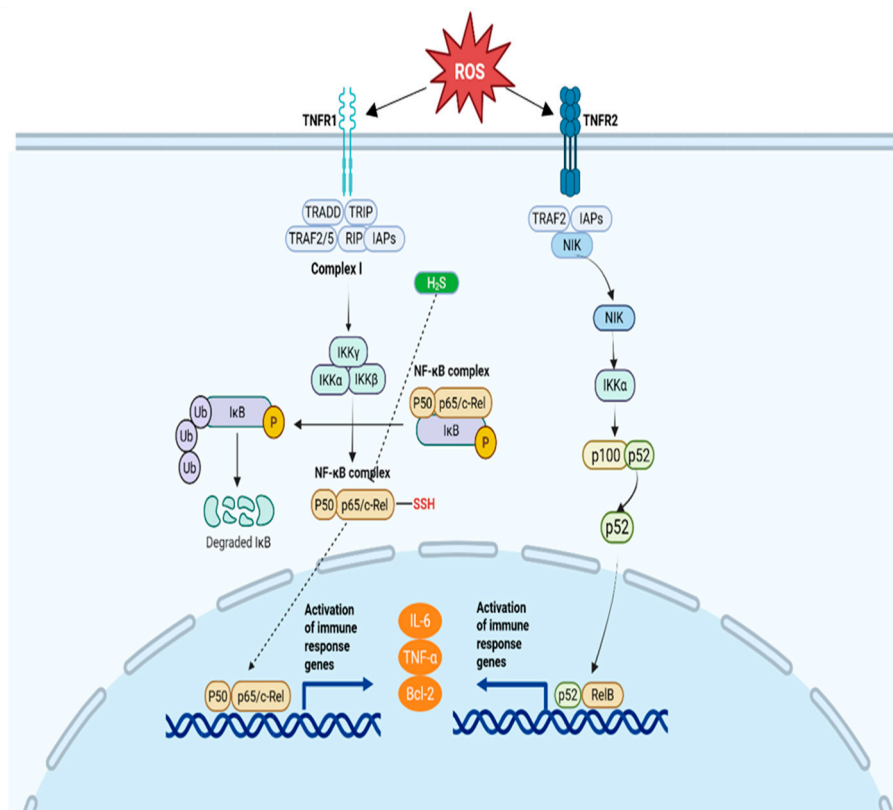
such as TNF- $\alpha$ , which in turn restimulates the NF- $\kappa$ B pathway. This creates a vicious cycle that continuously amplifies the inflammatory cascade. Numerous studies have indicated that H<sub>2</sub>S alleviates the body's inflammatory response through S-sulphydration modification [136].

Du et al. [137] established a macrophage atherosclerosis model and found that oxidized low-density lipoprotein (ox-LDL) increased the levels of monocyte chemoattractant protein 1 (MCP-1), TNF- $\alpha$ , and migration inhibition factor (MIF), whereas NaHS reversed this effect. Exogenous H<sub>2</sub>S S-sulphydrated the p65 subunit at Cys38, inhibiting its phosphorylation and I $\kappa$ B $\alpha$  activation, thereby suppressing NF- $\kappa$ B activity and alleviating inflammatory damage. Chen et al. [138] established a urogenital sepsis model in male rabbits and found that TNF- $\alpha$  and NF- $\kappa$ B expression in renal tissue were elevated in the sepsis group. NaHS treatment reduced TNF- $\alpha$  and NF- $\kappa$ B expression and also alleviated renal histopathological damage, with the mechanism potentially involving over-vulcanization modification. In an irritable bowel syndrome model, Yang et al. [139] found that the infection group exhibited increased levels of inflammatory cytokines including TNF- $\alpha$ , elevated MDA, and decreased T-AOC and GSH-Px activities. Infection also activated NF- $\kappa$ B and reduced I $\kappa$ B $\alpha$ /I $\kappa$ B $\beta$  levels. NaHS treatment significantly alleviated inflammatory infiltration, reduced the expression of certain inflammatory cytokines and TNF- $\alpha$ , inhibited NF- $\kappa$ B activity, and upregulated I $\kappa$ B $\alpha$ , ultimately attenuating the inflammatory response and oxidative damage. Liu et al. [140] established an acute myocardial ischemia model and found that exogenous H<sub>2</sub>S ameliorated the pathological damage induced by myocardial ischemia, including disordered myocardial fiber arrangement, mitochondrial swelling, and cristae loss while also reducing serum levels of TNF- $\alpha$  and other inflammatory cytokines. Furthermore, NF- $\kappa$ B expression was significantly increased in the ischemia group and NaHS treatment reduced its expression, suggesting that S-sulphydration modification may play an important role. Zhang et al. [141] used a monocrotaline-induced rat pulmonary hypertension model and found that H<sub>2</sub>S S-sulphydrated IKK $\beta$  at Cys179 and p65 at Cys38, directly inhibiting IKK $\beta$  activity and blocking the NF- $\kappa$ B signaling pathway, thereby reducing intracellular inflammatory cytokine and TNF- $\alpha$  levels. However, when the Cys179 site was mutated, the above changes did not occur. Thus, H<sub>2</sub>S can alleviate pulmonary artery endothelial cell inflammation, vascular remodeling, and pulmonary hypertension by S-sulphydrating the TNF- $\alpha$ /NF- $\kappa$ B signaling pathway (Figure 9).

#### 4.9. Biological Effects of H<sub>2</sub>S-Mediated Persulfidation of IL/NF- $\kappa$ B

Interleukins (ILs) are a family of cytokines secreted by immune cells and play essential roles in inflammatory responses, immune cell differentiation and activation, and the overall homeostasis of the immune system. To date, at least 38 interleukins have been identified and classified into six major families based on structure and function: IL-1, IL-2, IL-6, IL-10, IL-12, and IL-17 [142]. The IL-1 family is primarily derived from mononuclear phagocytes, as well as lymphocytes, endothelial cells, and keratinocytes. At low concentrations, IL-1 mainly exerts immunomodulatory effects, whereas at high concentrations it induces inflammatory responses [143]. The IL-2 family is mainly produced by CD4<sup>+</sup> T cells and plays an immunomodulatory role, suppressing immune responses at low concentrations and promoting them at high concentrations [144]. The IL-6 family is produced by monocytes/macrophages, endothelial cells, adipocytes, and hematopoietic cells, and plays critical roles in inflammation regulation, immune responses, and hematopoiesis. It transmits downstream signals through three pathways: classical signaling, trans signaling, and trans presentation signaling [145]. The IL-10 family is produced by Th2 cells, mononuclear macrophages, B cells, and epithelial cells. It exerts anti-inflammatory effects by inhibiting the secretion of proinflammatory cytokines from monocytes and macrophages and also

modulates immune responses by suppressing Th1 and Th17 cell responses while promoting Th2 responses [146]. The IL-12 family is produced by dendritic cells, macrophages, neutrophils, and human B lymphoblasts and plays important roles in regulating innate and adaptive immune responses [147]. The IL-17 family is secreted by CD4<sup>+</sup> T cells and primarily functions to activate tissue responses and guide neutrophil-mediated immune defense [148].



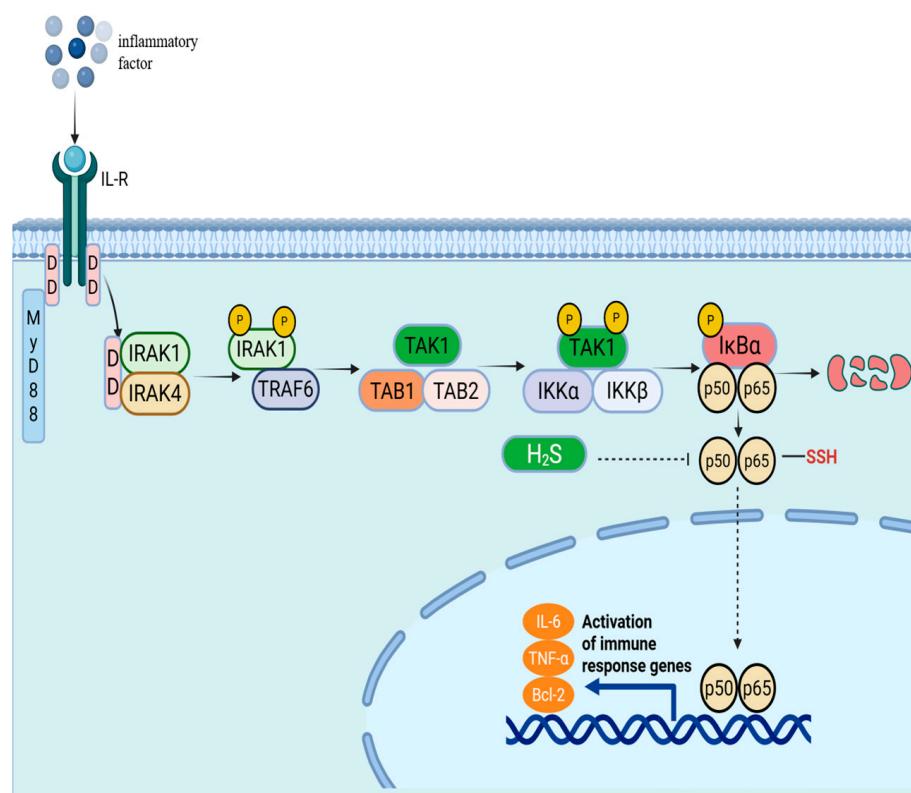
**Figure 9.** H<sub>2</sub>S regulates the TNF- $\alpha$ /NF- $\kappa$ B signaling pathway through persulfidation modification. Abbreviations: NF- $\kappa$ B: nuclear factor  $\kappa$ B; I $\kappa$ B: inhibitor of NF- $\kappa$ B; TNFR1: type I tumor necrosis factor receptor; TNFR2: type II tumor necrosis factor receptor. Created in BioRender. Liang, K. (2026) <https://BioRender.com/w2qn7za> (accessed on 22 July 2026).

ILs primarily activate the NF- $\kappa$ B signaling pathway through the canonical pathway. For example, IL-1 $\beta$  binds to the IL-1 receptor (IL-1R) and forms a complex with IRKA1 and IRKA4 by recruiting the death domain of the adaptor protein MyD88. IRKA4 phosphorylates IRKA1, which then dissociates from the complex and binds to TRAF6, thereby activating and synthesizing K63 ubiquitin chains. TAK1/TAB1/TAB2 use these ubiquitin chains as a scaffold to activate IKK, ultimately leading to the release of the p50/p65 dimer, which translocates into the nucleus and initiates gene transcription [149].

