



Heme oxygenase-1/CO signaling suppresses NLRP3 inflammasome-mediated stromal fibrosis in primary myelofibrosis

Lucia Longhitano^{a,1}, Enrico La Spina^{a,1}, Sebastiano Giallongo^{b,1}, Cesarina Giallongo^{a,*}, Jessica Ferrigno^a, Laura Orlando^a, Vittorio Del Fabro^b, Rosario Caltabiano^c, Giuseppe Nicolò Conti^a, Michelino Di Rosa^a, Giuseppe Broggi^c, Elisabetta P. Pricoco^d, Vittorio Calabrese^a, Andrea Duminuco^e, Giuseppe Alberto Palumbo^c, Gabriella Marsala^f, Giovanni Li Volti^a, Ignazio Alberto Barbagallo^{a,2}, Daniele Tibullo^{a,2}

^a Department of Biomedical and Biotechnological Sciences, Division of Medical Biochemistry, University of Catania, Catania, Italy

^b Department of Medicine and Surgery, University of Enna "Kore", Enna, Italy

^c Department of Medical and Surgical Sciences and Advanced Technologies "G.F. Ingrassia", Division of Anatomic Pathology, University of Catania, Catania, Italy

^d Anatomic Pathology, A.O.U. Policlinico "G. Rodolico-San Marco", Catania, Italy

^e Hematology Unit with BMT, A.O.U. Policlinico "G. Rodolico-San Marco", Catania, Italy

^f Drug Department, Azienda Sanitaria Provinciale (ASP) Catania, Catania, Italy

ARTICLE INFO

Keywords:

Heme oxygenase 1
Inflammasome NLRP3
Myelofibrosis
Fibrosis
Tumor microenvironment

ABSTRACT

Inflammation and oxidative stress are critical drivers of bone marrow (BM) fibrosis in primary myelofibrosis (MF). Here, we investigated the interplay between the heme oxygenase-1 (HO-1)/carbon monoxide (CO) pathway and NLRP3 inflammasome activation in MF microenvironment. Bioinformatic analysis of patient datasets (GSE53482 and GSE41812) revealed an approximately two-fold reduction of *HO-1* expression in CD34⁺ peripheral blood cells compared with healthy controls ($p < 0.01$). Consistently, JAK2V617F-mutated cell lines (HEL, SET2) displayed respectively lower HO-1 and higher NLRP3 expression than JAK2 wild-type UT7 cells. Ruxolitinib, a potent pharmacological inhibitor of JAK/STAT pathway, restored *HO-1* (≈ 1.7 -fold increase) and reduced *NLRP3* by $\sim 50\%$ ($p < 0.01$). Similarly, in mesenchymal stromal cells (HS-5), tumor-conditioned medium induced a two-fold increase in NLRP3 and Caspase-1 and a $\sim 40\%$ decrease in HO-1. Pharmacological induction of HO-1 by hemin (10 μM) or CO-releasing molecule CORM-A1 (25 μM) restored HO-1 (~ 1.8 -fold) and reduced NLRP3 activation ($\sim 50\%$), limiting αSMA expression and collagen deposition by ~ 55 – 60% . In a *TPO*^{high} zebrafish model, *nlrp3*, *caspase-a*, and *il1 β* were upregulated ≈ 2.5 -fold, while *hmx1a/b* decreased by $\sim 50\%$; CORM-A1 co-treatment reversed these effects, increasing *hmx1a* (~ 1.7 -fold) and decreasing *cd41* and *col1a1* ($\sim 45\%$). NLRP3 inhibition by MCC950 (1 μM) produced similar results. Consistently, BM biopsies from MF patients showed $\sim 65\%$ lower HO-1 expression ($p < 0.001$) and nuclear BACH1 localization in megakaryocytes. In conclusion, our results demonstrate that HO-1/CO signaling suppresses NLRP3 inflammasome activation and fibrotic remodeling, suggesting that this pathway may represent a potential therapeutic target in MF.

1. Introduction

Primary myelofibrosis (MF) is a clonal myeloproliferative neoplasm (MPN) arising from hematopoietic stem cells that acquire mutations in *JAK2*, *MPL*, or *CALR*, leading to constitutive activation of the JAK/STAT signaling pathway [1,2]. Clinically, the disease is characterized by

progressive bone marrow (BM) fibrosis, megakaryocytic proliferation, and extramedullary hematopoiesis, ultimately resulting in BM failure and splenomegaly [3,4]. The advent of JAK inhibitors dramatically changed the approach to this disease, improving the symptoms, quality of life and overall survival [5–7]. Chronic inflammation and oxidative stress are recognized as major drivers of MF pathogenesis and fibrotic

* Corresponding author.

E-mail address: cesarina.giallongo@unict.it (C. Giallongo).

¹ These authors contributed equally as co-First.

² These authors contributed equally as co-Last.

<https://doi.org/10.1016/j.freeradbiomed.2026.07.030>

Received 26 November 2025; Received in revised form 13 July 2026; Accepted 14 July 2026

Available online 20 July 2026

0891-5849/© 2026 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

progression, sustaining an aberrant microenvironment that favors clonal expansion, cytokine overproduction, and tissue remodeling [8]. In particular, inflammation promotes fibroblast activation and extracellular matrix (ECM) deposition through the release of proinflammatory mediators, including interleukins, chemokines, and growth factors such as TGF- β [9]. Among the key regulators of this process, the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome has emerged as a pivotal mediator of inflammatory signaling and fibrosis [10]. NLRP3 activation triggers caspase-1 cleavage and the release of IL-1 β and IL-18, amplifying the inflammatory cascade and contributing to stromal activation. Elevated inflammasome activity has been reported in several fibrotic diseases, including MF, linking innate immune dysregulation to tissue scarring and disease progression. To counterbalance this persistent inflammatory state, cells rely on endogenous cytoprotective systems that limit oxidative and inflammatory stress. Among them, the heme oxygenase-1 (HO-1) pathway plays a central role. HO-1 catalyzes the degradation of heme into biliverdin, iron, and carbon monoxide (CO), molecules with potent antioxidant and cytoprotective properties [11–13]. Reduced HO-1 expression or activity has been associated with enhanced oxidative stress, excessive cytokine production, and fibrosis in multiple tissues [14,15]. Notably, a reciprocal regulatory loop exists between HO-1 and the NLRP3 inflammasome [16]. In this regard, previous studies showed that HO-1 can suppress inflammasome activation, while excessive NLRP3 signaling can inhibit HO-1 transcription, perpetuating inflammation and tissue damage [17]. Despite the recognized role of inflammation in MF, the contribution of the HO-1/CO axis to the regulation of NLRP3 inflammasome activity in the myelofibrotic niche remains poorly understood. We therefore aimed to elucidate the molecular crosstalk between HO-1 signaling and NLRP3 inflammasome activation in MF, using integrated bioinformatic, in vitro, and in vivo approaches. We further explored whether pharmacological activation of HO-1 or its byproduct CO could counteract inflammasome-driven fibrotic remodeling in stromal cells and the BM microenvironment of MF.

2. Methods

2.1. Datasets selection and data processing

For our study, two datasets were selected. Norfo et al., 2014; Guglielmelli et al., 2013, profiled the expression of CD34⁺ cells in PMF patients through array experiments [18,19] (data accessible at NCBI GEO database [20] accession respectively GSE53482 and GSE41812). A total of 78 samples, comprising 16 healthy controls and 62 primary myelofibrosis (PMF) patients, were considered for expression analysis. All selection and analysis criteria refer to our previous manuscript [21]. The getGEO function, included in the GEOQuery R package [22], was used to retrieve both expression values and metadata of the involved datasets. Samples were stratified according to their JAK2 mutational status, following sample metadata.

A z-score scaling was computed on normalized expression values, to facilitate the comparison between the conditions among different datasets. The Kruskal-Wallis test, followed by Dunn's multiple comparison post-hoc test were computed to assess differential expression between groups.

2.2. Cell culture and pharmacological treatments

Healthy mesenchymal stromal cells (HS5), and erythroblast cell line (HEL), were purchased from ATCC (ATCC TIB-180TM, Milan, Italy), essential thrombocythemia cell line (SET-2) and acute myeloid leukemia cell line (UT7) were obtained from DSMZ (Braunschweig, Germany).

HEL and SET-2 cells were selected as JAK2V617F-mutated hematopoietic cell models to reproduce constitutive JAK2/STAT signaling associated with myeloproliferative neoplasms. UT7 cells were used as a JAK2 wild-type hematopoietic comparator, allowing the assessment of

differences associated with the JAK2V617F-mutated background. HS-5 cells were selected as a human bone marrow-derived mesenchymal stromal cell model to investigate the response of the stromal compartment to soluble factors released by JAK2V617F-mutated cells.

HS5 cells were maintained in culture medium Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 U/mL streptomycin. HEL, and SET2 were maintained in RPMI-1640 Medium supplemented with 10% or 20% FBS respectively, 100 U/mL penicillin, and 100 U/mL streptomycin. UT7 were grown in RPMI-1640 Medium supplemented with 20% FBS, 100 U/mL penicillin, and 100 U/mL streptomycin and 5 ng/ml GM-CSF, according to manufacturer's guidelines.

Conditioned medium from HEL and SET2 cell lines was collected 24 h after seeding 1×10^6 cells and subsequently used to incubate 5×10^6 HS-5 cells for 24 h, at a 1:1 medium ratio. The control condition consisted of a 1:1 mixture of fresh RPMI and fresh DMEM, prepared with the same serum concentration and the same medium ratio used for the conditioned-medium experiments. Ruxolitinib was obtained from Enzo Life Science (New York, USA). The doses of hemin (Sigma-Aldrich, Mylan, Italy), CORM-A1 (Sigma-Aldrich, Mylan, Italy) and MCC950 (InvivoGen, San Diego, CA, USA) used were 10 μ M, 25 μ M and 1 μ M respectively [23,24].

2.3. Real time PCR

Cellular RNA was extracted according to the TRIzol protocol (ThermoFisher scientific) [25]. cDNA was then synthesized with a High-Capacity cDNA Reverse Transcription kit (category no. 4368814, Applied Biosystems, Foster City, CA, USA) [26]. Quantitative real-time PCR was performed in Step-One Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) using the SYBR Green PCR MasterMix (category no. 4309155, Life Technologies, Monza, Italy). Primers were purchased by Metabion International AG (Planegg, Germany) and primers' sequences are shown in Table 1. The relative mRNA expression level was calculated using the threshold cycle (Ct) value of each PCR product and normalized to β -actin using a comparative $2^{-\Delta\Delta C_t}$ method.

2.4. Western blot analysis

Western blot analysis was performed as previously described [27]. Briefly, 30 μ g of protein extract was loaded onto a 12% polyacrylamide gel MiniPRO-TEAN[®] TGXTM (BIO-RAD, Milan, Italy) and electro-transferred to a nitrocellulose membrane TransBlot[®] TurboTM (BIO-RAD, Milan, Italy). Protein detection was performed by incubating with primary antibody against Nlrp3 (1:500; rabbit) (ab263899, Abcam, Cambridge, UK), Caspase-1 (1:500; rabbit) (ab179515 Abcam, Cambridge, UK), α -SMA (1:500; rabbit) (ab124964, Abcam, Cambridge, UK), HO-1 (1:1000; rabbit) (ab68477, Abcam, Cambridge, UK) and β -actin (1:2000; anti-mouse, (ab8226, Abcam, Cambridge, UK) overnight at 4 °C. Secondary antibody anti-mouse and anti-rabbit HRP-conjugated (Jackson, WestGrove, PA, USA) were used. Protein bands intensities were detected using C-DiGit[®] Blot Scanner (LI-COR Biotech), quantified and normalized to β -actin levels.