Xu et al. [150] established a high-glucose-induced injury model and found that glucose treatment significantly increased p-NF- $\kappa$ B p65 levels as well as IL-1 $\beta$  and IL-6 levels. NaHS pretreatment reversed this effect, increased cell viability, reduced the number of apoptotic cells and cleaved caspase 3 expression. In addition, exogenous H<sub>2</sub>S reduced ROS production and maintained mitochondrial membrane potential, thereby alleviating high-glucose-induced cardiac injury. In an aortic valve calcification model established by Éva and colleagues [151], IL-1 $\beta$  levels in calcified tissues were significantly higher than those in the control group, and NF- $\kappa$ B protein expression was also increased. H<sub>2</sub>S inhibited the activation of the NF- $\kappa$ B signaling pathway, preventing the nuclear translocation of the osteogenic transcription factor Runx2, thereby blocking the transformation of aortic valve

interstitial cells into osteoblast-like cells and inhibiting valve calcification. This process is speculated to involve persulfidation modification. In a study by Xu et al. [152], doxorubicin (DOX) upregulated NF- $\kappa$ B p65 expression in cardiomyocytes, significantly increased the secretion of IL-1 $\beta$  and IL-6, increased the number of apoptotic cells, and decreased cell viability. NaHS treatment suppressed the upregulation of p-p65 expression, alleviated DOX-induced inflammation and cell damage, reduced the secretion levels of IL-1 $\beta$  and IL-6, decreased the number of apoptotic cells, and increased cell survival. Zhong et al. [153] used a streptozotocin-induced type 1 diabetes model and found that, compared with the control group, the model group exhibited elevated serum levels of IL-1 $\beta$ , IL-8, and dsDNA, as well as increased expression of CXCR2, p-I $\kappa$ B $\alpha$ , and p-NF- $\kappa$ B. NaHS treatment significantly reduced these levels. In further in vitro experiments, glucose induced upregulation of Bax, IL-1 $\beta$ , and IL-8 expression and downregulation of Bcl-2 in endothelial cells, and exogenous H<sub>2</sub>S treatment reversed these changes. Furthermore, in a sepsis model established by cecal ligation and puncture, Xu et al. [154] found that NaHS increased IL-10 levels and inhibited NF- $\kappa$ B expression, while also reducing pathological damage. These findings suggest that exogenous H<sub>2</sub>S protects against sepsis-induced myocardial injury by inhibiting NF- $\kappa$ B expression and upregulating the anti-inflammatory cytokine IL-10, with the mechanism speculated to involve H<sub>2</sub>S-mediated persulfidation modification (Figure 10).

In summary, H<sub>2</sub>S-mediated persulfidation can regulate the NF- $\kappa$ B signaling pathway through multiple pathways, including STAT3, NLRP3, Nrf2, SIRT, MAPK, PI3K/Akt, TLR4, TNF- $\alpha$ , and IL, thereby achieving various biological effects such as anti-inflammatory, anti-apoptotic, and anti-oxidative stress responses, ultimately exerting protective effects (Table 3).



**Figure 10.** H<sub>2</sub>S regulates the IL/NF- $\kappa$ B signaling pathway through persulfidation modification. Abbreviations: NF- $\kappa$ B: nuclear factor  $\kappa$ B; I $\kappa$ B: inhibitor of NF- $\kappa$ B; IL: interleukin. Created in BioRender. Liang, K. (2026) <https://BioRender.com/w2qn7za> (accessed on 22 July 2026).

**Table 3.** Biological effects of H<sub>2</sub>S mediated by persulfidation modification.

| Signaling Pathway | Subject                             | Model                                               | H <sub>2</sub> S Donor    | Administration Method                                                                   | Mechanism of Action                                                                                                 | Reference |
|-------------------|-------------------------------------|-----------------------------------------------------|---------------------------|-----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|-----------|
| TLR4/NF-κB        | MAC-T cells                         | Inflammatory model                                  | NaHS                      | Cells were pre-incubated with NaHS for 2 h, followed by co-treatment with LPS.          | ROS, p-p65, TLR4↓                                                                                                   | [68]      |
|                   | SH-SY5Y cells                       | Parkinson's model                                   | NaHS                      | Directly added to the medium                                                            | TLR4, iNOS, p-IκB, p-p65 ↓                                                                                          | [69]      |
|                   | pregnant C57BL/6 mice; HUSMCs cells | Infectious preterm birth model                      | NaHS                      | Subcutaneous injection; directly added to the medium                                    | ASC, caspase 1, TLR4, p-p65 ↓                                                                                       | [70]      |
|                   | Dahl salt-sensitive rats            | Hypertension model                                  | GGY4137                   | Dissolved in artificial cerebrospinal fluid (aCSF) and administered via an osmotic pump | p-p65, p-IKKβ, TLR4, MyD88 ↓                                                                                        | [71]      |
|                   | SD rats                             | COPD model                                          | H <sub>2</sub> S          | Inhalation                                                                              | GSDMD-N, cleaved caspase 1, LDH, TLR4, p-p65 ↓                                                                      | [72]      |
|                   | Wistar male rats                    | Hepatotoxicity model                                | NaHS                      | IP                                                                                      | TBARS, Nox, TLR2, TLR4, p-JNK, p-p65 ↓; GSH ↑                                                                       | [73]      |
| PI3K/Akt/NF-κB    | KM mice                             | Alcoholic liver disease model                       | NaHS                      | IP                                                                                      | AST, ALT, MDA, TLR4, NF-κB ↓; GSH, CSE ↑                                                                            | [74]      |
|                   | SD rats                             | Sepsis model                                        | NaHS                      | IP                                                                                      | p-PI3, p-Akt, Bcl-2 ↑; NF-κB, Bax, caspase 3 ↓                                                                      | [80]      |
|                   | C57BL/6J mice; RAW 264.7 cells      | ALI model                                           | GGY4137                   | IP; directly added to the medium                                                        | p-PDGFRβ, p-Akt, p-p65 ↓                                                                                            | [81]      |
|                   | MAC-T cells                         | Inflammatory model                                  | NaHS                      | Directly added to the medium                                                            | p-Akt, NF-κB ↓                                                                                                      | [82]      |
|                   | Male white big-eared rabbits        | Portal hypertension model                           | NaHS                      | IP                                                                                      | p-AKT, NF-κB ↑; IκBα, Bcl-2 ↓                                                                                       | [83]      |
|                   | MC3T3-E1 cells; hFOB 1.19 cells     | Osteoblast injury model                             | NaHS or Na <sub>2</sub> S | NaHS was added 1 h prior to LPS treatment.                                              | p-Akt, p-IκB ↓                                                                                                      | [84]      |
|                   | Rat pancreatic acinar cells         | Acute pancreatitis model                            | NaHS or Na <sub>2</sub> S | Directly added to the medium                                                            | p-Akt, p-p65 ↓                                                                                                      | [85]      |
|                   | SD rats                             | Severe acute pancreatitis model                     | NaHS                      | IP                                                                                      | MPO, p-Akt, NF-κB ↓; IκBα ↑                                                                                         | [86]      |
|                   | C57BL/6J mice                       | Myocardial injury model                             | NaHS                      | IP                                                                                      | MDA, Bax/Bcl-2, cleaved caspase 3, PI3K/AKT, p-p65 ↓; SOD ↑                                                         | [87]      |
|                   | HL-1 cells                          | I-R injury                                          | NaHS                      | NaHS was added 30 min before hypoxia treatment.                                         | cleaved caspase 3, p-p65 ↓, Bcl-2, PI3K/Akt ↑                                                                       | [88]      |
| MAPK/NF-κB        | NRK-52E; Wistar rats                | Nephrotoxicity model; chronic renal failure model   | NaHS                      | Co-treatment with NaHS was performed for 24 h during gentamicin-induced injury. IP      | Bax, caspase 3, cleaved caspase 3, ROS, MDA, p-ERK, p-JNK, p-p38, p65 ↓; SOD, GSH-Px ↑                              | [94]      |
|                   | L6 cells; Wistar rats               | Inflammatory model; diaphragmatic dysfunction model | NaHS                      | NaHS was co-treated for 24 h during LPS stimulation. IP                                 | Bax, caspase 3, cleaved caspase 3, p-ERK, p-JNK, p-p38, ROS, p-IKKα/β, p-IκBα, p-p65 ↓ SOD, GSH-Px ↑                | [95]      |
|                   | Broilers                            | H <sub>2</sub> S gas exposure model                 | H <sub>2</sub> S          | Direct inhalation                                                                       | GSH, CAT, T-AOC, SOD, IκBα ↓; iNOS, NO, H <sub>2</sub> O <sub>2</sub> , MDA, p-IKKα/β, p-p65, p-ERK, p-JNK, p-p38 ↑ | [96]      |
|                   | C57BL/6J mice                       | Diabetes model                                      | NaHS                      | IP                                                                                      | p-p38MAPK, p-p65 ↓                                                                                                  | [97]      |

Table 3. Cont.

| Signaling Pathway | Subject                                                                                                 | Model                           | H <sub>2</sub> S Donor | Administration Method                                                                     | Mechanism of Action                                         | Reference |
|-------------------|---------------------------------------------------------------------------------------------------------|---------------------------------|------------------------|-------------------------------------------------------------------------------------------|-------------------------------------------------------------|-----------|
| MAPK/<br>NF-κB    | NIH3T3 cells                                                                                            | Cellular fibrosis model         | NaHS                   | Cells were pretreated with NaHS for 1 h, followed by treatment with TGF β1 for 24 h.      | p-p38MAPK, p-p65 ↓                                          | [98]      |
|                   | C57BL/6J mice;<br>MCF-7; SGC-7901                                                                       | HCC model                       | NaHS;<br>GYY4137       | IP; directly added to the medium                                                          | IDO1, p-STAT3, p-NF-κB ↓                                    | [102]     |
|                   | LLC-PK1;<br>C57BL/6J mice                                                                               | Nephrotoxicity model            | NaHS;<br>GYY4137       | Pretreatment with NaHS or GYY4137 was performed for 30 min before cisplatin addition.; IP | COX-2, p-STAT3, p-IKKβ, NF-κB ↓                             | [103]     |
| STAT3/<br>NF-κB   | Swiss albino mouse                                                                                      | Acute pancreatitis model        | DADS                   | IP                                                                                        | p-STAT3, p-p65 ↓;<br>PPARγ ↑                                | [104]     |
|                   | HUVECs                                                                                                  | Atherosclerosis model           | ADT-OH                 | Directly added to the medium                                                              | p-STAT3, p-p65 ↓; SOD ↑                                     | [105]     |
|                   | Wistar rat cardiomyocytes                                                                               | I–R model                       | NaHS                   | NaHS was added during reoxygenation.                                                      | MDA, ROS, p-p65, p-STAT3 ↓; SOD, CAT, GSH-Px↑               | [106]     |
| Nrf2/NF-κB        | SD rats                                                                                                 | Acute nephrotoxicity model      | NaHS                   | IP                                                                                        | p-p65, p-IκB, Nrf2, MDA ↓, SOD, CAT, GPx, GST ↑             | [110]     |
|                   | Wistar rats                                                                                             | Acute nephrotoxicity model      | NaHS                   | IP                                                                                        | NF-κB ↓; p-Nrf2, OH-1 ↑                                     | [111]     |
|                   | Wistar albino rats                                                                                      | Type I diabetes model           | NaHS                   | IP                                                                                        | MDA, NO, p-p65 ↓; SOD, GPx, CAT, Nrf2 ↑                     | [112]     |
|                   | GES-1 cells                                                                                             | I–R model                       | NaHS                   | Pretreatment with NaHS was performed for 30 min before ischemia.                          | ROS, MDA, p-p65, Nrf2 ↓; GSH ↑                              | [113]     |
|                   | C57BL/6J mice;<br>HUVECs;<br>EA.hy926 cells                                                             | Diabetes model                  | GYY4137                | IP; co-treatment with GYY4137 was performed for 24 h during HG+ox-LDL-induced injury.     | VCAM-1, ICAM-1, p-p65, Nrf2 ↓                               | [114]     |
| SIRT/NF-κB        | HT-22 cells                                                                                             | Diabetes model                  | NaHS                   | Before high-glucose treatment, cells were pretreated with NaHS for 30 min.                | p-p65 ↓; SIRT ↑                                             | [119]     |
|                   | RAW264.7 cells                                                                                          | Inflammatory model              | NaHS                   | Directly added to the medium                                                              | p-p65 ↓; SIRT1 ↑                                            | [120]     |
|                   | C57BL/6 mice                                                                                            | Acute demyelination model       | DADS                   | i.g.                                                                                      | NF-κB ↓; MBP, Olig2, CNTF, ULK1, beclin1, SIRT1, GSH, TAC ↑ | [121]     |
|                   | ApoE <sup>−/−</sup> mice;<br>HAECs cells;<br>HepG2; HEK-293 cells; primary mouse peritoneal macrophages | Atherosclerosis model           | NaHS;<br>GYY4137       | IP; co-treatment with NaHS was performed at the same time as cisplatin treatment.         | NF-κB ↓; SIRT1 ↑                                            | [122]     |
|                   | HUVECs                                                                                                  | Cellular senescence model       | NaHS                   | NaHS was co-treated with H <sub>2</sub> O <sub>2</sub> .                                  | p-p65 ↓; MnSOD, CAT, FOXO1, SIRT1 ↑                         | [123]     |
| NLRP3/<br>NF-κB   | C57BL/6 mice;<br>HK2 cells                                                                              | Acute kidney injury (AKI) model | NaHS                   | IP; directly added to the medium                                                          | SIRT ↑; ROS ↓                                               | [124]     |
|                   | HepG2 cells                                                                                             | Inflammatory mode               | NaHS                   | Before PA addition, cells were pretreated with various concentrations of NaHS for 2 h.    | NLRP3, p-IKKα/β, p-p65 ↓                                    | [129]     |
|                   | ICR mice                                                                                                | Depression model                | NaHS                   | IP                                                                                        | NLRP3, p-p65, ROS ↓; MMP, ATP ↑                             | [130]     |