2.5. Mallory staining

Mallory staining was performed using a previously published method [28]. Briefly, cells cultured on chamber slides were stained using Mallory's Trichrome kit (REF010227, Diapath). Stained cells were examined under a light microscope for cytological analysis.

2.6. In vivo zebrafish model

2.6.1. Ethics statement

The experimental procedures were approved by the Animal Studies Committee of Ministero della Salute Italy (Approval code: 30/2022-PR).

Table 1
Primers' list (human).

Gene	Forward 5' → 3'	Reverse 5' → 3'	Accession Number
<i>NLRP3</i>	CTGGCATCTGGGGAAACCT	TCCTTAGGCTTCGGTCCACA	NM_001079821.3
<i>CASP-1</i>	GAGAAAAGCCATGGCCGACAA	TACCTTCACCCATGGAACGG	NM_001223.5
<i>IL1β</i>	GCCAATCTTCATTCCTCAAGTGT	AGCCATCATTTCACTGGCGA	NM_000576.3
<i>IL18</i>	TGACCAAGGAAATCGGCCTC	GGTCCGGGGTGCAATTATCTCT	NM_001243211.2
<i>HMOX1</i>	AAGACTGCGTTCCTGCTCAA	GGGCAGAATCTTGCACTTTGT	NM_002133.3
<i>β-Actin</i>	CCTTTGCCGATCCGCCG	AACATGATCTGGGTCATCTTCTCGC	NM_001101.5

All the procedures were performed according to the relevant regulations.

2.6.2. Zebrafish husbandry and treatment

Adult (5–8-months-old) and larvae (2 dpf) zebrafish (*Danio rerio*) were used for this study. Fishes were housed at a density of five fishes per tank in mixed-sex groups in 2.5 L tanks on a recirculating system in 28 °C water in a room with a 14:10 h light:dark cycle. System water was carbon-filtered municipal tap water, filtered through a 20 µm pleated particulate filter, and exposed to 40 W UV light [29]. Standard feeding protocol was three meals daily of Tetra-Min (Tetra) in the CAPIR (University of Catania) facility. Zebrafish fertilized eggs were obtained from natural spawning of fish held at the facilities following standard husbandry practices.

As previous described [28], TPO^{high} adult zebrafish cohort was exposed to 100 µg/kg Eltrombopag Olamine (Selleckchem, Cologne, Germany), a TPO receptor agonist (TPO-RA), every 3 days.

Control and TPO^{high} zebrafish were anaesthetized after 15 days with 0.02% tricaine before whole kidney marrow (WKM) collection. After a ventral, midline incision was made, WKM was dissected and placed into ice-cold PBS containing 5% FBS. Larvae (2dpf) were treated for 3 days with 6 µg/mL Eltrombopag Olamine and/or with 25 µM CORM-A1 or 1 µM MCC950 for 72 h.

2.6.3. RT-qPCR

Total RNA was extracted from WKM or larvae using TRIzol reagent. After reverse transcription, we evaluated expression of the following zebrafish mRNA using primers' sequences showed in Table 2. For each sample, the relative expression levels of each studied mRNA were normalized using gapdh.

2.7. Immunohistochemistry analysis

Upon treatment, the embryo medium was carefully removed using a micropipette, and the embryos were fixed in 4% paraformaldehyde (PFA) for 2 h at room temperature with gentle rocking, then washed three times in 1 × PBS containing 1% Triton-X (PBTrition) for 5 min each. Permeabilization was achieved by digesting the embryos in Proteinase K for 15 min, followed by rinsing in PBTrition and re-fixation in 4% PFA for 20 min at room temperature. After three washes in PBTrition, blocking was performed in blocking solution for 3 h at room temperature. For immunostaining, embryos were incubated overnight at 4 °C in primary antibody (col1a1, sc-59772) (1:500) diluted in blocking solution,

washed five times in PBTrition for 10 min each, and then incubated for 2 h at room temperature with goat-anti-mouse Alexa488 (1:500) diluted in 1% goat serum in PBTrition. After three final washes in PBTrition, images were acquired using a fluorescence stereomicroscope (Carl Zeiss, Oberkochen, Germany). The immunohistochemical analyses on Bone marrow biopsies were performed as previously described [30]; samples were collected from MF patients and age-matched controls after written informed consent (Azienda Ospedaliero-Universitaria Policlinico “G. Rodolico-San Marco”, n. 54/2022/PO). After deparaffinization and pretreatments, control or PMF slides were incubated with primary antibodies against HO-1 (1:200) (ab68477, Abcam, Cambridge, UK) and BACH1 (1:200) (ab151509, Abcam, Cambridge, UK), counterstained with hematoxylin, and evaluated with a light microscope (Carl Zeiss, Oberkochen, Germany). Clinical data of patients included in this study are shown in Table 3.

2.8. Statistical analysis

For statistical analyses, a two-tailed unpaired Student's t-test was used for comparison of n = 2 groups. For comparison of n ≥ 3 groups, one-way analysis of variance (ANOVA), for multiple comparisons was used. Data are presented as the mean ± SD of biological replicates. Data analysis was performed using GraphPad Prism software. A value of p < 0.05 was considered statistically significant and symbols used to indicate statistical differences are described in figure legends.

Table 3

Clinical characteristic of PMF patients (n = 23) included in the study.

Median years of age (range)	67 (40–76)
Sex	16 M (69%), 7 F (31%)
Blood count	
-Hb, g/dl (range)	10.2 (6.1–17.2)
-WBC, x10 ³ /mmc (range)	9.7 (1.2–149.1)
-Platelets, x10 ³ /mmc (range)	349 (70–1016)
IPSS/MYSEC-PM	
-Low	2 (8.7%)
-Intermediate-1	6 (26.08%)
-Intermediate-2	10 (43.48%)
-High	5 (21.74%)

Hb: hemoglobin; WBC = white blood cells.

IPSS/MYSEC-PM = International Prognostic Scoring System/MYSEC Prognostic Model Risk Calculator.

Table 2
Genes of interest (*Danio rerio*).

Gene	Forward 5' → 3'	Reverse 5' → 3'	Accession Number
<i>nlrp3</i>	TGTAAACTGAAGACACTCAAACTG	TAGAGTCACCTTTCTGTCTCG	XM_068222147.1
<i>caspase-1a</i>	GGCATGTGTCAGAAATGGAACG	CGAAACGCTTTAGAACCTTCCTG	NM_131505.2
<i>caspase-1b</i>	ACAATGCCGGGTCTGAGGAT	CTCCTCGGTGTGTAATATCGTCT	NM_152884.2
<i>il1β</i>	GTAACCTGTACCTGGCCTGC	AGACCCGCTGATCTCCTTGAG	NM_212844.2
<i>hmox1a</i>	TGGACAGAAACGACAGACCAC	GACTGCTCTTGCCAATCTCTCT	NM_001127516.1
<i>hmox1b</i>	GAGAGCACCCGGAGTATCTG	TGCTTGAAGAGTTTGGGGCT	NM_205671.1
<i>cd41</i>	CTGAAGGCACTAACGTCAC	TCCTTCTCTGACCAAGATTA	NM_001003857.1
<i>col1a1</i>	ACCCTTAAGTCCCTGAGCCA	AATCCAGTACTCGCGCTCT	NM_199214.1
<i>gapdh</i>	AGTGTCCAGACGACAGAGGCT	GCCAATGCGACCGAATCCGTTA	NM_001115114.1

3. Results

3.1. Expression of HMOX1 in JAK2V617F mutated cells

To evaluate the expression of HO-1 in MF, we performed a bioinformatic analysis using two publicly available datasets (GSE53482 and GSE41812). Comparison of peripheral blood CD34⁺ cells from MF patients and healthy controls revealed a significant reduction in HO-1 expression (Fig. 1A). To further explore whether the expression pattern of HO-1 and inflammasome-related genes was influenced by the JAK2^{V617F} mutation, we analyzed JAK2 wild-type (UT7) and JAK2^{V617F}-mutated (SET2 and HEL) cell lines. HMOX1 expression was markedly higher in UT7 cells compared with HEL and SET2 (Fig. 1B), suggesting that HO-1 downregulation may be associated with the proinflammatory milieu driven by JAK2^{V617F} signaling. Accordingly, NLRP3 expression was significantly upregulated in JAK2^{V617F}-mutated cells relative to wild-type counterparts (Fig. 1C). Treatment with the JAK2 inhibitor ruxolitinib restored HMOX1 expression (≈ 1.7 -fold increase) and concomitantly reduced NLRP3 levels by approximately 50% (Fig. 1D and E). Collectively, these findings indicate that the JAK2^{V617F} mutation promotes NLRP3 inflammasome activation while suppressing HMOX1 expression, thereby linking aberrant JAK2 signaling to oxidative and inflammatory dysregulation in myelofibrosis.

3.2. Tumor cells activate NLRP3 inflammasome in stromal cells, eventually decreasing HO-1 levels

Tumor cell-derived inflammasomes are known to generate a proinflammatory microenvironment that promotes fibrotic transformation. To investigate the potential contribution of NLRP3 inflammasome activation to bone marrow (BM) fibrosis, we exposed healthy mesenchymal stromal cells (HS-5) to conditioned medium (CM) obtained from JAK2^{V617F}-mutated HEL and SET2 cell lines (Fig. 2A). Exposure to tumor CM induced a significant upregulation of NLRP3 and Caspase-1 transcripts compared with untreated controls (Fig. 2B–E). Western blot analysis confirmed increased NLRP3 protein levels and the conversion of pro-caspase-1 to its active form, consistent with inflammasome activation (Fig. 2F). Notably, these changes were accompanied by a marked downregulation of HO-1 expression (Fig. 2G). Together, these data indicate that tumor-derived soluble factors promote NLRP3 inflammasome activation in stromal cells while suppressing HO-1 antioxidant defenses, thereby contributing to the establishment of a proinflammatory and profibrotic microenvironment.