Table 3. Cont.

| Signaling Pathway | Subject                                     | Model                            | H <sub>2</sub> S Donor | Administration Method                                                      | Mechanism of Action                               | Reference |
|-------------------|---------------------------------------------|----------------------------------|------------------------|----------------------------------------------------------------------------|---------------------------------------------------|-----------|
| NLRP3/<br>NF-κB   | L02 cells                                   | Inflammatory mode                | NaHS                   | Cells were pretreated with NaHS for 30 min prior to LPS treatment.         | NLRP3, NF-κB ↓                                    | [131]     |
|                   | H9c2 cells                                  | Cardiotoxicity model             | NaHS                   | Before high-glucose treatment, cells were pretreated with NaHS for 30 min. | NLRP3, p-p65, ROS ↓; MMP ↑                        | [132]     |
|                   | C57BL/6J mice                               | Acute lung injury (ALI) model    | GY4137                 | IP                                                                         | NLRP3, p-p65 ↓                                    | [81]      |
|                   | RAW 264.7 cells; primary mouse chondrocytes | Macrophage pyroptosis model      | GY4137                 | Before stimulation with LPS/ATP, cells were pretreated with GY4137.        | MMP-13, ROS, MDA, p-IκBα, p-p65 ↓; SOD ↑          | [133]     |
| TNF-α/<br>NF-κB   | THP-1 cells; RAW 264.7 cells                | Inflammatory mode                | NaHS                   | Before ox-LDL stimulation, cells were pretreated with NaHS for 30 min.     | MCP-1, TNF-α, MIF, p-p65 ↓                        | [137]     |
|                   | Rabbit                                      | Urosepsis model                  | NaHS                   | injection into the marginal ear vein                                       | TNF-α, NF-κB ↓                                    | [138]     |
|                   | KM mice; Caco-2 cells                       | IBS model; Inflammatory mode     | NaHS                   | IP; directly added to the medium                                           | MDA, p-p65, TNF-α ↓; IκBα, SOD1, Gpx1, Gpx4 ↑     | [139]     |
|                   | SD rats                                     | Myocardial ischemia model        | NaHS                   | IP                                                                         | TNF-α, NF-κB ↓                                    | [140]     |
|                   | Human PAECs cells; Wistar rats              | PAH model                        | NaHS                   | Directly added to the medium; IP                                           | p-IKKβ, p-IκBα, TNF-α ↓                           | [141]     |
| IL/NF-κB          | H9c2 cells                                  | High-glucose injury model        | NaHS                   | Cells were pretreated with NaHS prior to high-glucose (HG) exposure        | p-p65, ROS, MMP, IL-1β, IL-6 ↓                    | [150]     |
|                   | AVICs cells; C57BL/6J mice                  | Aortic valve calcification model | AP27                   | Directly added to the medium; IP                                           | p-p65, IL-1β ↓                                    | [151]     |
|                   | H9c2 cells                                  | Inflammatory mode                | NaHS                   | Pretreatment with NaHS for 30 min.                                         | p-p65, IL-1β, IL-6 ↓                              | [152]     |
|                   | Wistar rats; RAECs                          | Type 1 diabetes model            | NaHS                   | IP; directly added to the medium                                           | IL-1β, IL-8, dsDNA, CXCR2, p-IκBα, p-NF-κB, ROS ↓ | [153]     |
|                   | SD rats                                     | Sepsis model                     | NaHS                   | IP                                                                         | NF-κB ↓; IL-10 ↑                                  | [154]     |

Note: ↑ represents increase, ↓ represents decrease.

## 5. Conclusions and Outlook

As a key gaseous signaling molecule in the body, H<sub>2</sub>S widely participates in the functional regulation of the NF-κB signaling pathway through persulfidation, an important post-translational protein modification. It plays an irreplaceable role in maintaining cellular homeostasis, suppressing excessive inflammation, alleviating OS, ameliorating apoptosis and pyroptosis, and protecting organ structure and function. Existing studies have systematically demonstrated that H<sub>2</sub>S can S-sulphydrate specific cysteine residues on core proteins such as IKKβ, p65, Keap1, and SIRT1/3, thereby inhibiting IKK complex activation, reducing IκBα degradation, and blocking p65 phosphorylation and nuclear translocation. This upstream inhibition of NF-κB pathway overactivation downregulates the expression of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and MCP-1 and halts the inflammatory cascade. Furthermore, through cross-regulation of multiple signaling pathways, including STAT3, NLRP3, Nrf2, SIRT, MAPK, PI3K/Akt, TLR4, TNF-α, and IL-related cascades, H<sub>2</sub>S exerts pleiotropic protective effects in various disease models, such

as atherosclerosis, sepsis, acute kidney injury, acute myocardial ischemia, acute pancreatitis, pulmonary hypertension, osteoarthritis, neuroinflammation, diabetic complications, OS, and ischemia–reperfusion injury. These effects include anti-inflammation, antioxidation, antiapoptosis, inhibition of pyroptosis, improvement of mitochondrial function, and attenuation of histopathological damage. Key modification sites identified to date include p65 Cys38, IKK $\beta$  Cys179, Keap1 Cys151, and multiple sites on SIRT1/3, providing a stable and reliable experimental basis for understanding the molecular mechanisms by which H<sub>2</sub>S regulates inflammation.

Despite significant progress in this field, several limitations remain that constrain the depth of mechanistic understanding and the pace of clinical translation. First, the molecular mechanisms have not been systematically or thoroughly elucidated. Most studies remain at the level of phenotypic observation of signaling pathways, and further work is needed to precisely determine the modification sites of persulfidation on key proteins, modification efficiency, conformational changes, alterations in binding affinity, and the details of upstream and downstream cascade transmission. Second, research on the physiological regulation of endogenous H<sub>2</sub>S is lacking. Existing findings rely largely on interventions with exogenous H<sub>2</sub>S donors, whereas how endogenous H<sub>2</sub>S synthesizing enzymes such as CBS, CSE, and 3 MST dynamically regulate the NF- $\kappa$ B pathway and maintain immune homeostasis under physiological and pathological conditions remains unclear. Third, the mechanisms underlying the bidirectional regulatory effects of H<sub>2</sub>S are unknown. H<sub>2</sub>S exhibits either activating or inhibitory effects on NF- $\kappa$ B and its related pathways depending on the model, concentration, and disease stage. The molecular switches, microenvironment-dependent mechanisms, and dose–effect relationships underlying this dual regulation have not been elucidated. Fourth, studies on cross talk among post-translational modifications are insufficient. How persulfidation antagonizes or coordinates with phosphorylation, acetylation, ubiquitination, and S nitrosylation to regulate NF- $\kappa$ B activity lacks systematic validation. Fifth, the foundation for clinical translation is weak. Most studies are based on cell and animal models, lacking validation using clinical tissue samples. The administration route, dosage range, intervention timing, safety, targeting, and pharmacokinetic properties of H<sub>2</sub>S donors have not been standardized. Sixth, the limitations of current detection techniques are evident. Existing methods for detecting S-sulfhydration suffer from insufficient specificity, high false-positive rates, inability to perform real-time in situ dynamic monitoring, and difficulty in achieving high-throughput mass spectrometry-based screening, which impairs the accuracy of mechanistic studies.

In summary, regulation of the NF- $\kappa$ B signaling pathway by H<sub>2</sub>S through persulfidation modification represents a critical node connecting gaseous signaling molecules, post-translational protein modifications, and inflammatory regulation. As research continues to advance, this axis is expected to provide novel molecular targets and therapeutic strategies for inflammation related diseases, cardiovascular diseases, metabolic disorders, neurodegenerative diseases, and organ injury, holding significant scientific value and broad prospects for clinical translation. It is worth noting that the biological effects of H<sub>2</sub>S exhibit a strict biphasic nature: physiological concentrations of H<sub>2</sub>S exert cytoprotective effects, whereas excessively high concentrations may induce reductive stress, leading to metabolic reprogramming and cellular dysfunction. Future studies are needed to further define the concentration thresholds, molecular mechanisms, and pathophysiological significance of H<sub>2</sub>S-mediated reductive stress.

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## Abbreviations

The following abbreviations are used in this manuscript.

|        |                                                  |
|--------|--------------------------------------------------|
| NF-κB  | nuclear factor κB                                |
| (TLR4) | Toll-like receptor 4                             |
| PI3K   | phosphoinositide 3-kinase                        |
| Akt    | protein kinase B                                 |
| MAPKs  | mitogen-activated protein kinases                |
| STAT   | signal transducer and activator of transcription |
| Nrf2   | nuclear factor erythroid 2-related factor 2      |
| SIRT6  | sirtuins                                         |
| NLRP3  | NOD-like receptor protein 3                      |
| TNF-α  | tumor necrosis factor alpha                      |
| ILs    | interleukins                                     |
| TRAF2  | TNF receptor-associated factor 2                 |
| TMAO   | trimethylamine N-oxide                           |
| CNTF   | ciliary neurotrophic factor                      |
| MBP    | myelin basic protein                             |
| Olig2  | oligodendrocyte transcription factor 2           |
| DADS   | diallyl disulfide                                |
| SAM    | S-adenosyl L-methionine                          |
| OA     | osteoarthritis                                   |
| MCP-1  | monocyte chemoattractant protein 1               |
| MIF    | migration inhibition factor                      |

## References

1. Murphy, B.; Bhattacharya, R.; Mukherjee, P. Hydrogen Sulfide Signaling in Mitochondria and Disease. *FASEB J.* **2019**, *33*, 13098–13125. [[CrossRef](#)] [[PubMed](#)]
2. Ge, X.; Sun, J.; Fei, A.; Gao, C.; Pan, S.; Wu, Z. Hydrogen Sulfide Treatment Alleviated Ventilator-Induced Lung Injury through Regulation of Autophagy and Endoplasmic Reticulum Stress. *Int. J. Biol. Sci.* **2019**, *15*, 2872–2884. [[CrossRef](#)] [[PubMed](#)]
3. Benedetti, F.; Curreli, S.; Krishnan, S.; Davinelli, S.; Cocchi, F.; Scapagnini, G.; Gallo, R.C.; Zella, D. Anti-Inflammatory Effects of H<sub>2</sub>S during Acute Bacterial Infection: A Review. *J. Transl. Med.* **2017**, *15*, 100. [[CrossRef](#)] [[PubMed](#)]
4. Hine, C.; Ponti, A.K.; Cáliz-Molina, M.Á.; Martín-Montalvo, A. H<sub>2</sub>S Serves as the Immunoregulatory Essence of Apoptotic Cell Death. *Cell Metab.* **2024**, *36*, 3–5. [[CrossRef](#)] [[PubMed](#)]

5. Mun, J.; Kang, H.-M.; Jung, J.; Park, C. Role of Hydrogen Sulfide in Cerebrovascular Alteration during Aging. *Arch. Pharmacol. Res.* **2019**, *42*, 446–454. [[CrossRef](#)] [[PubMed](#)]
6. Mei, Y.-D.; Jin, X.-X.; Huang, L.-Q. S-sulfhydration: A New Post-Translational Modification of Proteins. *Chin. J. Biochem. Mol. Biol.* **2018**, *34*, 911–920.
7. Yang, Y.; Xi, J.H.; Wang, Z.W.; Tang, Z.L.; Huang, K.T.; Liang, K.K.; Li, J.Y.; Du, Y.; Zhang, C.A. New progress in the study of NF- $\kappa$ B signal pathway involved in the regulation of pathological characteristics of bronchial asthma. *J. Lanzhou Univ. (Med. Sci.)* **2023**, *8*, 76–81.
8. Sen, N.; Paul, B.D.; Gadalla, M.M.; Mustafa, A.K.; Sen, T.; Xu, R.; Kim, S.; Snyder, S.H. Hydrogen Sulfide-Linked Sulfhydration of NF- $\kappa$ B Mediates Its Antiapoptotic Actions. *Mol. Cell* **2012**, *45*, 13–24. [[CrossRef](#)] [[PubMed](#)]
9. Yang, H.Z.; Su, S.S.; Chen, H.W. Summary of nuclear factor- $\kappa$ B family members: A review. *J. Tradit. Chin. Vet. Med.* **2025**, *44*, 40–46.
10. Giridharan, S.; Srinivasan, M. Mechanisms of NF- $\kappa$ B P65 and Strategies for Therapeutic Manipulation. *J. Inflamm. Res.* **2018**, *11*, 407–419. [[CrossRef](#)] [[PubMed](#)]
11. Downton, P.; Bagnall, J.S.; England, H.; Spiller, D.G.; Humphreys, N.E.; Jackson, D.A.; Paszek, P.; White, M.R.H.; Adamson, A.D. Overexpression of I $\kappa$ B $\alpha$  Modulates NF- $\kappa$ B Activation of Inflammatory Target Gene Expression. *Front. Mol. Biosci.* **2023**, *10*, 1187187. [[CrossRef](#)] [[PubMed](#)]
12. Mikuda, N.; Kolesnichenko, M.; Beaudette, P.; Popp, O.; Uyar, B.; Sun, W.; Tufan, A.B.; Perder, B.; Akalin, A.; Chen, W.; et al. The I $\kappa$ B Kinase Complex Is a Regulator of mRNA Stability. *EMBO J.* **2018**, *37*, EMBJ201798658. [[CrossRef](#)] [[PubMed](#)]
13. Li, C.; Moro, S.; Shostak, K.; O'Reilly, F.J.; Donzeau, M.; Graziadei, A.; McEwen, A.G.; Desplancq, D.; Poussin-Courmontagne, P.; Bachelart, T.; et al. Molecular Mechanism of IKK Catalytic Dimer Docking to NF- $\kappa$ B Substrates. *Nat. Commun.* **2024**, *15*, 7692. [[CrossRef](#)] [[PubMed](#)]
14. Göktuna, S.I.; Diamanti, M.A.; Chau, T.L. IKK  $\alpha$  and Tumor Cell Plasticity. *FEBS J.* **2018**, *285*, 2161–2181. [[CrossRef](#)] [[PubMed](#)]
15. Woods, J.S.; Dieguez-Acuña, F.J.; Ellis, M.E.; Kushleika, J.; Simmonds, P.L. Attenuation of Nuclear Factor Kappa B (NF- $\kappa$ B) Promotes Apoptosis of Kidney Epithelial Cells: A Potential Mechanism of Mercury-Induced Nephrotoxicity. *Environ. Health Perspect.* **2002**, *110*, 819–822. [[CrossRef](#)] [[PubMed](#)]
16. Gasparini, C.; Celeghini, C.; Monasta, L.; Zauli, G. NF- $\kappa$ B Pathways in Hematological Malignancies. *Cell. Mol. Life Sci.* **2014**, *71*, 2083–2102. [[CrossRef](#)] [[PubMed](#)]
17. Liu, X.T.; Jiang, G.F.; Huang, W.Q.; Zhou, Y.M. Advances in the Action Mechanism of Classical Pathways IKK $\alpha$  and IKK $\beta$  in Hematological Tumors and Drug Therapy Blocking Their Effect—Review. *J. Exp. Hematol.* **2021**, *29*, 306–310.
18. Ma, H.Y.; Feng, Z.H.; Ren, Q.Y.; Gao, H.X.; Liu, M. Mechanism of Traditional Chinese Medicine Monomers in Improving Diabetic Peripheral Neuropathy Based on NF- $\kappa$ B Signaling Pathway. *Shaanxi J. Tradit. Chin. Med.* **2024**, *45*, 1565–1569.
19. Yu, H.; Lin, L.; Zhang, Z.; Zhang, H.; Hu, H. Targeting NF- $\kappa$ B Pathway for the Therapy of Diseases: Mechanism and Clinical Study. *Signal Transduct. Target. Ther.* **2020**, *5*, 209. [[CrossRef](#)] [[PubMed](#)]
20. Xing, Y.; Zhou, F.; Wang, J. Subset of Genes Targeted by Transcription Factor NF- $\kappa$ B in TNF $\alpha$ -Stimulated Human HeLa Cells. *Funct. Integr. Genom.* **2012**, *13*, 143–154. [[CrossRef](#)] [[PubMed](#)]
21. Sun, S.-C. Non-Canonical NF- $\kappa$ B Signaling Pathway. *Cell Res.* **2010**, *21*, 71–85. [[CrossRef](#)] [[PubMed](#)]
22. Liu, W.; Lu, X.; Shi, P.; Yang, G.; Zhou, Z.; Li, W.; Mao, X.; Jiang, D.; Chen, C. TNF- $\alpha$  Increases Breast Cancer Stem-like Cells through up-Regulating TAZ Expression via the Non-Canonical NF- $\kappa$ B Pathway. *Sci. Rep.* **2020**, *10*, 1804. [[CrossRef](#)] [[PubMed](#)]
23. Razani, B.; Reichardt, A.D.; Cheng, G. Non-canonical NF- $\kappa$ B Signaling Activation and Regulation: Principles and Perspectives. *Immunol. Rev.* **2011**, *244*, 44–54. [[CrossRef](#)] [[PubMed](#)]
24. Nam, J.S.; Dixon, M.S.; Chio, I.I.C. Hydrogen Sulfide: A Whiff of Trouble for Cancer Cell Survival. *Mol. Cell* **2024**, *84*, 3865–3867. [[CrossRef](#)] [[PubMed](#)]
25. Kanagy, N.L.; Szabo, C.; Papapetropoulos, A. Vascular Biology of Hydrogen Sulfide. *Am. J. Physiol.-Cell Physiol.* **2017**, *312*, C537–C549. [[CrossRef](#)] [[PubMed](#)]
26. Song, Y.; Wu, S.; Zhang, R.; Zhong, Q.; Zhang, X.; Sun, X. Therapeutic Potential of Hydrogen Sulfide in Osteoarthritis Development. *Front. Pharmacol.* **2024**, *15*, 1336693. [[CrossRef](#)] [[PubMed](#)]
27. Dilek, N.; Papapetropoulos, A.; Toliver-Kinsky, T.; Szabo, C. Hydrogen Sulfide: An Endogenous Regulator of the Immune System. *Pharmacol. Res.* **2020**, *161*, 105119. [[CrossRef](#)] [[PubMed](#)]
28. Kamoun, P. Endogenous Production of Hydrogen Sulfide in Mammals. *Amino Acids* **2004**, *26*, 243–254. [[CrossRef](#)] [[PubMed](#)]
29. Myszkowska, J.; Derevenkov, I.; Makarov, S.V.; Spiekerkoetter, U.; Hannibal, L. Biosynthesis, Quantification and Genetic Diseases of the Smallest Signaling Thiol Metabolite: Hydrogen Sulfide. *Antioxidants* **2021**, *10*, 1065. [[CrossRef](#)] [[PubMed](#)]
30. Pedre, B.; Barayeu, U.; Ezeriņa, D.; Dick, T.P. The Mechanism of Action of N-Acetylcysteine (NAC): The Emerging Role of H<sub>2</sub>S and Sulfane Sulfur Species. *Pharmacol. Ther.* **2021**, *228*, 107916. [[CrossRef](#)] [[PubMed](#)]
31. Bianco, C.L.; Chavez, T.A.; Sosa, V.; Saund, S.; Nguyen, S.; Tantillo, Q.N.N.; Ichimura, D.J.; Toscano, A.S.; Fukuto, J.P. The Chemical Biology of the Persulfide (RSSH)/Perthiyl (RSS $\cdot$ ) Redox Couple and Possible Role in Biological Redox Signaling. *Free Radic. Biol. Med.* **2016**, *101*, 20–31. [[CrossRef](#)] [[PubMed](#)]