3.3. HO-1/CO pathway inhibits tumor-induced NLRP3 inflammasome activation in stromal cells

Given that HO-1 activity can be pharmacologically modulated, we next investigated whether activation of the HO-1/carbon monoxide (CO) pathway could counteract tumor-induced inflammasome activation in stromal cells. HS-5 cells exposed to tumor-conditioned medium

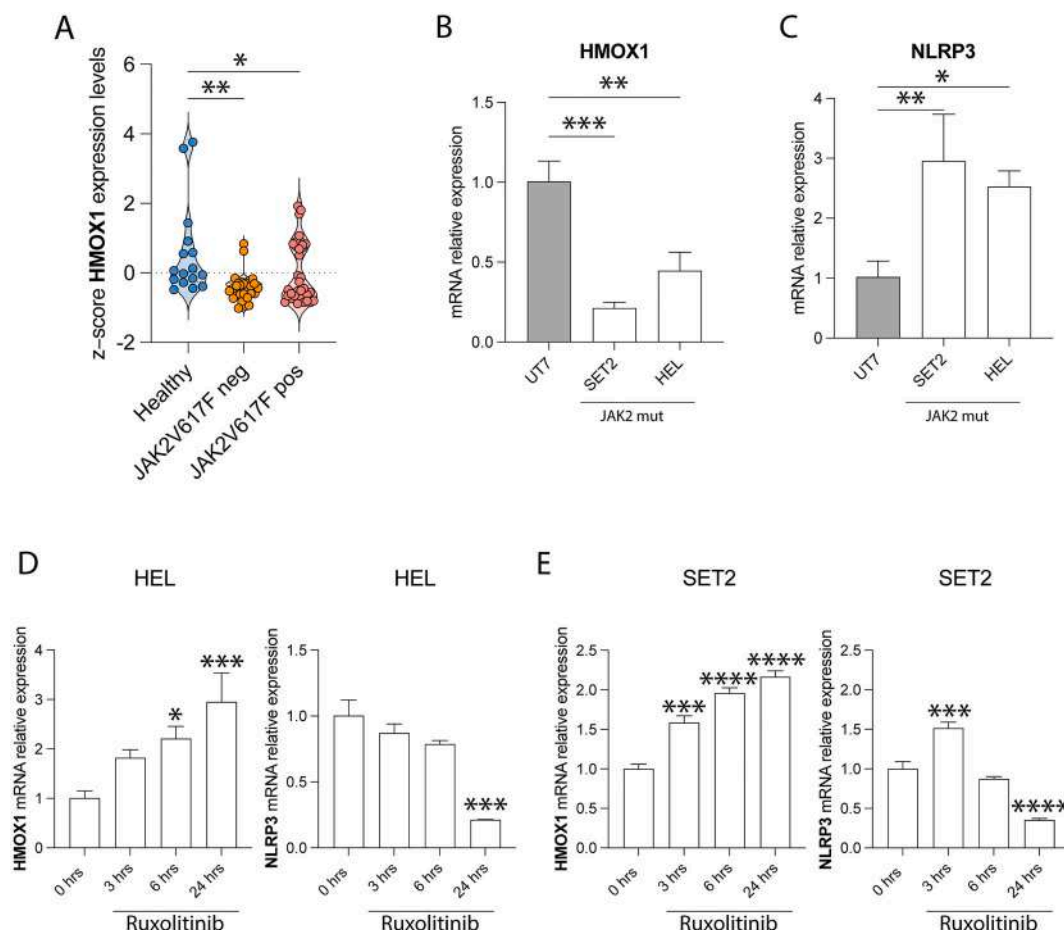


Fig. 1. Analysis of HMOX1 genes in PMF patients and JAK2V617F mutated cell lines. (A) Z-score expression levels of HMOX1 in PB CD34⁺ cells from healthy and JAK2^{V617F} negative and positive PMF patients. (B) HMOX1 and (C) NLRP3 mRNA expression levels in SET2, HEL and UT7 cell lines. HMOX1 and NLRP3 mRNA expression in HEL (D) and SET2 (E) cells after treatment respectively with 300 nM and 50 nM ruxolitinib. β -actin was used as housekeeping gene. Calculated value of $2^{-\Delta\Delta Ct}$ in control was 1. Data are presented as mean $\pm$ SD of three independent experiments. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. **** $p < 0.0001$.

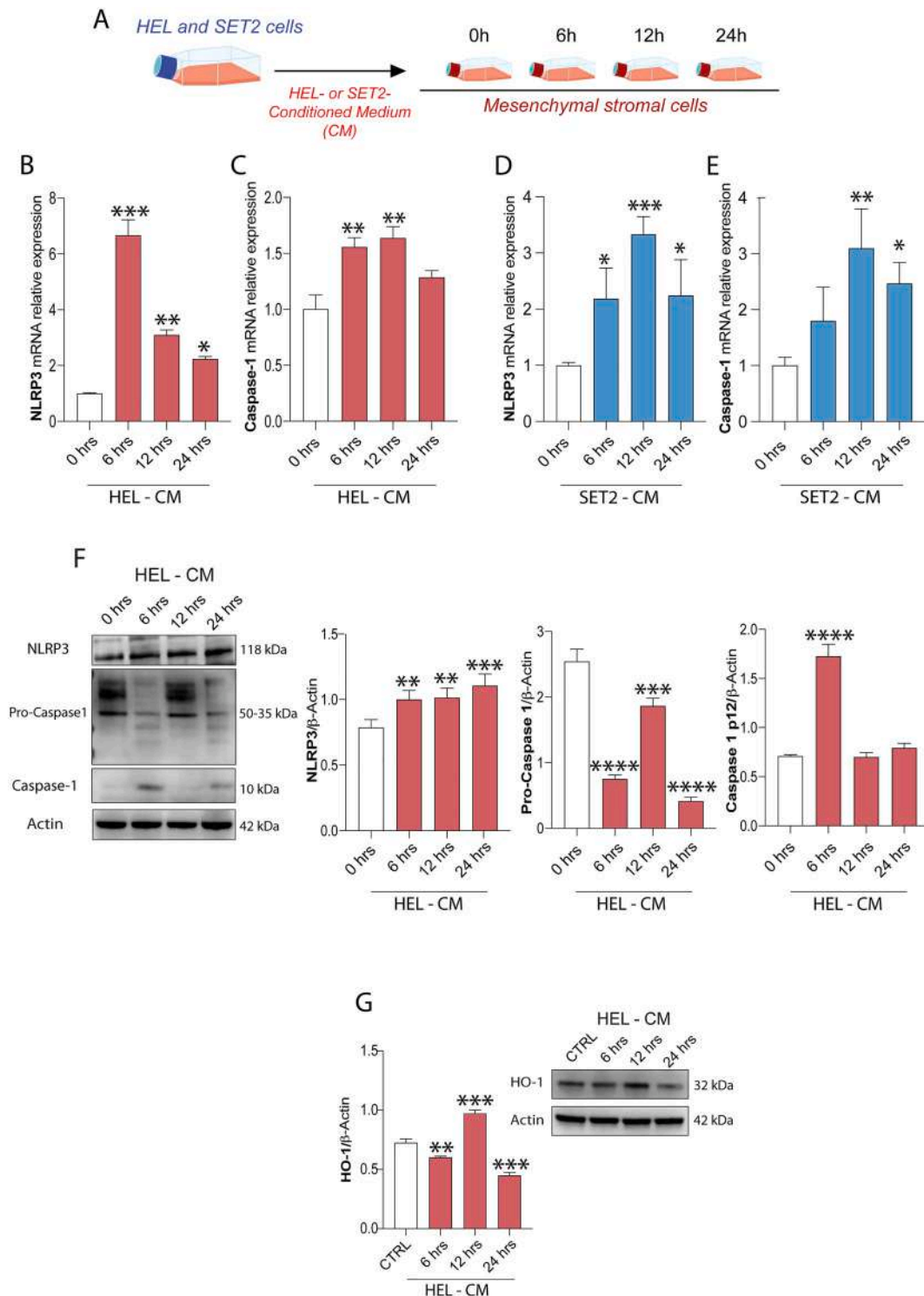


Fig. 2. HS-5 cells activated NLRP3 inflammasome after exposure to tumor conditioned medium. (A) Schematic representation of the experimental design. Stromal cells were cultured in CM from HEL or SET2 cell lines for different time points. (B–E) Gene expression analysis of NLRP3 and Caspase-1 in HS-5 cells. β -actin was used as housekeeping gene. Calculated value of $2^{-\Delta\Delta C_t}$ in control was 1. (F–G) Western blot analysis of NLRP3, pro-caspase-1, Caspase-1, HO-1 in HS-5 cells after exposure to HEL-CM. β -actin was used as total protein loading reference. Data are presented as mean $\pm$ SD of three independent experiments. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. **** $p < 0.0001$.

(CM) were treated with the HO-1 inducer hemin (10 μ M) or the CO-releasing molecule CORM-A1 (25 μ M). Both treatments significantly restored *HMOX1* expression and reduced *NLRP3* mRNA levels, effectively reversing the CM-induced proinflammatory response (Fig. 3A and B). Western blot analysis corroborated these findings, showing increased HO-1 protein and decreased NLRP3 levels following hemin or CORM-A1

exposure (Fig. 3C and D). These results demonstrate that pharmacological activation of the HO-1/CO axis attenuates NLRP3 inflammasome signaling in stromal cells. Since CO is a biologically active byproduct of HO-1 enzymatic activity with established anti-inflammatory and cytoprotective properties, these data suggest that CO may mediate the inhibitory effects of HO-1 on tumor-induced inflammasome activation.

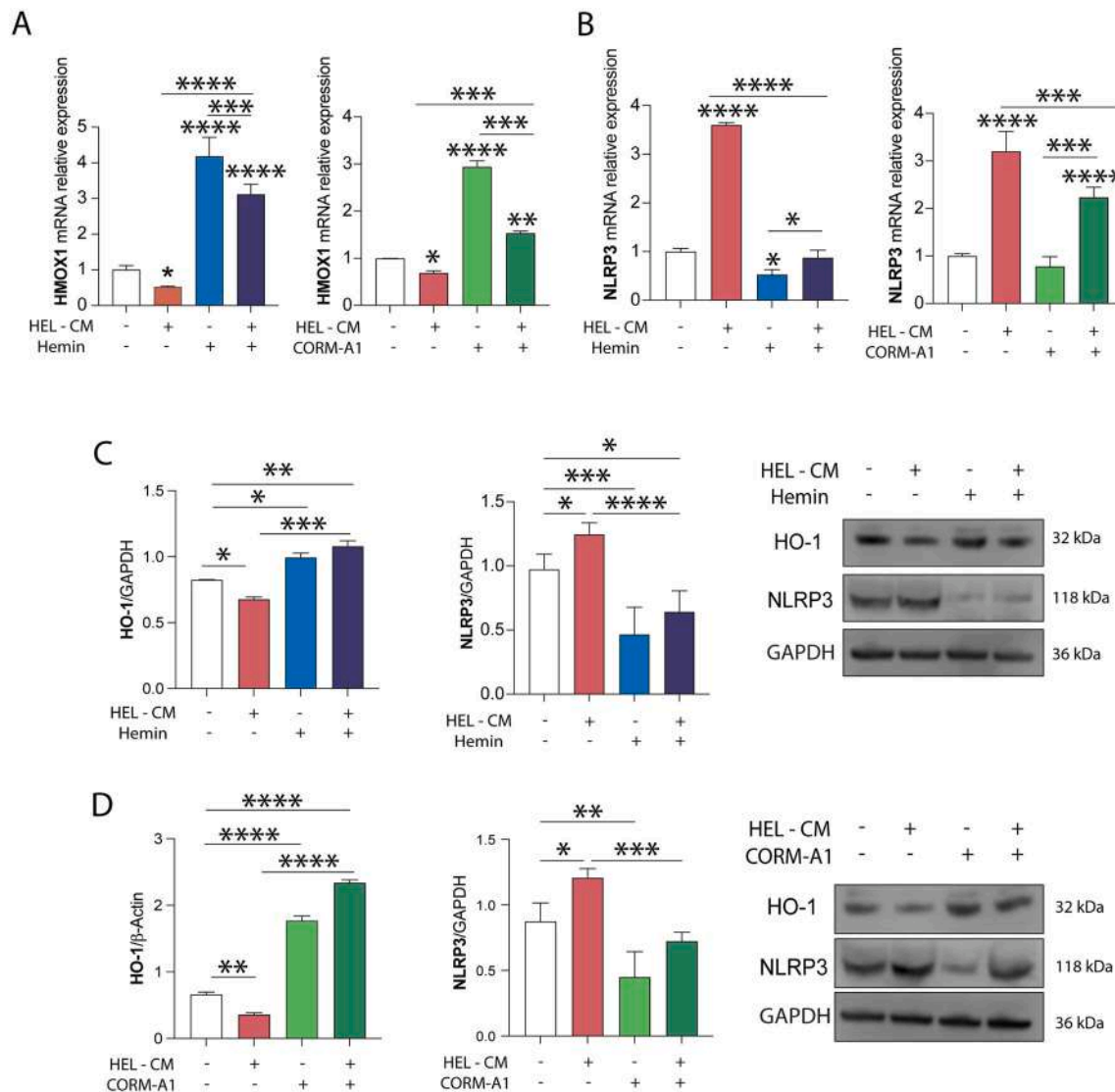


Fig. 3. Tumor-induced NLRP3 inflammasome was suppressed by HO-1/CO pathway activation. (A) Gene expression of HMOX1 and (B) NLRP3 in HS-5 cells after Hemin (10 μ M) and CORM-A1 (25 μ M) exposure with or without HEL-CM medium for 24 h. β -actin was used as housekeeping gene. Calculated value of $2^{-\Delta\Delta C_t}$ in control was 1. (C-D) Western blot analysis of HO-1 and NLRP3 in HS-5 cells following Hemin (10 μ M) and CORM-A1 (25 μ M) exposure with or without HEL-CM medium. Data are presented as mean $\pm$ SD of three independent experiments. * p < 0.05. ** p < 0.01. *** p < 0.001. **** p < 0.0001.

3.4. Hemin and CORM-A1 restrain tumor cells induced pro-fibrotic phenotype in stromal cells

Inflammation is a key driver of fibrosis onset and progression in myelofibrosis. To determine whether HO-1 activation could mitigate tumor-induced fibrotic changes, we evaluated the effects of hemin and CORM-A1 on the stromal phenotype. Exposure of HS-5 cells to tumor-conditioned medium (CM) from HEL cells markedly increased α -smooth muscle actin (α SMA) expression, consistent with a profibrotic activation (Fig. 4C and D). Treatment with either hemin (10 μ M) or CORM-A1 (25 μ M) significantly reduced α SMA accumulation by approximately 55–60%, indicating inhibition of myofibroblastic differentiation. To further assess extracellular matrix deposition, Mallory trichrome staining was performed. CM exposure led to a robust increase in collagen deposition, which was almost completely prevented by hemin or CORM-A1 supplementation (Fig. 4E and F). These in vitro findings were supported by analysis of a TPO^{high} zebrafish model, which recapitulates inflammatory and fibrotic features of myelofibrosis. In this model, *nlrp3*, *caspase-a*, and *il1 β* transcripts were upregulated approximately 2.5-fold, whereas *hmox1a/b* expression decreased by ~50%

(p < 0.01) (Fig. 4A and B). Together, these results demonstrate that pharmacological activation of the HO-1/CO pathway counteracts tumor-induced stromal activation and collagen accumulation, highlighting its protective role against fibrotic transformation within the bone marrow microenvironment.

3.5. CO modulates NLRP3 and HO-1 expression in a TPO^{high} zebrafish model

To further investigate the regulatory role of the HO-1/CO axis in vivo, we employed a TPO^{high} zebrafish larvae model (2 dpf) treated with a thrombopoietin receptor agonist (TPO-RA) and/or CORM-A1 (25 μ M) for three days (Fig. 5A). TPO-RA treatment induced a strong inflammatory and fibrotic response, as indicated by a $\approx$ 2.5-fold upregulation of *nlrp3*, *caspase-a*, and *il1 β* transcripts (p < 0.01) (Fig. 5B). Notably, co-treatment with CORM-A1 markedly reduced the expression of these genes, restoring inflammatory balance.

In parallel, *hmox1a* expression increased by approximately 1.7-fold in larvae co-treated with CORM-A1, confirming activation of the HO-1/CO pathway (Fig. 5C). TPO-RA also induced a significant rise in the

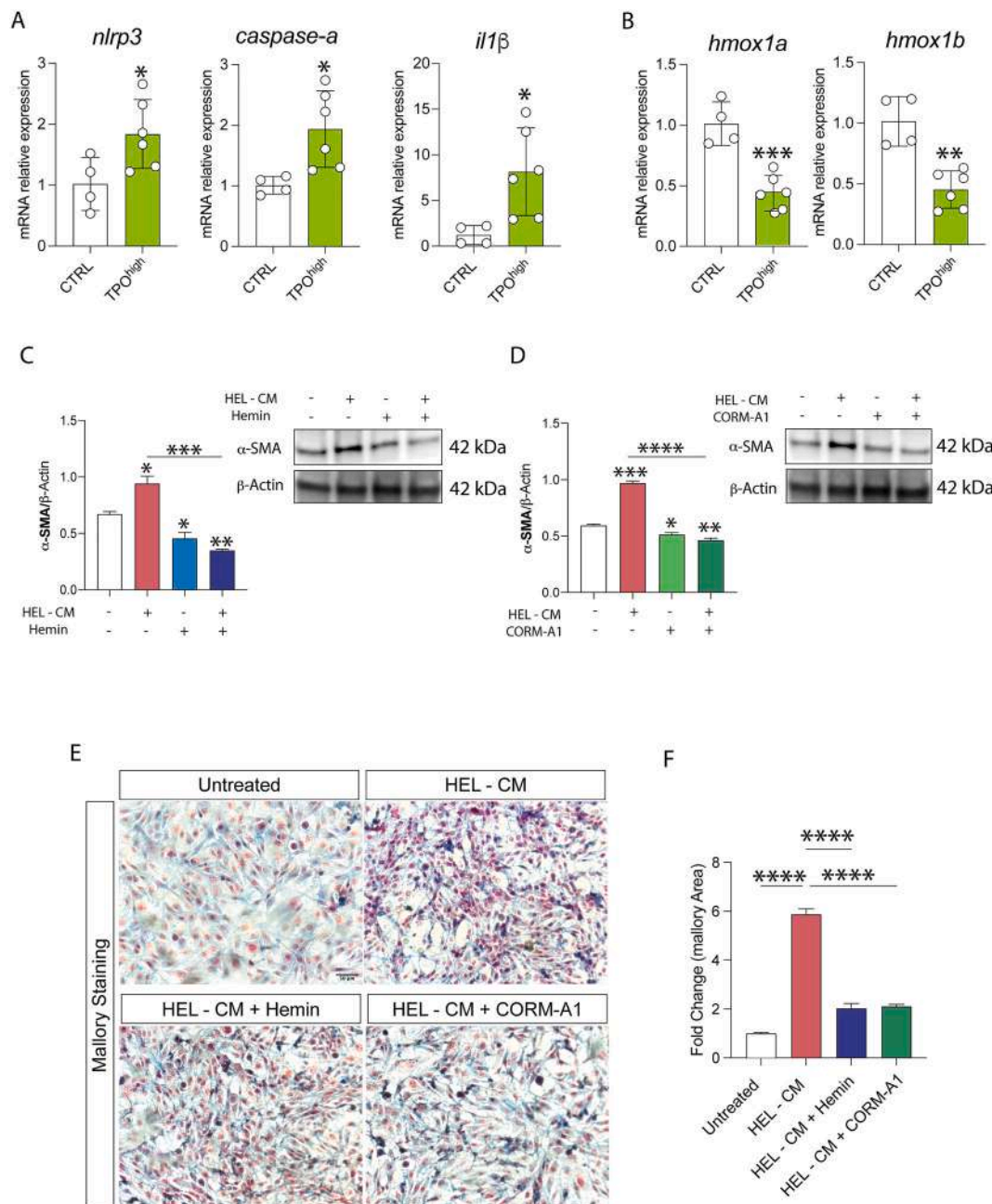


Fig. 4. Tumor activated pro-fibrotic phenotype in HS-5 cells was reversed by HO-1 inducers hemin and CORM-1. (A) Gene expression of *nlrp3*, *caspase-a*, *il1β*, (B) *hmox1a* and *hmox1b*, in WKM from adult TPO^{high} zebrafish. Gapdh was used as housekeeping gene. Calculated value of $2^{-\Delta\Delta C_t}$ in control was 1. (C–D) Western blot analysis of α -SMA protein following 24 h of Hemin or CORM-A1 treatment. β -actin was used as total protein loading reference. (E) Representative images of Mallory staining and (F) quantification of Mallory Area of HS-5 cells exposed to HEL-CM w/o Hemin or CORM-A1. Scale bar: 50 μ m. Data are presented as mean $\pm$ SD of three independent experiments. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. **** $p < 0.0001$.

megakaryocytic marker *cd41* and the profibrotic gene *col1a1* ($\approx 45\%$ increase), both of which were strongly downregulated following CORM-A1 exposure (Fig. 5D and E). Immunofluorescence analysis corroborated these results, showing reduced Col1a1 staining and collagen accumulation in CORM-A1-treated animals (Fig. 5F and G).

Collectively, these data demonstrate that CO, a biologically active product of HO-1 activity, effectively suppresses NLRP3 inflammasome activation and fibrotic remodeling in vivo. The results further substantiate the protective role of HO-1/CO signaling in limiting inflammation-driven fibrosis within the myelofibrotic microenvironment.

3.6. MCC950-mediated NLRP3 inhibition limits tumor-induced pro-fibrotic changes in stromal cells

To confirm the involvement of the NLRP3 inflammasome in tumor-induced stromal activation, we used the selective NLRP3 inhibitor MCC950. Treatment of HS-5 cells exposed to tumor-conditioned medium (HEL-CM) with MCC950 (1 μ M) significantly restored HMOX1 expression and reduced NLRP3 transcript levels by approximately 50% (Fig. 6A and B). Western blot analysis corroborated these findings, showing increased HO-1 protein and decreased NLRP3 accumulation following MCC950 treatment (Fig. 6C). In addition, transient NLRP3 silencing by siRNA confirmed effective genetic modulation of NLRP3 expression in HS-5 cells. These findings provide genetic support for the