32. Petrovic, D.; Slade, L.; Paikopoulos, Y.; D'Andrea, D.; Savic, N.; Stancic, A.; Miljkovic, J.L.; Vignane, T.; Drekolia, M.K.; Mladenovic, D.; et al. Ergothioneine Improves Healthspan of Aged Animals by Enhancing cGPDH Activity through CSE-Dependent Persulfidation. *Cell Metab.* **2025**, *37*, 789. [[CrossRef](#)] [[PubMed](#)]
33. Pedre, B.; Talwar, D.; Barayeu, U.; Schilling, D.; Luzarowski, M.; Sokolowski, M.; Glatt, S.; Dick, T.P. 3-Mercaptopyruvate Sulfur Transferase Is a Protein Persulfidase. *Nat. Chem. Biol.* **2023**, *19*, 507–517. [[CrossRef](#)] [[PubMed](#)]
34. Guenther, E.A.; Johnson, K.S.; Coale, K.H. Direct Ultraviolet Spectrophotometric Determination of Total Sulfide and Iodide in Natural Waters. *Anal. Chem.* **2001**, *73*, 3481–3487. [[CrossRef](#)] [[PubMed](#)]
35. Ubuka, T.; Abe, T.; Kajikawa, R.; Morino, K. Determination of Hydrogen Sulfide and Acid-Labile Sulfur in Animal Tissues by Gas Chromatography and Ion Chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* **2001**, *757*, 31–37. [[CrossRef](#)] [[PubMed](#)]
36. Goodwin, L.R.; Francom, D.; Dieken, F.P.; Taylor, J.D.; Warenycia, M.W.; Reiffenstein, R.J.; Dowling, G. Determination of Sulfide in Brain Tissue by Gas Dialysis/Ion Chromatography: Postmortem Studies and Two Case Reports. *J. Anal. Toxicol.* **1989**, *13*, 105–109. [[CrossRef](#)] [[PubMed](#)]
37. Furne, J.; Saeed, A.; Levitt, M.D. Whole Tissue Hydrogen Sulfide Concentrations Are Orders of Magnitude Lower than Presently Accepted Values. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* **2008**, *295*, R1479–R1485. [[CrossRef](#)] [[PubMed](#)]
38. Ishigami, M.; Hiraki, K.; Umemura, K.; Ogasawara, Y.; Ishii, K.; Kimura, H. A Source of Hydrogen Sulfide and a Mechanism of Its Release in the Brain. *Antioxid. Redox Signal.* **2009**, *11*, 205–214. [[CrossRef](#)] [[PubMed](#)]
39. Jung, Y.-J.; Oh, M.S.; Eo, H.; Kwon, H.-J.; Hong, S.-P. Reversed-Phase Ion-Pair High Performance Liquid Chromatography with Electrochemical Detection for Simultaneous Quantification of Neurotransmitters in Mice Brain Tissues. *J. Liq. Chromatogr. Relat. Technol.* **2024**, *47*, 275–283. [[CrossRef](#)]
40. Krijt, J.; Kopecká, J.; Hnízda, A.; Moat, S.; Kluijtmans, L.A.J.; Mayne, P.; Kožich, V. Determination of Cystathionine Beta-synthase Activity in Human Plasma by LC-MS/MS: Potential Use in Diagnosis of CBS Deficiency. *J. Inher. Metab. Dis.* **2011**, *34*, 49–55. [[CrossRef](#)]
41. Tan, X.-X.; Lian, K.-Q.; Li, X.; Li, N.; Wang, W.; Kang, W.-J.; Shi, H.-M. Development of a Derivatization Method for the Quantification of Hydrogen Sulfide and Its Application in Vascular Calcification Rats. *J. Chromatogr. B* **2017**, *1055–1056*, 8–14. [[CrossRef](#)] [[PubMed](#)]
42. Liu, C.; Pan, J.; Li, S.; Zhao, Y.; Wu, L.Y.; Berkman, C.E.; Whorton, A.R.; Xian, M. Capture and Visualization of Hydrogen Sulfide by a Fluorescent Probe. *Angew. Chem. Int. Ed.* **2011**, *50*, 10327–10329. [[CrossRef](#)] [[PubMed](#)]
43. Fang, Q.; Xiong, H.; Yang, L.; Wang, B.; Song, X. An Instantaneous Fluorescent Probe for Detecting Hydrogen Sulfide in Biological Systems. *New J. Chem.* **2019**, *43*, 13594–13599. [[CrossRef](#)]
44. Pluth, M.D.; Montoya, L.A.; Bailey, T.S.; Hammers, M.D. OP01 Visible and Chemiluminescent Methods for H<sub>2</sub>S Detection and Quantification. *Nitric Oxide* **2013**, *31*, S19. [[CrossRef](#)]
45. Ranjana, M.; Shrilaxmi, M.S.; Nag, P.; Mitra, P.K.; Sunil, D.; Dastidar, S.G.; Sudhakar, Y.N.; Vennapusa, S.R.; Raju, R.; Titttonen, I.; et al. A Versatile Electrochemical, Colorimetric, and Visible Light Excitable Turn-on Fluorescent Probe for Stress-Induced H<sub>2</sub>S Detection. *Photochem. Photobiol. Sci.* **2025**, *24*, 975–990. [[CrossRef](#)] [[PubMed](#)]
46. Ryu, H.G.; Singha, S.; Jun, Y.W.; Reo, Y.J.; Ahn, K.H. Two-Photon Fluorescent Probe for Hydrogen Sulfide Based on a Red-Emitting Benzocoumarin Dye. *Tetrahedron Lett.* **2018**, *59*, 49–53. [[CrossRef](#)]
47. Wu, M.; Wang, S.; Hai, W.; Lu, X.; Li, P. Development of a H<sub>2</sub>S-Responsive NIR Fluorescent Probe for H<sub>2</sub>S Detection and H<sub>2</sub>S Releasing Monitoring From Prodrug. *J. Fluoresc.* **2023**, *33*, 1853–1860. [[CrossRef](#)] [[PubMed](#)]
48. Li, H.; Peng, W.; Feng, W.; Wang, Y.; Chen, G.; Wang, S.; Li, S.; Li, H.; Wang, K.; Zhang, J. A Novel Dual-Emission Fluorescent Probe for the Simultaneous Detection of H<sub>2</sub>S and GSH. *Chem. Commun.* **2016**, *52*, 4628–4631. [[CrossRef](#)] [[PubMed](#)]
49. Searcy, D.G.; Peterson, M.A. Hydrogen Sulfide Consumption Measured at Low Steady State Concentrations Using a Sulfidostat. *Anal. Biochem.* **2004**, *324*, 269–275. [[CrossRef](#)] [[PubMed](#)]
50. Doeller, J.E.; Isbell, T.S.; Benavides, G.; Koenitzer, J.; Patel, H.; Patel, R.P.; Lancaster, J.R.; Darley-Usmar, V.M.; Kraus, D.W. Polarographic Measurement of Hydrogen Sulfide Production and Consumption by Mammalian Tissues. *Anal. Biochem.* **2005**, *341*, 40–51. [[CrossRef](#)] [[PubMed](#)]
51. Nagy, L.; Filotás, D.; Pozsgai, G.; Pintér, E.; Nagy, G. OP02 Amperometric Microcell for Subcutaneous Detection of Hydrogen Sulfide in Anesthetized Experimental Animals. *Nitric Oxide* **2013**, *31*, S19–S20. [[CrossRef](#)]
52. Hu, Q.; Zhang, B.; Liu, Y.; Guo, Y.; Zhang, T.; Nie, R.; Ke, X.; Dong, X. The Effect of Fluid Shear Stress in Hydrogen Sulphide Production and Cystathionine  $\gamma$ -Lyase Expression in Human Early Endothelial Progenitor Cells. *Ann. Transl. Med.* **2020**, *8*, 1318. [[CrossRef](#)] [[PubMed](#)]
53. Kimura, Y.; Toyofuku, Y.; Koike, S.; Shibuya, N.; Nagahara, N.; Lefer, D.; Ogasawara, Y.; Kimura, H. Identification of H<sub>2</sub>S<sub>3</sub> and H<sub>2</sub>S Produced by 3-Mercaptopyruvate Sulfurtransferase in the Brain. *Sci. Rep.* **2015**, *5*, 14774. [[CrossRef](#)] [[PubMed](#)]
54. Liu, Y.; Tang, Q.; Zhang, Y.; Xu, M.; Yao, Y.; Qin, Y.; Liu, Q.; Wu, L. A Ratiometric Fluorescent Probe with Reversible Redox-Response for Monitoring Dynamic Release of H<sub>2</sub>S Donors. *Anal. Chem.* **2025**, *97*, 10611–10618. [[CrossRef](#)] [[PubMed](#)]