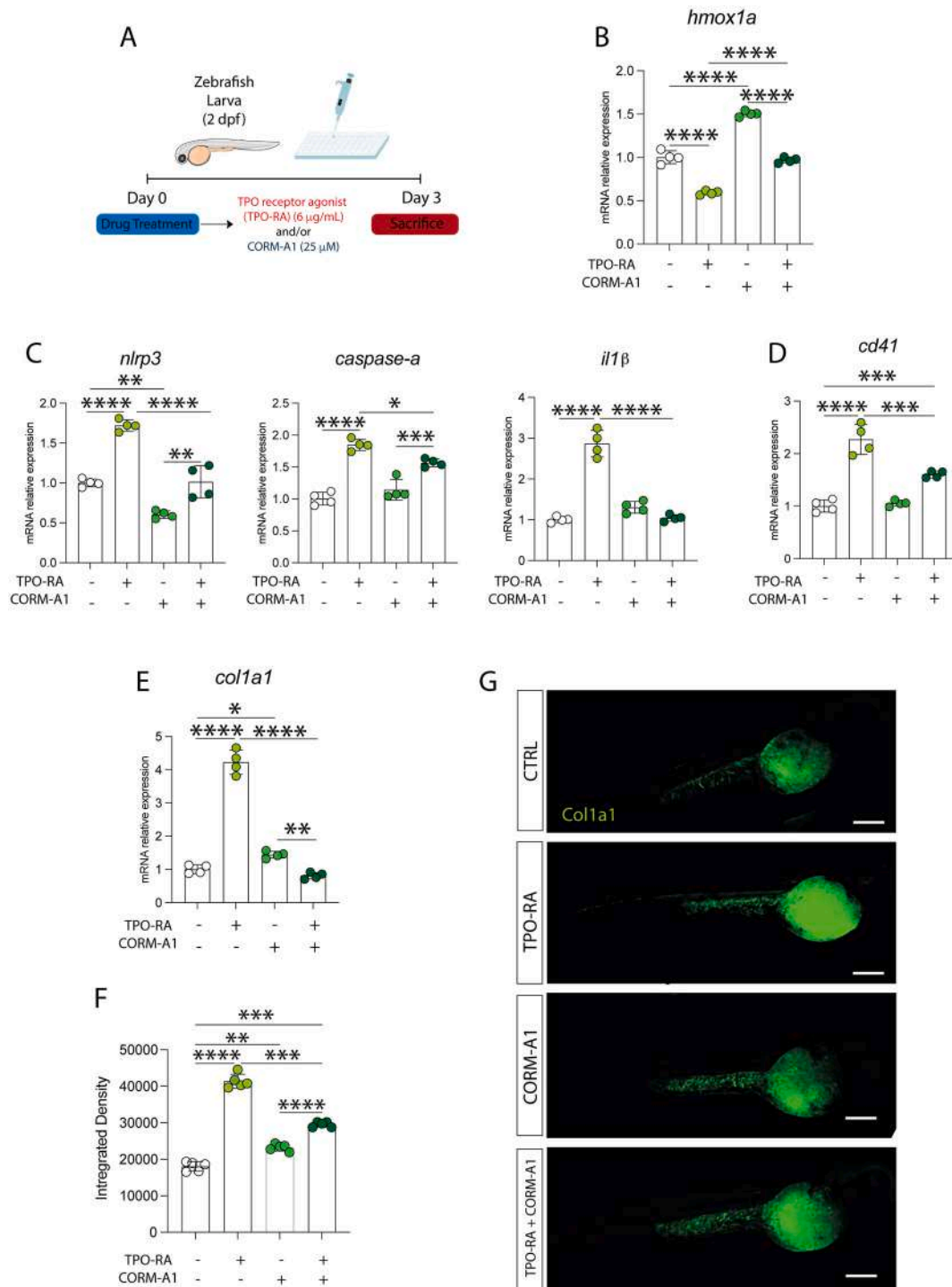


Fig. 5. Effect of CORM-A1 in TPO receptor agonist-treated zebrafish model. (A) Schematic representation of the experimental design in zebrafish larvae. (B) Gene expression of *hmx1a*, (C) *nlrp3*, *caspase-a*, *il1β*, (D) *cd41* and (E) *col1a1* in zebrafish larvae following 3 days of TPO receptor agonist (TPO-RA) and/or 25 µM CORM-A1 treatment. Gapdh was used as housekeeping gene. Calculated value of $2^{-\Delta\Delta Ct}$ in control was 1. (F-G) Immunofluorescence analysis for Col1a1 in zebrafish larvae following 3 days of TPO-RA and/or 25 µM CORM-A1 treatment. Scale bar in G: 100 µm. Data are presented as mean ± SD of four independent experiments. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. **** $p < 0.0001$.

reciprocal regulation between HO-1 and NLRP3 signaling in stromal cells (Supplementary Fig. 1). To investigate on the potential mechanism behind this crosstalk, we silenced HMOX1 in HS-5 stromal cells by shRNA, observing a significant increase of NLRP3 protein expression in both silenced and control HS-5 cells treated with HEL-conditioned media (Supplementary Fig. 2).

Functionally, MCC950 markedly attenuated α SMA expression and

reduced collagen deposition by ~60%, as confirmed by Mallory tri-chrome staining (Fig. 6D and E).

To validate these observations in vivo, *TPO*^{high} zebrafish larvae were treated with MCC950 (1 µM) for three days. The inhibitor induced a significant upregulation of *hmx1a* while suppressing *nlrp3*, *caspase-a*, and *il1β* expression (Fig. 6F). Immunofluorescence and gene expression analyses revealed a substantial reduction in *col1a1* expression and

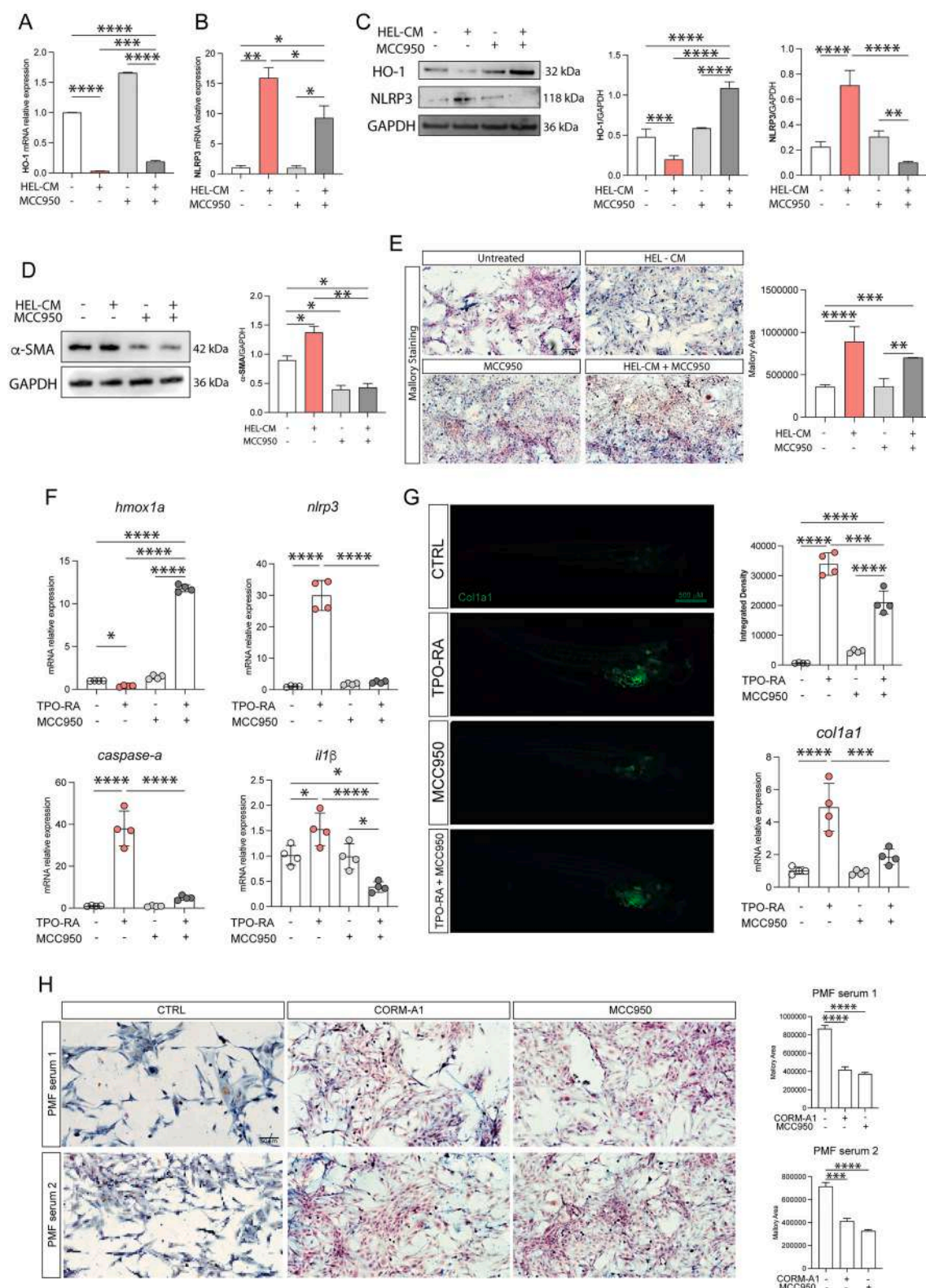


Fig. 6. Inhibition of NLRP3 by MCC950 counteracts stromal pro-fibrotic activation. in HS-5 cells after MCC950 (1 μ M) exposure with or without HEL-CM medium for 24 h. β -actin was used as housekeeping gene. Calculated value of $2^{-\Delta\Delta Ct}$ in control was 1. (C-D) Western blot analysis of HO-1, NLRP3 and α -SMA in HS-5 cells following MCC950 (1 μ M) exposure with or without HEL-CM medium. Representative images of Mallory staining and (E) quantification of Mallory Area of HS-5 cells exposed to HEL-CM w/o MCC950. Scale bar: 50 μ M. (F) Gene expression of *hmx1a*, *nlrp3*, *caspace-a* and *il1β* in zebrafish larvae following 3 days of TPO receptor agonist (TPO-RA) and/or 1 μ M MCC950 treatment. Gapdh was used as housekeeping gene. Calculated value of $2^{-\Delta\Delta Ct}$ in control was 1. (G) Immunofluorescence analysis and gene expression analysis for Col1a1 in zebrafish larvae following 3 days of TPO-RA and/or 1 μ M MCC950 treatment. Scale bar in G: 500 μ m. (H) Representative images of Mallory staining and quantification of Mallory Area of HS-5 cells exposed to sera from PMF patients w/o CORM-A1 or MCC950. Scale bar: 50 μ M. Data are presented as mean $\pm$ SD of four independent experiments. * p < 0.05. ** p < 0.01. *** p < 0.001. **** p < 0.0001.

collagen accumulation compared with TPO-RA-treated controls (Fig. 6G).

Finally, exposure of HS-5 cells to sera from patients with MF led to enhanced collagen deposition, which was significantly decreased by both MCC950 and CORM-A1 treatments (Fig. 6H).

Collectively, these results demonstrate that pharmacological inhibition of NLRP3 restores HO-1 expression and counteracts stromal profibrotic remodeling both in vitro and in vivo. These findings further support a mechanistic link between NLRP3 activation, oxidative imbalance, and fibrotic transformation in the myelofibrotic microenvironment.

3.7. HO-1 expression is decreased in BM biopsies from PMF patients

To validate our experimental findings in patient samples, we assessed HO-1 expression in bone marrow (BM) biopsies from individuals with MF and healthy controls. Immunohistochemical analysis revealed a marked reduction of HO-1 staining in PMF specimens, with approximately 65% lower expression compared with controls ($p < 0.001$) (Fig. 7A and B). Notably, HO-1 immunoreactivity was primarily confined to the cytoplasm of hematopoietic and stromal cells in healthy BM, whereas in PMF samples, residual HO-1 staining was weak

and localized to scattered stromal elements.

We next examined the subcellular distribution of BACH1, a transcriptional repressor of HMOX1. In PMF biopsies, BACH1 showed a predominant nuclear localization in megakaryocytes, in contrast to the cytoplasmic pattern observed in healthy controls (Fig. 7A–C). This nuclear accumulation is consistent with a transcriptionally repressive state and likely contributes to the reduced HO-1 expression observed in PMF.

Collectively, these ex vivo findings corroborate our in vitro and in vivo data, indicating that HO-1 downregulation is a hallmark of the myelofibrotic bone marrow microenvironment. The inverse relationship between HO-1 and NLRP3 activation observed across experimental models and patient tissues underscores a pathogenic link between impaired antioxidant defense, chronic inflammasome activation, and fibrotic remodeling in myelofibrosis.

4. Discussion

MF is a clonal myeloproliferative neoplasm characterized by chronic inflammation, oxidative stress, and progressive BM fibrosis that ultimately led to BM failure, extramedullary hematopoiesis, and reduced survival (ranging from 2 to more than 10 years) [31]. Our study reveals a mechanistic link between repression of the HO-1/CO pathway and

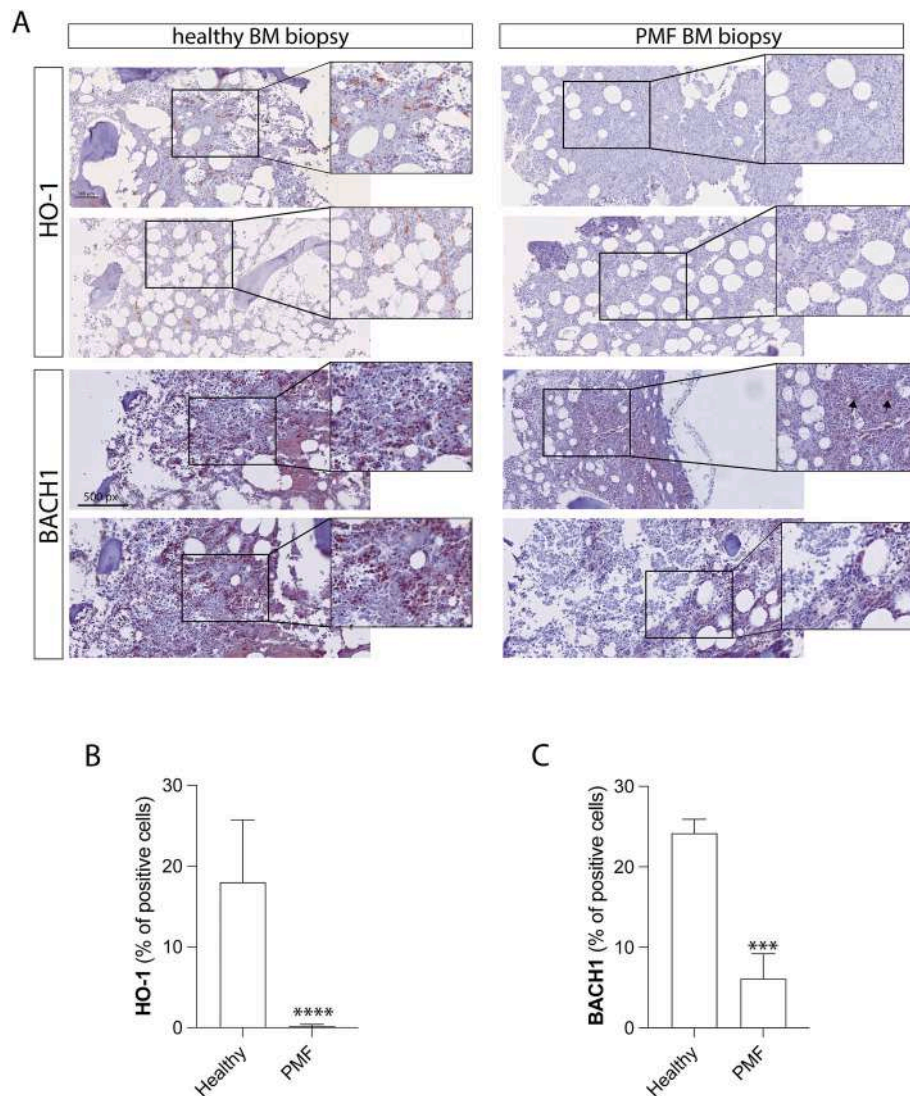


Fig. 7. HO-1 expression in PMF biopsies. (A) Representative images of HO-1 and BACH1 staining in healthy and PMF BM biopsies. Scale bar in A 100 μ m, original magnifications 150x. (B) Quantification of HO-1 and (C) BACH1 (% of positive cells) in BM biopsies. Data are presented as mean $\pm$ SD of independent experiments. *** $p < 0.001$; **** $p < 0.0001$.

activation of the NLRP3 inflammasome in the fibrotic transformation of the myelofibrotic microenvironment. By integrating bioinformatic, in vitro, in vivo, and patient-derived data, we demonstrate that activation of the HO-1/CO axis suppresses inflammasome activity, mitigates stromal activation, and limits collagen deposition, providing a rationale for therapeutic targeting of this pathway in MF.

In the first set of experiments by bioinformatic analysis, we showed that *HMOX1* expression is significantly reduced in CD34⁺ cells from MF patients compared with healthy controls. This result is consistent with previous transcriptomic studies reporting downregulation of antioxidant and detoxification genes in MF and other myeloproliferative neoplasms, which identified decreased Nrf2/HO-1 signaling as a hallmark of oxidative stress and disease progression [11]. Similar findings have been observed in other fibrotic diseases, where reduced HO-1 correlates with tissue damage and poor prognosis [32,33]. Furthermore, the inverse relationship we observed between *HMOX1* and *NLRP3* expression suggests that impaired antioxidant defenses may favor inflammasome activation, consistent with previous observations demonstrating that oxidative stress is a key trigger of NLRP3 assembly in macrophages and fibroblasts [34]. The pathogenic relevance of HO-1 repression in myelofibrosis is supported by the human HO-1 deficiency cases described by Yachie et al. [14] and Ohta et al. [15]. In both patients, loss of HO-1 function caused severe systemic inflammation, endothelial injury, iron overload, and progressive fibrosis, underscoring its essential cytoprotective role. Similar to these genetic conditions, our data show that partial, acquired HO-1 downregulation in MF promotes inflammasome activation, oxidative stress, and stromal fibrosis. Thus, myelofibrosis may represent an acquired form of functional HO-1 deficiency, highlighting the therapeutic potential of restoring the HO-1/CO pathway. To further explore the molecular connection between oncogenic signaling and oxidative imbalance, we compared JAK2^{V617F}-mutated (HEL, SET2) and JAK2 wild-type (UT7) cell lines. Mutated cells exhibited lower *HMOX1* and higher *NLRP3* expression, confirming that constitutive JAK2 activation drives an inflammatory phenotype. This observation extends previous findings by Fleischman and Kleppe, who reported that JAK2^{V617F} mutations sustain cytokine production and ROS accumulation through STAT3 and STAT5 signaling. Notably, ruxolitinib treatment restored *HMOX1* and reduced *NLRP3* expression, supporting the concept that JAK/STAT inhibition not only attenuates inflammation but also indirectly rebalances redox signaling.

Although the ability of HO-1 to modulate inflammasome activation has been previously described [17], our findings extend this concept to the specific context of primary myelofibrosis, where aberrant JAK2V617F-associated signaling, stromal remodeling, and chronic inflammation converge within the bone marrow niche. In this setting, HO-1 repression does not simply represent a generic loss of antioxidant defense but appears to be integrated into a disease-specific inflammatory circuit characterized by increased NLRP3 activation and profibrotic stromal conversion. JAK2V617F-mutant cells are known to promote inflammatory and redox-dependent signaling, including ROS accumulation, which may contribute to NLRP3 inflammasome priming and activation. Consistently, recent evidence indicates that NLRP3 inflammasome activity contributes to systemic inflammation and disease development in JAK2V617F-mutant myeloproliferative neoplasms. Therefore, our observation that ruxolitinib restores *HMOX1* while reducing *NLRP3* supports a model in which aberrant JAK2 signalling intersects with the HO-1/CO pathway at the level of inflammatory and redox regulation. Nevertheless, the precise molecular intermediates connecting JAK2V617F to HO-1 repression and NLRP3 activation, including ROS generation, NRF2 activation, and BACH1 nuclear retention, remain to be fully defined.

While earlier studies have shown that ruxolitinib reduces systemic cytokine levels in MF patients, our results identify *HMOX1* upregulation as a novel component of its anti-inflammatory mechanism.

The interaction between malignant and stromal compartments is a major determinant of MF progression. When healthy mesenchymal

stromal cells (HS-5) were exposed to conditioned medium (CM) from JAK2^{V617F}-mutated cells, they exhibited increased *NLRP3* and Caspase-1 expression and decreased *HMOX1*, confirming that tumor-derived soluble factors can activate the inflammasome and suppress antioxidant defenses. These findings align with prior reports showing that malignant megakaryocytes and myeloid cells release inflammatory cytokines and danger-associated molecular patterns (DAMPs), such as IL-1 β , TNF- α , and ATP, which activate NF- κ B and inflammasome signaling in stromal cells, as described by Leimkühler and colleagues [35]. The observed evidence supports the concept that the myelofibrotic bone marrow niche should be interpreted as a dynamic tumor microenvironment in which malignant hematopoietic cells, myeloid/inflammatory cells, megakaryocytes, endothelial elements, and mesenchymal stromal cells engage in reciprocal crosstalk. In this setting, stromal cells are not merely passive recipients of malignant hematopoiesis but actively respond to tumor-derived inflammatory mediators and contribute to extracellular matrix remodeling. Recent systematic analyses of the tumor microenvironment further support the relevance of integrating stromal, myeloid, and immune compartments to understand cancer-associated tissue remodeling and therapeutic response. Moreover, the suppression of *HMOX1* by tumor CM resembles mechanisms described in pulmonary and hepatic fibrosis, where cytokines such as TGF- β repress HO-1 transcription and promote extracellular matrix deposition [36].

Interestingly, pharmacologic induction of HO-1 with hemin or supplementation with the CO-releasing molecule CORM-A1 reversed CM-induced inflammasome activation, restoring *HMOX1* and reducing *NLRP3* expression. These results are consistent with studies showing that HO-1 and its products exert anti-inflammatory actions by limiting mitochondrial ROS and caspase-1 cleavage, as previously reported [37]. In our model, both hemin and CORM-A1 reduced α SMA expression and collagen accumulation by more than 50%, confirming that HO-1 activation exerts a potent antifibrotic effect. Similar results have been obtained in models of cardiac, renal, and hepatic fibrosis, where HO-1 or CO administration inhibited fibroblast activation and extracellular matrix deposition through suppression of TGF- β /Smad signaling [38,39]. Together, these data support a conserved mechanism whereby HO-1/CO signaling maintains stromal quiescence and prevents fibrotic remodeling.

Notably, exposure to a TPO mimetic enhances zebrafish susceptibility to develop fibrosis within the whole kidney marrow (WKM), which serves as the primary hematopoietic organ in adult fish. This observation is in line with findings from TPO-high mouse models, which reproduce several hallmarks of myelofibrosis, such as bone marrow fibrosis, megakaryocyte expansion, and splenomegaly. Upon interaction with its receptor MPL, thrombopoietin promotes megakaryocyte development and stimulates the expansion of hematopoietic progenitor cells via activation of the JAK/STAT pathway. In addition, the use of thrombopoietin receptor agonists has been associated with an increased likelihood of reticulin accumulation in the bone marrow [28,40,41].

Consistent with this rationale, our in vivo experiments using the *TPO*^{high} zebrafish model confirmed that inflammation and fibrosis are accompanied by *hmox1a/b* downregulation. Consistent with findings in mammalian models of MF and fibrosis, we observed upregulation of *nlrp3*, *caspase-a*, and *il1 β* and a corresponding increase in *cd41* and *colla1* expression, indicating both inflammatory activation and extracellular matrix deposition. Treatment with CORM-A1 reversed these changes, restoring *hmox1a*, suppressing *nlrp3*, *cd41*, and *colla1*, and reducing collagen accumulation. These data corroborate previous reports suggesting that CO donors attenuate inflammation and fibrosis in vivo by inhibiting NLRP3 inflammasome assembly and promoting Nrf2-driven antioxidant responses [42]. Likewise, the effects of MCC950, a selective NLRP3 inhibitor, in our model confirm that inflammasome signaling plays a causal role in fibrotic remodeling, consistent with evidence from liver and lung fibrosis studies in which NLRP3 blockade reduced collagen accumulation and improved tissue architecture [43, 44]. From a translational perspective, these observations also highlight

the potential value of multi-omics and network-based pharmacology approaches to further dissect the regulatory architecture of the HO-1/CO-NLRP3 pathway. Although the present study was not designed as a comprehensive multi-omics investigation, the integration of transcriptomic, proteomic, and single-cell approaches could help identify upstream regulators of HO-1 repression, BACH1 nuclear localization, inflammasome activation, and stromal fibrotic conversion in hematological malignancies. Similarly, network pharmacology and omics-guided drug discovery may support the identification of additional drug-targetable nodes and rational combination strategies involving JAK inhibitors, inflammasome inhibitors, and HO-1/CO pathway modulators.