55. Kim, T.J.; Lee, Y.J.; Ahn, Y.J.; Lee, G.-J. Characterization of H<sub>2</sub>S Releasing Properties of Various H<sub>2</sub>S Donors Utilizing Microplate Cover-Based Colorimetric Assay. *Anal. Biochem.* **2019**, *574*, 57–65. [[CrossRef](#)] [[PubMed](#)]
56. Filipovic, M.R. Persulfidation (S-Sulfhydration) and H<sub>2</sub>S. In *Chemistry, Biochemistry and Pharmacology of Hydrogen Sulfide*; Moore, P.K., Whiteman, M., Eds.; Springer International Publishing: Cham, Switzerland, 2015; Volume 230, pp. 29–59.
57. Zhang, B.; Pan, J.Y.; Liu, Z.Q.; Zheng, Y.G. Research progress in protein persulfidation. *Acta Microbiol. Sin.* **2024**, *64*, 1348–1363.
58. Zhang, Z.; Quan, X.Y.; Tang, Z.S. Hydrogen Sulfide-Mediated S-Sulfhydration and Its Chemical Detection Techniques. *Chin. J. Biochem. Mol. Biol.* **2024**, *40*, 44–53.
59. Zhang, D.; Du, J.; Tang, C.; Huang, Y.; Jin, H. H<sub>2</sub>S-Induced Sulfhydration: Biological Function and Detection Methodology. *Front. Pharmacol.* **2017**, *8*, 608. [[CrossRef](#)] [[PubMed](#)]
60. Pan, J.; Carroll, K.S. Persulfide Reactivity in the Detection of Protein S-Sulfhydration. *ACS Chem. Biol.* **2013**, *8*, 1110–1116. [[CrossRef](#)] [[PubMed](#)]
61. Park, C.-M.; Macinkovic, I.; Filipovic, M.R.; Xian, M. Use of the “Tag-Switch” Method for the Detection of Protein S-Sulfhydration. In *Methods in Enzymology*; Elsevier: Amsterdam, The Netherlands, 2015; pp. 39–56.
62. Krishnan, N.; Fu, C.; Pappin, D.J.; Tonks, N.K. H<sub>2</sub>S-Induced Sulfhydration of the Phosphatase PTP1B and Its Role in the Endoplasmic Reticulum Stress Response. *Sci. Signal.* **2011**, *4*, 203. [[CrossRef](#)] [[PubMed](#)]
63. Longen, S.; Richter, F.; Köhler, Y.; Wittig, I.; Beck, K.-F.; Pfeilschifter, J. Quantitative Persulfide Site Identification (qPerS-SID) Reveals Protein Targets of H<sub>2</sub>S Releasing Donors in Mammalian Cells. *Sci. Rep.* **2016**, *6*, 29808. [[CrossRef](#)] [[PubMed](#)]
64. Luo, S.; Kong, C.; Ye, D.; Liu, X.; Wang, Y.; Meng, G.; Han, Y.; Xie, L.; Ji, Y. Protein Persulfidation: Recent Progress and Future Directions. *Antioxid. Redox Signal.* **2023**, *39*, 829–852. [[CrossRef](#)] [[PubMed](#)]
65. Huang, R.; Sun, Z.; Xian, S.; Song, D.; Chang, Z.; Yan, P.; Zhang, J.; Yin, H.; Zheng, Z.; Hu, P.; et al. The Role of Toll-like Receptors (TLRs) in Pan-Cancer. *Ann. Med.* **2022**, *54*, 1918–1937. [[CrossRef](#)] [[PubMed](#)]
66. Kim, H.-J.; Kim, H.; Lee, J.-H.; Hwangbo, C. Toll-like Receptor 4 (TLR4): New Insight Immune and Aging. *Immun. Ageing* **2023**, *20*, 67. [[CrossRef](#)] [[PubMed](#)]
67. Zhang, Y.; Liang, X.; Bao, X.; Xiao, W.; Chen, G. Toll-like Receptor 4 (TLR4) Inhibitors: Current Research and Prospective. *Eur. J. Med. Chem.* **2022**, *235*, 114291. [[CrossRef](#)] [[PubMed](#)]
68. Sun, L.; Chen, L.; Wang, F.; Zheng, X.; Yuan, C.; Niu, Q.; Li, Z.; Deng, L.; Zheng, B.; Li, C.; et al. Exogenous Hydrogen Sulfide Prevents Lipopolysaccharide-Induced Inflammation by Blocking the TLR4/NF-κB Pathway in MAC-T Cells. *Gene* **2019**, *710*, 114–121. [[CrossRef](#)] [[PubMed](#)]
69. Cao, X.; Huang, R.L.; Chen, X.W. H<sub>2</sub>S Alleviates MPP<sup>+</sup>-induced Inflammation by Inhibiting TLR4/NF-κB Pathway. *J. Huazhong Univ. Sci. Technol. (Med. Sci.)* **2020**, *4*, 56.
70. Chen, Z.; Zhang, M.; Zhao, Y.; Xu, W.; Xiang, F.; Li, X.; Zhang, T.; Wu, R.; Kang, X. Hydrogen Sulfide Contributes to Uterine Quiescence Through Inhibition of NLRP3 Inflammasome Activation by Suppressing the TLR4/NF-κB Signalling Pathway. *J. Inflamm. Res.* **2021**, *14*, 2753–2768. [[CrossRef](#)] [[PubMed](#)]
71. Liang, Y.F.; Chen, S.Y.; Ren, Y.B.; Zheng, Y.T.; Su, J.L.; Qin, A.; Wang, X.; Yuan, B.Y.; Zhang, D.D. Effects of H<sub>2</sub>S in paraventricular nucleus on salt-sensitive hypertensive rats via TLR4/MyD88/NF-κB signaling pathway. *Tianjin Med. J.* **2024**, *4*, 457–465.
72. Wang, L.; Meng, J.; Wang, C.; Wang, Y.; Yang, C.; Li, Y. Hydrogen Sulfide Attenuates Cigarette Smoke-induced Pyroptosis through the TLR4/NF-κB Signaling Pathway. *Int. J. Mol. Med.* **2022**, *49*, 5112. [[CrossRef](#)] [[PubMed](#)]
73. Abdel-latif, R.; Heeba, G.H.; Hassanin, S.O.; Waz, S.; Amin, A. TLRs-JNK/NF-κB Pathway Underlies the Protective Effect of the Sulfide Salt Against Liver Toxicity. *Front. Pharmacol.* **2022**, *13*, 850066. [[CrossRef](#)] [[PubMed](#)]
74. Chen, R.; Shao, C.F.; Qu, H.L.; Chen, Y.L.; Chen, J.Q. Effect of Exercise Intervention on the Improvement of Alcoholic Liver Injury through CSE/H<sub>2</sub>S Regulating TLR4/NF-κB Signaling Pathway. *Chin. J. Biochem. Mol. Biol.* **2022**, *38*, 341–353.
75. Xie, Y.; Shi, X.; Sheng, K.; Han, G.; Li, W.; Zhao, Q.; Jiang, B.; Feng, J.; Li, J.; Gu, Y. PI3K/Akt Signaling Transduction Pathway, Erythropoiesis and Glycolysis in Hypoxia (Review). *Mol. Med. Rep.* **2018**, *19*, 783–791. [[PubMed](#)]
76. Deng, R.; Zhou, J. The Role of PI3K/AKT Signaling Pathway in Myocardial Ischemia-Reperfusion Injury. *Int. Immunopharmacol.* **2023**, *123*, 110714. [[CrossRef](#)] [[PubMed](#)]
77. Khezri, M.R.; Jafari, R.; Yousefi, K.; Zolbanin, N.M. The PI3K/AKT Signaling Pathway in Cancer: Molecular Mechanisms and Possible Therapeutic Interventions. *Exp. Mol. Pathol.* **2022**, *127*, 104787. [[CrossRef](#)] [[PubMed](#)]
78. Zhang, X.Y.; Song, R.P.; Wei, D.D. Research Progress on Regulation of PI3K/AKT/NF-κB Signaling Pathway in TCM Prevention and Treatment of Diabetic Nephropathy. *J. Tradit. Chin. Med.* **2025**, *11*, 2362–2369.
79. Fruman, D.A.; Chiu, H.; Hopkins, B.D.; Bagrodia, S.; Cantley, L.C.; Abraham, R.T. The PI3K Pathway in Human Disease. *Cell* **2017**, *170*, 605–635. [[CrossRef](#)] [[PubMed](#)]
80. Liu, J.; Li, J.; Tian, P.; Guli, B.; Weng, G.; Li, L.; Cheng, Q. H<sub>2</sub>S Attenuates Sepsis-induced Cardiac Dysfunction via a PI3K/Akt-dependent Mechanism. *Exp. Ther. Med.* **2019**, *17*, 4064–4072. [[CrossRef](#)] [[PubMed](#)]
81. Li, J.; Ma, J.; Li, M.; Tao, J.; Chen, J.; Yao, C.; Yao, S. GYY4137 Alleviates Sepsis-Induced Acute Lung Injury in Mice by Inhibiting the PDGFRβ/Akt/NF-κB/NLRP3 Pathway. *Life Sci.* **2021**, *271*, 119192. [[CrossRef](#)] [[PubMed](#)]

82. Wang, Y.; Zhang, C.; Xu, C.; Feng, L.; Li, A.; Jin, X.; Guo, S.; Jiao, X.; Liu, J.; Guo, Y.; et al. H<sub>2</sub>S Mediates Apoptosis in Response to Inflammation through PI3K/Akt/NF-κB Signaling Pathway. *Biotechnol. Lett.* **2020**, *42*, 375–387. [\[CrossRef\]](#) [\[PubMed\]](#)
83. Wang, C.; Han, J.; Li, D.; Yang, Z.; Zhang, L. Protective Effects of Hydrogen Sulfide on Portal Hypertensive Vasculopathy in Rabbits by Activating AKT-NF-κB Pathway. *J. Huazhong Univ. Sci. Technol. Med. Sci.* **2017**, *37*, 348–351. [\[CrossRef\]](#) [\[PubMed\]](#)
84. Wang, H.; Li, X.; Zhu, Z.; Wang, H.; Wei, B.; Bai, X. Hydrogen Sulfide Promotes Lipopolysaccharide-Induced Apoptosis of Osteoblasts by Inhibiting the AKT/NF-κB Signaling Pathway. *Biochem. Biophys. Res. Commun.* **2020**, *524*, 832–838. [\[CrossRef\]](#) [\[PubMed\]](#)
85. Fu, L.Y. Effect of Hydrogen Sulfide on Caerulein-Induced TNF-α, IL-1β, IL-10 in Isolated Rat Pancreatic Acinar Cells Through PI<sub>3</sub>K/AKT-NF-κB Pathway. Master's Thesis, Third Military Medical University, Chongqing, China, May 2013.
86. Rao, C.-Y.; Fu, L.-Y.; Hu, C.-L.; Chen, D.-X.; Gan, T.; Wang, Y.-C.; Zhao, X.-Y. H<sub>2</sub>S Mitigates Severe Acute Pancreatitis through the PI<sub>3</sub>K/AKT-NF-κB Pathway in Vivo. *World J. Gastroenterol.* **2015**, *21*, 4555–4563. [\[CrossRef\]](#) [\[PubMed\]](#)
87. Zheng, X.-B.; Wang, C.; Zhang, M.; Yao, B.-Q.; Wu, H.-Y.; Hou, S.-X. Exogenous H<sub>2</sub>S Targeting PI3K/AKT/mTOR Pathway Alleviates Chronic Intermittent Hypoxia-Induced Myocardial Damage through Inhibiting Oxidative Stress and Enhancing Autophagy. *Sleep Breath.* **2024**, *29*, 43. [\[CrossRef\]](#) [\[PubMed\]](#)
88. Zheng, X.B.; Wang, C.; Wu, H.Y.; Yao, B.Q.; Hou, S.X. Alleviation of hypoxia/reoxygenation-induced HL-1 cardiomyocyte injury with hydrogen sulfide through regulating Nrf2/HO-1 signaling pathway mediated by PI3K/AKT. *J. Cardiovasc. Med.* **2024**, *1*, 32–38.
89. Wang, L.J.; Si, W.T. Traditional Chinese Medicine Treats Tumors via MAPK/NF-κB Signaling Pathway: A Review. *Chin. J. Exp. Tradit. Med. Formulae* **2024**, *30*, 262–270.
90. Zheng, W.X.; Xu, L.; Jiang, Z.X.; Zhao, Z.Y.; Qin, G.Z. Research progress on action mechanism of MAPK/NF-κB signaling pathway mediating traditional Chinese medicine and pharmacy in the intervention of chronic prostatitis. *China Med. Her.* **2025**, *22*, 168–172.
91. Yue, J.; López, J.M. Understanding MAPK Signaling Pathways in Apoptosis. *Int. J. Mol. Sci.* **2020**, *21*, 2346. [\[CrossRef\]](#) [\[PubMed\]](#)
92. Dahm, K.; Vijayarangakannan, P.; Wollscheid, H.; Schild, H.; Rajalingam, K. Atypical MAPK s in Cancer. *FEBS J.* **2025**, *292*, 2173–2188. [\[CrossRef\]](#) [\[PubMed\]](#)
93. Nakano, R.; Kitanaka, T.; Namba, S.; Kitanaka, N.; Suwabe, Y.; Konno, T.; Yamazaki, J.; Nakayama, T.; Sugiya, H. Non-Transcriptional and Translational Function of Canonical NF-κB Signaling in Activating ERK1/2 in IL-1β-Induced COX-2 Expression in Synovial Fibroblasts. *Front. Immunol.* **2020**, *11*, 579266. [\[CrossRef\]](#) [\[PubMed\]](#)
94. Wu, D.; Luo, N.; Wang, L.; Zhao, Z.; Bu, H.; Xu, G.; Yan, Y.; Che, X.; Jiao, Z.; Zhao, T.; et al. Hydrogen Sulfide Ameliorates Chronic Renal Failure in Rats by Inhibiting Apoptosis and Inflammation through ROS/MAPK and NF-κB Signaling Pathways. *Sci. Rep.* **2017**, *7*, 455. [\[CrossRef\]](#) [\[PubMed\]](#)
95. Zhang, G.-Y.; Lu, D.; Duan, S.-F.; Gao, Y.-R.; Liu, S.-Y.; Hong, Y.; Dong, P.-Z.; Chen, Y.-G.; Li, T.; Wang, D.-Y.; et al. Hydrogen Sulfide Alleviates Lipopolysaccharide-Induced Diaphragm Dysfunction in Rats by Reducing Apoptosis and Inflammation through ROS/MAPK and TLR4/NF-κB Signaling Pathways. *Oxidative Med. Cell. Longev.* **2018**, *2018*, 9647809. [\[CrossRef\]](#) [\[PubMed\]](#)
96. Chi, Q.; Wang, D.; Hu, X.; Li, S.; Li, S. Hydrogen Sulfide Gas Exposure Induces Necroptosis and Promotes Inflammation through the MAPK/NF-κB Pathway in Broiler Spleen. *Oxidative Med. Cell. Longev.* **2019**, *2019*, 8061823. [\[CrossRef\]](#) [\[PubMed\]](#)
97. Song, B.; Ding, L. Effects and mechanisms of exogenous hydrogen sulfide on inhibiting the activation of p38MAPK/NF-κB p65 pathway to improve diabetic myocardial fibrosis. *Med. J. Chin. People's Lib. Army* **2016**, *41*, 902–908.
98. Li, Z.H. Exogenous Hydrogen Sulfide Inhibits Proliferation of Mouse Pulmonary Fibroblasts by Down-Regulating TGF-β1/P38MAPK/NF-κB Signaling Pathway. Master's Thesis, University of South China, Hengyang, China, May 2021.
99. Zhao, J.; Qi, Y.-F.; Yu, Y.-R. STAT3: A Key Regulator in Liver Fibrosis. *Ann. Hepatol.* **2021**, *21*, 100224. [\[CrossRef\]](#) [\[PubMed\]](#)
100. Kaminskiy, Y.; Melenhorst, J.J. STAT3 Role in T-Cell Memory Formation. *Int. J. Mol. Sci.* **2022**, *23*, 2878. [\[CrossRef\]](#) [\[PubMed\]](#)
101. Hu, Y.; Dong, Z.; Liu, K. Unraveling the Complexity of STAT3 in Cancer: Molecular Understanding and Drug Discovery. *J. Exp. Clin. Cancer Res.* **2024**, *43*, 23. [\[CrossRef\]](#) [\[PubMed\]](#)
102. Yang, D.; Li, T.; Li, Y.; Zhang, S.; Li, W.; Liang, H.; Xing, Z.; Du, L.; He, J.; Kuang, C.; et al. H<sub>2</sub>S Suppresses Indoleamine 2,3-Dioxygenase 1 and Exhibits Immunotherapeutic Efficacy in Murine Hepatocellular Carcinoma. *J. Exp. Clin. Cancer Res.* **2019**, *38*, 88. [\[CrossRef\]](#) [\[PubMed\]](#)
103. Sun, H.-J.; Leng, B.; Wu, Z.-Y.; Bian, J.-S. Polysulfide and Hydrogen Sulfide Ameliorate Cisplatin-Induced Nephrotoxicity and Renal Inflammation through Persulfidating STAT3 and IKKβ. *Int. J. Mol. Sci.* **2020**, *21*, 7805. [\[CrossRef\]](#) [\[PubMed\]](#)
104. Marimuthu, M.K.; Moorthy, A.; Ramasamy, T. Diallyl Disulfide Attenuates STAT3 and NF-κB Pathway Through PPAR-γ Activation in Cerulein-Induced Acute Pancreatitis and Associated Lung Injury in Mice. *Inflammation* **2022**, *45*, 45–58. [\[CrossRef\]](#) [\[PubMed\]](#)
105. Ou, X.; Xia, T.; Yang, C.; Yu, C.; Zhang, S.; Huang, R.; Chen, C.; Zhou, C. Novel H<sub>2</sub>S Donor Proglumide-ADT-OH Protects HUVECs from Ox-LDL-Induced Injury through NF-κB and JAK/SATA Pathway. *Open Med.* **2021**, *16*, 1318–1327. [\[CrossRef\]](#) [\[PubMed\]](#)