To validate our results in a clinical setting, we analyzed BM biopsies from individuals with primary MF. Similarly, HO-1 expression was markedly reduced compared with healthy controls, confirming our experimental observations. Importantly, we identified predominant nuclear localization of BACH1, a transcriptional repressor of *HMOX1*, in megakaryocytes from MF patients, whereas it remained cytoplasmic in healthy BM. BACH1 forms heterodimers with small MAF proteins and binds MAF recognition elements (MAREs) in the *HMOX1* enhancer to inhibit transcription, as originally described [45,46]. Similarly, nuclear accumulation of BACH1 has been reported in various pathological contexts, including cancer, heme deficiency, and chronic inflammation, where it suppresses antioxidant genes and promotes oxidative stress [47, 48]. Our findings suggest that in MF, persistent inflammatory signaling and altered heme metabolism favor BACH1 nuclear retention, thereby maintaining transcriptional repression of *HMOX1* and perpetuating oxidative imbalance.

The upstream mechanisms responsible for BACH1 nuclear accumulation in PMF megakaryocytes remain to be fully elucidated. However, a plausible link may involve the chronic inflammatory and redox imbalance generated by constitutive JAK2V617F signaling [49]. JAK2V617F-mutant hematopoietic cells have been reported to accumulate reactive oxygen species (ROS), which contribute to oxidative DNA damage, genomic instability, and disease progression in myeloproliferative neoplasms [50]. Consistently, our data show that JAK2V617F-mutated HEL and SET2 cells display reduced HMOX1 expression and increased NLRP3 levels compared with JAK2 wild-type UT7 cells, while pharmacological JAK inhibition with ruxolitinib restores HMOX1 expression and reduces NLRP3 activation. These findings suggest that aberrant JAK2 signaling contributes not only to inflammatory activation, but also to impairment of the antioxidant HO-1 response.

Silencing experiments targeting both NLRP3 and HMOX1 shed more light on the potential mechanism behind their crosstalk, with a significant increase in HO-1 protein expression after NLRP3 silencing. In contrast, no effects on NLRP3 protein expression have been observed after HMOX1 silencing, suggesting an unidirectional regulation of NLRP3 and HO-1 expression.

At the transcriptional level, HMOX1 expression is controlled by the dynamic balance between NRF2, which promotes antioxidant response element-driven transcription, and BACH1, which acts as a competitive transcriptional repressor. Under physiological stress conditions, oxidative or electrophilic stimuli promote NRF2 activation and BACH1 inactivation/displacement from HMOX1 regulatory regions, thereby allowing HO-1 induction [51]. Conversely, persistence of BACH1 within the nucleus may maintain HMOX1 in a transcriptionally repressed state. In this regard, the nuclear accumulation of BACH1 observed in PMF megakaryocytes provides a potential explanation for the reduced HO-1 expression detected in patient biopsies [52].

Although acute oxidative stress generally favors BACH1 inactivation and NRF2-mediated HMOX1 transcription, chronic oncogenic and inflammatory signaling may produce a maladaptive redox response in which NRF2 activation is insufficient, transient, or functionally uncoupled from effective HO-1 induction. In this scenario, persistent JAK2V617F-driven inflammatory signaling, ROS accumulation, altered

heme metabolism, and inflammasome activation may converge to favor BACH1 nuclear retention and sustained HMOX1 repression [53]. This interpretation is also consistent with the reciprocal relationship observed in our models, where NLRP3 activation was associated with HO-1 downregulation, whereas HO-1/CO pathway activation or NLRP3 inhibition restored a less inflammatory and less fibrotic phenotype.

Nevertheless, the present study does not directly demonstrate the complete causal sequence linking JAK2V617F signaling to BACH1 nuclear localization. In particular, ROS levels, NRF2 phosphorylation or nuclear translocation, and the effects of specific ROS/NRF2/BACH1 modulators on BACH1 subcellular distribution were not assessed. Therefore, the proposed JAK2V617F–ROS/NRF2–BACH1–HO-1 axis should be interpreted as a mechanistic model supported by our integrated bioinformatic, in vitro, in vivo, and patient-derived observations, rather than as a fully established linear pathway. Future studies using JAK2 inhibitors, ROS scavengers, NRF2 modulators, and BACH1 loss-of-function approaches in megakaryocytic models will be required to define the precise upstream and downstream relationships governing BACH1-mediated HMOX1 repression in PMF.

The fibrotic process observed in PMF also shares several conserved features with fibrosis in other organs, including persistent inflammation, oxidative stress, stromal activation, myofibroblast differentiation, and excessive extracellular matrix deposition. For example, Galectin-3 has been implicated in liver fibrosis as a mediator of inflammatory and profibrotic responses, while pulmonary fibrosis involves multiple therapeutic targets related to oxidative stress, inflammatory signaling, extracellular matrix turnover, and fibroblast activation [54]. These comparisons suggest that inflammatory-stromal circuits are partially conserved across fibrotic diseases. However, the specificity of PMF resides in the central contribution of clonal hematopoiesis, aberrant JAK/STAT signaling, megakaryocytic activation, and bone marrow niche remodeling. In this disease-specific context, our data indicate that HO-1 repression and NLRP3 inflammasome activation are interconnected mechanisms contributing to inflammatory and fibrotic remodeling of the myelofibrotic microenvironment.

Finally, although microbiome-driven immune modulation was not investigated in the present study, emerging evidence indicates that the microbiome can shape tumor immunity, inflammatory tone, and therapeutic response [55]. This may represent an additional future perspective for understanding how systemic inflammatory cues influence the bone marrow immune microenvironment and fibrosis progression in myeloproliferative neoplasms.

5. Conclusion

In conclusion, our study establishes that HO-1 downregulation and NLRP3 inflammasome activation are interdependent drivers of inflammation and fibrosis in myelofibrosis. Our findings support a model in which BACH1-mediated transcriptional repression of HMOX1 may contribute to the loss of antioxidant capacity in PMF megakaryocytes, thereby amplifying inflammasome signaling and extracellular matrix deposition. Pharmacologic reactivation of the HO-1/CO pathway or inhibition of NLRP3 restores redox balance, suppresses proinflammatory signaling, and limits stromal fibrotic remodeling both in vitro and in vivo. These findings demonstrate that the HO-1/CO–NLRP3 axis is a critical regulatory checkpoint in myelofibrotic pathogenesis and provide a strong rationale for therapeutic strategies that combine JAK inhibition with modulation of cellular cytoprotection to prevent or reverse BM fibrosis in MF.

Availability of data and materials

The datasets used and/or analyzed in this study are reported within the manuscript and/or additional files and are available from the corresponding authors.

Funding

This work was founded by the PRIN 2022 project entitled “The biochemical and clinical significance of lactate metabolism in patients with myelofibrosis: a new target to improve the clinical outcome”, project code 2022C4 × 4W4, proposals D.D. n. 104 of 2 February 2022 M4C2I1.1 funded by the European Union – NextGenerationEU.

CRediT authorship contribution statement

Lucia Longhitano: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **Enrico La Spina:** Conceptualization, Investigation. **Sebastiano Giallongo:** Conceptualization, Investigation, Writing – original draft. **Cesarina Giallongo:** Conceptualization, Data curation, Investigation, Visualization, Writing – original draft. **Jessica Ferrigno:** Methodology. **Laura Orlando:** Methodology. **Vittorio Del Fabro:** Project administration, Supervision. **Rosario Caltabiano:** Supervision, Validation, Visualization. **Giuseppe Nicolò Conti:** Software, Visualization, Writing – original draft. **Micholino Di Rosa:** Software. **Giuseppe Broggi:** Software, Validation. **Elisabetta P. Pricoco:** Methodology. **Vittorio Calabrese:** Data curation, Supervision, Writing – review & editing. **Andrea Duminuco:** Validation, Visualization. **Giuseppe Alberto Palumbo:** Supervision, Visualization. **Gabriella Marsala:** Visualization. **Giovanni Li Volti:** Funding acquisition, Project administration, Resources, Writing – original draft, Writing – review & editing. **Ignazio Alberto Barbagallo:** Investigation, Software. **Daniele Tibullo:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

This study has been supported in part by A.I.L. (Associazione Italiana contro le Leucemie) sezione di Catania and by FON.CA.NE.SA. (Fondazione Catanese per lo Studio delle Malattie Neoplastiche del Sangue).

List of Abbreviations

αSMA	Alpha-smooth muscle actin
BACH1	BTB and CNC homology 1
BM	Bone marrow
BMF	Bone marrow fibrosis
CASP1	Caspase 1
CM	Conditioned medium
CO	Carbon Monoxide
CORMs	CO-releasing molecules
HMOX1	Heme oxygenase-1
HO-1	Heme oxygenase-1
HSCs	Hematopoietic stem cells
JAK2	Janus Kinase 2
MF	Myelofibrosis
MPNs	Myeloproliferative neoplasms
MSCs	Mesenchymal stromal cells
NLRP3	NOD-LRR- and pyrin-domain-containing protein 3
PMF	Primary Myelofibrosis
TPO-RA	Thrombopoietin receptor agonist
WKM	Whole kidney marrow

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.freeradbiomed.2026.07.030>.