106. Li, L.; Li, M.; Li, Y.; Sun, W.; Wang, Y.; Bai, S.; Li, H.; Wu, B.; Yang, G.; Wang, R.; et al. Exogenous H<sub>2</sub>S Contributes to Recovery of Ischemic Post-Conditioning-Induced Cardioprotection by Decrease ROS Level Via Down-Regulation of NF- $\kappa$ B and JAK2-STAT3 Pathways in the Aging Cardiomyocytes. *Cell Biosci.* **2016**, *6*, 26. [\[CrossRef\]](#) [\[PubMed\]](#)
107. Tonelli, C.; Chio, I.I.C.; Tuveson, D.A. Transcriptional Regulation by Nrf2. *Antioxid. Redox Signal.* **2018**, *29*, 1727–1745.
108. Sajadimajd, S.; Khazaei, M. Oxidative Stress and Cancer: The Role of Nrf2. *Curr. Cancer Drug Targets* **2018**, *18*, 538–557. [\[CrossRef\]](#) [\[PubMed\]](#)
109. Wang, Z.; Fang, C.; Yao, M.; Wu, D.; Chen, M.; Guo, T.; Mo, J. Research Progress of NF- $\kappa$ B Signaling Pathway and Thrombosis. *Front. Immunol.* **2023**, *14*, 1257988. [\[CrossRef\]](#) [\[PubMed\]](#)
110. Zheng, J.; Zhao, T.; Yuan, Y.; Hu, N.; Tang, X. Hydrogen Sulfide (H<sub>2</sub>S) Attenuates Uranium-Induced Acute Nephrotoxicity through Oxidative Stress and Inflammatory Response via Nrf2-NF- $\kappa$ B Pathways. *Chem.-Biol. Interact.* **2015**, *242*, 353–362. [\[CrossRef\]](#) [\[PubMed\]](#)
111. Waz, S.; Heeba, G.H.; Hassanin, S.O.; Abdel-latif, R.G. Nephroprotective Effect of Exogenous Hydrogen Sulfide Donor against Cyclophosphamide-Induced Toxicity Is Mediated by Nrf2/HO-1/NF- $\kappa$ B Signaling Pathway. *Life Sci.* **2021**, *264*, 118630. [\[CrossRef\]](#) [\[PubMed\]](#)
112. Abdel-Hamid, H.A.; Marey, H.; Fouli Gaber Ibrahim, M. Hydrogen Sulfide Protects the Endometrium in a Rat Model of Type 1 Diabetes via Modulation of PPAR $\gamma$ /mTOR and Nrf-2/NF-Kb Pathways. *Arch. Physiol. Biochem.* **2024**, *130*, 909–920. [\[CrossRef\]](#) [\[PubMed\]](#)
113. Guo, C.; Liang, F.; Shah Masood, W.; Yan, X. Hydrogen Sulfide Protected Gastric Epithelial Cell from Ischemia/Reperfusion Injury by Keap1 s-Sulfhydration, MAPK Dependent Anti-Apoptosis and NF- $\kappa$ B Dependent Anti-Inflammation Pathway. *Eur. J. Pharmacol.* **2014**, *725*, 70–78. [\[CrossRef\]](#) [\[PubMed\]](#)
114. Xie, L.; Gu, Y.; Wen, M.; Zhao, S.; Wang, W.; Ma, Y.; Meng, G.; Han, Y.; Wang, Y.; Liu, G.; et al. Hydrogen Sulfide Induces Keap1 S-Sulfhydration and Suppresses Diabetes-Accelerated Atherosclerosis via Nrf2 Activation. *Diabetes* **2016**, *65*, 3171–3184. [\[CrossRef\]](#) [\[PubMed\]](#)
115. Bai, X.; Yao, L.; Ma, X.; Xu, X. Small Molecules as SIRT Modulators. *Mini-Rev. Med. Chem.* **2018**, *18*, 1151–1157. [\[CrossRef\]](#) [\[PubMed\]](#)
116. Chen, M.; Tan, J.; Jin, Z.; Jiang, T.; Wu, J.; Yu, X. Research Progress on Sirtuins (SIRT) Family Modulators. *Biomed. Pharmacother.* **2024**, *174*, 116481. [\[CrossRef\]](#) [\[PubMed\]](#)
117. Zhao, X.; Xue, X.; Wang, C.; Wang, J.; Peng, C.; Li, Y. Emerging Roles of Sirtuins in Alleviating Alcoholic Liver Disease: A Comprehensive Review. *Int. Immunopharmacol.* **2022**, *108*, 108712. [\[CrossRef\]](#) [\[PubMed\]](#)
118. Smimmo, M.; Casale, V.; D'Andrea, D.; Bello, I.; Iaccarino, N.; Romano, F.; Brancalone, V.; Panza, E.; d'Emmanuele Di Villa Bianca, R.; Katsouda, A.; et al. Defective Protein Persulfidation Is Involved in Obesity Associated Skeletal Muscle Dysfunction: Role of SIRT-1. *Redox Biol.* **2025**, *83*, 103645. [\[CrossRef\]](#) [\[PubMed\]](#)
119. Li, X.; Yu, P.; Yu, Y.; Xu, T.; Liu, J.; Cheng, Y.; Yang, X.; Cui, X.; Yin, C.; Liu, Y. Hydrogen Sulfide Ameliorates High Glucose-Induced pro-Inflammation Factors in HT-22 Cells: Involvement of SIRT1-mTOR/NF- $\kappa$ B Signaling Pathway. *Int. Immunopharmacol.* **2021**, *95*, 107545. [\[CrossRef\]](#) [\[PubMed\]](#)
120. Liu, M.-H.; Lin, X.-L.; Xiao, L.-L. Hydrogen Sulfide Attenuates TMAO-induced Macrophage Inflammation through Increased SIRT1 Sulfhydration. *Mol. Med. Rep.* **2023**, *28*, 13016. [\[CrossRef\]](#) [\[PubMed\]](#)
121. El-Boghdady, N.A.; Elsayed, E.I.; Samir, A.; Abdelmonem, M. SAME and DADS Attenuated Cuprizone-Induced Demyelination via Modulating H<sub>2</sub>S/AMPK/SIRT1/ULK1/Beclin1 Signaling. *Chem.-Biol. Interact.* **2025**, *418*, 111617. [\[CrossRef\]](#) [\[PubMed\]](#)
122. Du, C.; Lin, X.; Xu, W.; Zheng, F.; Cai, J.; Yang, J.; Cui, Q.; Tang, C.; Cai, J.; Xu, G.; et al. Sulfhydrated Sirtuin-1 Increasing Its Deacetylation Activity Is an Essential Epigenetics Mechanism of Anti-Atherogenesis by Hydrogen Sulfide. *Antioxid. Redox Signal.* **2019**, *30*, 184–197. [\[CrossRef\]](#) [\[PubMed\]](#)
123. Yuan, C.; Luo, X.Y.; Xiong, S.S.; Xie, N.; Ren, Z.; Wu, J.; Jiang, Z.S. The antagonist role of H<sub>2</sub>S against senescence of HUVEC may involve activation of SIRT1 S-sulfhydration and reduction of FOXO1 acetylation. *Chin. J. Arterioscler.* **2023**, *31*, 746–753.
124. Yuan, Y.; Zhu, L.; Li, L.; Liu, J.; Chen, Y.; Cheng, J.; Peng, T.; Lu, Y. S-Sulfhydration of SIRT3 by Hydrogen Sulfide Attenuates Mitochondrial Dysfunction in Cisplatin-Induced Acute Kidney Injury. *Antioxid. Redox Signal.* **2019**, *31*, 1302–1319. [\[CrossRef\]](#) [\[PubMed\]](#)
125. Chen, Y.; Ye, X.; Escames, G.; Lei, W.; Zhang, X.; Li, M.; Jing, T.; Yao, Y.; Qiu, Z.; Wang, Z.; et al. The NLRP3 Inflammasome: Contributions to Inflammation-Related Diseases. *Cell. Mol. Biol. Lett.* **2023**, *28*, 51. [\[CrossRef\]](#) [\[PubMed\]](#)
126. Henedak, N.T.; El-Abhar, H.S.; Soubh, A.A.; Abdallah, D.M. NLRP3 Inflammasome: A Central Player in Renal Pathologies and Nephropathy. *Life Sci.* **2024**, *351*, 122813. [\[CrossRef\]](#) [\[PubMed\]](#)
127. Gupta, S.; Cassel, S.L.; Sutterwala, F.S.; Dagvadorj, J. Regulation of the NLRP3 Inflammasome by Autophagy and Mitophagy. *Immunol. Rev.* **2025**, *329*, e13410. [\[CrossRef\]](#) [\[PubMed\]](#)
128. Mo, B.; Ding, Y.; Ji, Q. NLRP3 Inflammasome in Cardiovascular Diseases: An Update. *Front. Immunol.* **2025**, *16*, 1550226. [\[CrossRef\]](#) [\[PubMed\]](#)

129. Wang, Y.-D.; Li, J.-Y.; Qin, Y.; Liu, Q.; Liao, Z.-Z.; Xiao, X.-H. Exogenous Hydrogen Sulfide Alleviates-Induced Intracellular Inflammation in HepG2 Cells. *Exp. Clin. Endocrinol. Diabetes* **2019**, *128*, 137–143. [\[CrossRef\]](#)
130. Bao, P.; Gong, Y.; Wang, Y.; Xu, M.; Qian, Z.; Ni, X.; Lu, J. Hydrogen Sulfide Prevents LPS-Induced Depression-like Behavior through the Suppression of NLRP3 Inflammasome and Pyroptosis and the Improvement of Mitochondrial Function in the Hippocampus of Mice. *Biology* **2023**, *12*, 1092. [\[CrossRef\]](#) [\[PubMed\]](#)
131. Wu, D.; Zhong, P.; Wang, J.; Wang, H. Exogenous Hydrogen Sulfide Mitigates LPS + ATP-Induced Inflammation by Inhibiting NLRP3 Inflammasome Activation and Promoting Autophagy in L02 Cells. *Mol. Cell. Biochem.* **2019**, *457*, 145–156. [\[CrossRef\]](#) [\[PubMed\]](#)
132. Huang, Z.; Zhuang, X.; Xie, C.; Hu, X.; Dong, X.; Guo, Y.; Li, S.; Liao, X. Exogenous Hydrogen Sulfide Attenuates High Glucose-Induced Cardiotoxicity by Inhibiting NLRP3 Inflammasome Activation by Suppressing TLR4/NF- $\kappa$ B Pathway in H9c2 Cells. *Cell. Physiol. Biochem.* **2016**, *40*, 1578–1590. [\[CrossRef\]](#) [\[PubMed\]](#)
133. Ma, J.; Yang, P.; Zhou, Z.; Song, T.; Jia, L.; Ye, X.; Yan, W.; Sun, J.; Ye, T.; Zhu, L. GYY4137-Induced P65 Sulphydration Protects Synovial Macrophages against Pyroptosis by Improving Mitochondrial Function in Osteoarthritis Development. *J. Adv. Res.* **2025**, *71*, 173–188. [\[CrossRef\]](#) [\[PubMed\]](#)
134. García-Domínguez, M. The Role of TNF- $\alpha$  in Neuropathic Pain: An Immunotherapeutic Perspective. *Life* **2025**, *15*, 785. [\[CrossRef\]](#) [\[PubMed\]](#)
135. Jang, D.; Lee, A.-H.; Shin, H.-Y.; Song, H.-R.; Park, J.-H.; Kang, T.-B.; Lee, S.-R.; Yang, S.-H. The Role of Tumor Necrosis Factor Alpha (TNF- $\alpha$ ) in Autoimmune Disease and Current TNF- $\alpha$  Inhibitors in Therapeutics. *Int. J. Mol. Sci.* **2021**, *22*, 2719. [\[CrossRef\]](#) [\[PubMed\]](#)
136. Yang, S.S.; Wang, H.; Liu, Y.; Hu, Y.J.; Ma, D.H. Expression of NF- $\kappa$ B and TNF- $\alpha$  in Endometrial Polyps and Their Clinical Significance. *Prog. Mod. Biomed.* **2025**, *25*, 2881–2889.
137. Du, J.; Huang, Y.; Yan, H.; Zhang, Q.; Zhao, M.; Zhu, M.; Liu, J.; Chen, S.X.; Bu, D.; Tang, C.; et al. Hydrogen Sulfide Suppresses Oxidized Low-Density Lipoprotein (Ox-LDL)-Stimulated Monocyte Chemoattractant Protein 1 Generation from Macrophages via the Nuclear Factor  $\kappa$ B (NF- $\kappa$ B) Pathway. *J. Biol. Chem.* **2014**, *289*, 9741–9753. [\[CrossRef\]](#) [\[PubMed\]](#)
138. Chen, X.; Xu, W.; Wang, Y.; Luo, H.; Quan, S.; Zhou, J.; Yang, N.; Zhang, T.; Wu, L.; Liu, J.; et al. Hydrogen Sulfide Reduces Kidney Injury Due to Urinary-Derived Sepsis by Inhibiting NF- $\kappa$ B Expression, Decreasing TNF- $\alpha$  Levels and Increasing IL-10 Levels. *Exp. Ther. Med.* **2014**, *8*, 464–470. [\[CrossRef\]](#) [\[PubMed\]](#)
139. Yang, S.; Deng, D.; Luo, Y.; Wu, Y.; Zhu, R.; Xue, K.; Zhou, Y. NaHS Inhibits NF- $\kappa$ B Signal against Inflammation and Oxidative Stress in Post-Infectious Irritable Bowel Syndrome. *RSC Adv.* **2016**, *6*, 64208–64214. [\[CrossRef\]](#)
140. Liu, F.; Liu, G.-J.; Liu, N.; Zhang, G.; Zhang, J.-X.; Li, L.-F. Effect of Hydrogen Sulfide on Inflammatory Cytokines in Acute Myocardial Ischemia Injury in Rats. *Exp. Ther. Med.* **2015**, *9*, 1068–1074. [\[CrossRef\]](#) [\[PubMed\]](#)
141. Zhang, D.; Wang, X.; Chen, S.; Chen, S.; Yu, W.; Liu, X.; Yang, G.; Tao, Y.; Tang, X.; Bu, D.; et al. Endogenous Hydrogen Sulfide Sulphydrates IKK $\beta$  at Cysteine 179 to Control Pulmonary Artery Endothelial Cell Inflammation. *Clin. Sci.* **2019**, *133*, 2045–2059. [\[CrossRef\]](#) [\[PubMed\]](#)
142. Lin, J.-X.; Leonard, W.J. The Common Cytokine Receptor  $\gamma$  Chain Family of Cytokines. *Cold Spring Harb. Perspect. Biol.* **2017**, *10*, a028449. [\[CrossRef\]](#) [\[PubMed\]](#)
143. Mantovani, A.; Barajon, I.; Garlanda, C. IL -1 and IL -1 Regulatory Pathways in Cancer Progression and Therapy. *Immunol. Rev.* **2018**, *281*, 57–61. [\[CrossRef\]](#) [\[PubMed\]](#)
144. Jiang, T.; Zhou, C.; Ren, S. Role of IL-2 in Cancer Immunotherapy. *OncoImmunology* **2016**, *5*, e1163462. [\[CrossRef\]](#) [\[PubMed\]](#)
145. Hirano, T. IL-6 in Inflammation, Autoimmunity and Cancer. *Int. Immunol.* **2020**, *33*, 127–148. [\[CrossRef\]](#) [\[PubMed\]](#)
146. Yarmohammadi, R.; Najafi, K.; Noroozbeygi, M.; Didehvar, K.; Rastin, A.; Ataei, F.; Atashzar, M.R.; Shushtari, S.S. The Role of IL-6, IL-10 and CRP in Gastrointestinal Cancers. *Cell Biol. Int.* **2025**, *49*, 1061–1078. [\[CrossRef\]](#) [\[PubMed\]](#)
147. van der Heijden, T.; Bot, I.; Kuiper, J. The IL-12 Cytokine Family in Cardiovascular Diseases. *Cytokine* **2019**, *122*, 154188. [\[CrossRef\]](#) [\[PubMed\]](#)
148. Kurschus, F.C.; Moos, S. IL-17 for Therapy. *J. Dermatol. Sci.* **2017**, *87*, 221–227. [\[CrossRef\]](#) [\[PubMed\]](#)
149. Cayrol, C.; Girard, J. Interleukin-33 (IL-33): A Nuclear Cytokine from the IL -1 Family. *Immunol. Rev.* **2018**, *281*, 154–168. [\[CrossRef\]](#) [\[PubMed\]](#)
150. Xu, W.; Chen, J.; Lin, J.; Liu, D.; Mo, L.; Pan, W.; Feng, J.; Wu, W.; Zheng, D. Exogenous H<sub>2</sub>S Protects H9c2 Cardiac Cells against High Glucose-Induced Injury and Inflammation by Inhibiting the Activation of the NF- $\kappa$ B and IL-1 $\beta$  Pathways. *Int. J. Mol. Med.* **2015**, *35*, 177–186. [\[CrossRef\]](#) [\[PubMed\]](#)
151. Éva Sikura, K.; Combi, Z.; Potor, L.; Szerafin, T.; Hendrik, Z.; Méhes, G.; Gergely, P.; Whiteman, M.; Beke, L.; Fürtös, I.; et al. Hydrogen Sulfide Inhibits Aortic Valve Calcification in Heart via Regulating RUNX2 by NF- $\kappa$ B, a Link between Inflammation and Mineralization. *J. Adv. Res.* **2021**, *27*, 165–176. [\[CrossRef\]](#) [\[PubMed\]](#)

152. Xu, W.M.; Guo, R.M.; Chen, J.F.; Chen, G.; Guo, R.X.; Feng, J.Q.; Liao, X.X. Hydrogen sulfide inhibits doxorubicin-induced inflammation and cytotoxicity in rat cardiomyocytes by modulating activation of NF- $\kappa$ B pathway. *Chin. J. Pathophysiol.* **2013**, *29*, 2651–2656.
153. Zhong, X.; Zhao, X.; Zhang, L.; Liu, N.; Shi, S.; Wang, Y. Sodium Hydrosulfide Inhibiting Endothelial Cells Injury and Neutrophils Activation via IL-8/CXCR2/ROS/NF- $\kappa$ B Axis in Type 1 Diabetes Mellitus Rat. *Biochem. Biophys. Res. Commun.* **2022**, *606*, 1–9. [[CrossRef](#)] [[PubMed](#)]
154. Xu, C.; Zhao, H.Y. Effect and Mechanism of Hydrogen Sulfide on Septic Rats with Myocardial Injury. *J. Sichuan Univ. (Med. Sci.)* **2018**, *49*, 540–545.

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