References

- [1] Y.C. Chia, M.J. Siti Asmaa, M. Ramli, P.Y. Woon, M.F. Johan, R. Hassan, M. A. Islam, Molecular genetics of thrombotic myeloproliferative neoplasms: implications in precision oncology, *Diagnostics (Basel)* 13 (1) (2023).
- [2] A.J. Mead, A. Mullally, Myeloproliferative neoplasm stem cells, *Blood* 129 (12) (2017) 1607–1616.
- [3] T. Barbui, J. Thiele, H. Gisslinger, A. Orazi, A.M. Vannucchi, U. Gianelli, C. Beham-Schmid, A. Tefferi, Comments on pre-fibrotic myelofibrosis and how should it be managed, *Br. J. Haematol.* 186 (2) (2019) 358–360.
- [4] E. La Spina, S. Giallongo, C. Giallongo, N. Vicario, A. Duminuco, R. Parenti, R. Giuffrida, L. Longhitano, G. Li Volti, D. Cambria, F. Di Raimondo, G. Musumeci, A. Romano, G.A. Palumbo, D. Tibullo, Mesenchymal stromal cells in tumor microenvironment remodeling of BCR-ABL negative myeloproliferative diseases, *Front. Oncol.* 13 (2023) 1141610.
- [5] A. Duminuco, C. Vetro, C. Giallongo, G.A. Palumbo, The pharmacotherapeutic management of patients with myelofibrosis: looking beyond JAK inhibitors, *Expert Opin. Pharmacother.* 24 (13) (2023) 1449–1461.
- [6] A. Duminuco, E. Torre, G.A. Palumbo, C. Harrison, A journey through JAK inhibitors for the treatment of myeloproliferative diseases, *Curr. Hematol. Malig. Rep.* 18 (5) (2023) 176–189.
- [7] A. Duminuco, H.T. Chifotides, S. Giallongo, C. Giallongo, D. Tibullo, G.A. Palumbo, ACVR1: a novel therapeutic target to treat anemia in myelofibrosis, *Cancers (Basel)* 16 (1) (2023).
- [8] H.F.E. Gleitz, A. Benabid, R.K. Schneider, Still a burning question: the interplay between inflammation and fibrosis in myeloproliferative neoplasms, *Curr. Opin. Hematol.* 28 (5) (2021) 364–371.
- [9] S. Koschmieder, T.I. Mughal, H.C. Hasselbalch, G. Barosi, P. Valent, J.J. Kiladjian, G. Jerczynski, H. Gisslinger, J.S. Jutzi, H.L. Pahl, R. Hehlmann, A. Maria Vannucchi, F. Cervantes, R.T. Silver, T. Barbui, Myeloproliferative neoplasms and inflammation: whether to target the malignant clone or the inflammatory process or both, *Leukemia* 30 (5) (2016) 1018–1024.
- [10] C.M. Artlett, The mechanism and regulation of the NLRP3 inflammasome during fibrosis, *Biomolecules* 12 (5) (2022).
- [11] G. Li Volti, D. Tibullo, L. Vanella, C. Giallongo, F. Di Raimondo, S. Forte, M. Di Rosa, S.S. Signorelli, I. Barbagallo, The heme oxygenase system in hematological malignancies, *Antioxid. Redox Signaling* 27 (6) (2017) 363–377.
- [12] T. Kushida, G. Li Volti, S. Quan, A. Goodman, N.G. Abraham, Role of human heme oxygenase-1 in attenuating TNF-alpha-mediated inflammation injury in endothelial cells, *J. Cell. Biochem.* 87 (4) (2002) 377–385.
- [13] D. Sacerdoti, C. Colombrita, M.H. Ghattas, E.F. Ismaeil, G. Scapagnini, M. Bolognesi, G. Li Volti, N.G. Abraham, Heme oxygenase-1 transduction in endothelial cells causes downregulation of monocyte chemoattractant protein-1 and of genes involved in inflammation and growth, *Cell. Mol. Biol. (Noisy-Le-Grand)* 51 (4) (2005) 363–370.
- [14] A. Yachie, Y. Niida, T. Wada, N. Igarashi, H. Kaneda, T. Toma, K. Ohta, Y. Kasahara, S. Koizumi, Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency, *J. Clin. Invest.* 103 (1) (1999) 129–135.
- [15] K. Ohta, A. Yachie, K. Fujimoto, H. Kaneda, T. Wada, T. Toma, A. Seno, Y. Kasahara, H. Yokoyama, H. Seki, S. Koizumi, Tubular injury as a cardinal pathologic feature in human heme oxygenase-1 deficiency, *Am. J. Kidney Dis.* 35 (5) (2000) 863–870.
- [16] S.H. Vitali, A. Fernandez-Gonzalez, J. Nadkarni, A. Kwong, C. Rose, S.A. Mitsialis, S. Kourembanas, Heme oxygenase-1 dampens the macrophage sterile inflammasome response and regulates its components in the hypoxic lung, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 318 (1) (2020) L125–L134.
- [17] M.Z. Ratajczak, M. Adamiak, J. Ratajczak, M. Kucia, Heme oxygenase 1 (HO-1) as an inhibitor of trafficking of normal and malignant hematopoietic stem cells - clinical and translational implications, *Stem Cell Rev. Rep.* 17 (3) (2021) 821–828.
- [18] R. Norfo, R. Zini, V. Pennucci, E. Bianchi, S. Salati, P. Guglielmelli, C. Bogani, T. Fanelli, C. Mannarelli, V. Rosti, D. Pietra, S. Salmoiraghi, A. Bisognin, S. Ruberti, S. Rontautoli, G. Sacchi, Z. Prudente, G. Barosi, M. Cazzola, A. Rambaldi, S. Bortoluzzi, S. Ferrari, E. Tagliafico, A.M. Vannucchi, R. Manfredini, I. Associazione, Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative, miRNA-mRNA integrative analysis in primary myelofibrosis CD34+ cells: role of miR-155/JARID2 axis in abnormal megakaryopoiesis, *Blood* 124 (13) (2014) e21–e32.
- [19] A.M. Vannucchi, T.L. Lasho, P. Guglielmelli, F. Biamonte, A. Pardanani, A. Pereira, C. Finke, J. Score, N. Gangat, C. Mannarelli, R.P. Ketterling, G. Rotunno, R. A. Knudson, M.C. Susini, R.R. Laborde, A. Spolverini, A. Pancrazzi, L. Pieri, R. Manfredini, E. Tagliafico, R. Zini, A. Jones, K. Zoi, A. Reiter, A. Duncombe, D. Pietra, E. Rumi, F. Cervantes, G. Barosi, M. Cazzola, N.C. Cross, A. Tefferi, Mutations and prognosis in primary myelofibrosis, *Leukemia* 27 (9) (2013) 1861–1869.
- [20] R. Edgar, M. Domrachev, A.E. Lash, Gene expression omnibus: NCBI gene expression and hybridization array data repository, *Nucleic Acids Res.* 30 (1) (2002) 207–210.
- [21] M. Di Rosa, C. Giallongo, A. Romano, D. Tibullo, G. Li Volti, G. Musumeci, I. Barbagallo, R. Imbesi, P. Castrogiovanni, G.A. Palumbo, Immunoproteasome genes are modulated in CD34(+) JAK2(V617F) mutated cells from primary myelofibrosis patients, *Int. J. Mol. Sci.* 21 (8) (2020).

- [22] K. Iida, J. Kondo, J.N. Wibisana, M. Inoue, M. Okada, ASURAT: functional annotation-driven unsupervised clustering of single-cell transcriptomes, *Bioinformatics* 38 (18) (2022) 4330–4336.
- [23] L. Longhitano, G. Broggi, S. Giallongo, M. Failla, L. Puzzo, T. Avitabile, D. Tibullo, A. Distefano, V. Pittala, M. Reibaldi, G.N. Zanghi, A. Longo, A. Russo, R. Caltabiano, G.L. Volti, N. Musso, Heme Oxygenase-1 overexpression promotes uveal melanoma progression and is associated with poor clinical outcomes, *Antioxidants (Basel)* 11 (10) (2022).
- [24] A. Meszaros, K. Molnar, C. Fazakas, B. Nogradi, A. Luvi, T. Dudas, L. Tiszlavicz, A. E. Farkas, I.A. Krizbai, I. Wilhelm, Inflammasome activation in peritumoral astrocytes is a key player in breast cancer brain metastasis development, *Acta Neuropathol. Commun.* 11 (1) (2023) 155.
- [25] L. Longhitano, N. Vicario, S. Forte, C. Giallongo, G. Broggi, R. Caltabiano, G.M. V. Barbagallo, R. Altieri, G. Raciti, M. Di Rosa, M. Caruso, R. Parenti, A. Liso, F. Busi, M. Lolicato, M.C. Mione, G. Li Volti, D. Tibullo, Lactate modulates microglia polarization via IGFBP6 expression and remodels tumor microenvironment in glioblastoma, *Cancer Immunol. Immunother.* 72 (1) (2023) 1–20.
- [26] D. Cambria, L. Longhitano, E. La Spina, S. Giallongo, L. Orlando, R. Giuffrida, D. Tibullo, P. Fontana, I. Barbagallo, V.G. Nicoletti, G.L. Volti, V.D. Fabro, A.R. D. Coda, A. Liso, G.A. Palumbo, IGFBP6 alters mesenchymal stromal cell phenotype driving dasatinib resistance in chronic myeloid leukemia, *Life (Basel)* 13 (2) (2023).
- [27] C. Giallongo, I. Dulcamare, D. Tibullo, V. Del Fabro, N. Vicario, N. Parrinello, A. Romano, G. Scandura, G. Lazzarino, C. Conticello, G. Li Volti, A.M. Amorini, G. Musumeci, M. Di Rosa, F. Polito, R. Oteri, M. Aguenouz, R. Parenti, F. Di Raimondo, G.A. Palumbo, CXCL12/CXCR4 axis supports mitochondrial trafficking in tumor myeloma microenvironment, *Oncogenesis* 11 (1) (2022) 6.
- [28] M. Spampinato, C. Giallongo, S. Giallongo, E. Spina, A. Duminuco, L. Longhitano, R. Caltabiano, L. Salvatorelli, G. Broggi, E.P. Pricoco, V. Del Fabro, I. Dulcamare, D. I.M. Am, A. Romano, F. Di Raimondo, G. Li Volti, G.A. Palumbo, D. Tibullo, Lactate accumulation promotes immunosuppression and fibrotic transformation of bone marrow microenvironment in myelofibrosis, *J. Transl. Med.* 23 (1) (2025) 69.
- [29] O. Ivanovski, K. Kulkeaw, M. Nakagawa, T. Sasaki, C. Mizuuchi, Y. Horio, T. Ishitani, D. Sugiyama, Characterization of kidney marrow in zebrafish (*Danio rerio*) by using a new surgical technique, *Prilozi* 30 (2) (2009) 71–80.
- [30] G. Broggi, A. Lo Giudice, M. Di Mauro, M.G. Asmundo, E. Pricoco, E. Piombino, R. Caltabiano, G. Morgia, G.L. Russo, SRSF-1 and microvessel density immunohistochemical analysis by semi-automated tissue microarray in prostate cancer patients with diabetes (DIAMOND study), *Prostate* 81 (12) (2021) 882–892.
- [31] A. Duminuco, A. Nardo, G. Giuffrida, S. Leotta, U. Markovic, C. Giallongo, D. Tibullo, A. Romano, F. Di Raimondo, G.A. Palumbo, Myelofibrosis and survival prognostic models: a journey between past and future, *J. Clin. Med.* 12 (6) (2023).
- [32] Z. Zhang, J. Qu, C. Zheng, P. Zhang, W. Zhou, W. Cui, X. Mo, L. Li, L. Xu, J. Gao, Nrf2 antioxidant pathway suppresses Numb-mediated epithelial-mesenchymal transition during pulmonary fibrosis, *Cell Death Dis.* 9 (2) (2018) 83.
- [33] H.J. Kim, Y. Joe, S.K. Kim, S.U. Park, J. Park, Y. Chen, J. Kim, J. Ryu, G.J. Cho, Y. J. Surh, S.W. Ryter, U.H. Kim, H.T. Chung, Carbon monoxide protects against hepatic steatosis in mice by inducing sestrin-2 via the PERK-eIF2alpha-ATF4 pathway, *Free Radic. Biol. Med.* 110 (2017) 81–91.
- [34] X.X. Guan, H.H. Yang, W.J. Zhong, J.X. Zhong, C.Y. Zhang, H.L. Jiang, Y. Xiang, Y. Zhou, C.X. Guan, Fn14 exacerbates acute lung injury by activating the NLRP3 inflammasome in mice, *Mol. Med.* 28 (1) (2022) 85.
- [35] N.B. Leimkuhler, H.F.E. Gleitz, L. Ronghui, I.A.M. Snoeren, S.N.R. Fuchs, J. S. Nagai, B. Banjanin, K.H. Lam, T. Vogl, C. Kuppe, U.S.A. Stalman, G. Busche, H. Kreipe, I. Gutemann, P. Krebs, Y. Banz, P. Boor, E.W. Tai, T.H. Brummendorf, S. Koschmieder, M. Crysandt, E. Bindels, R. Kramann, I.G. Costa, R.K. Schneider, Heterogeneous bone-marrow stromal progenitors drive myelofibrosis via a druggable alarmin axis, *Cell Stem Cell* 28 (4) (2021) 637–652 e8.
- [36] M. Correa-Costa, P. Smedo, A.P. Monteiro, R.C. Silva, R.L. Pereira, G. M. Goncalves, G.D. Marques, M.A. Cenedeze, A.C. Faleiros, A.C. Keller, M. H. Shimizu, A.C. Seguro, M.A. Reis, A. Pacheco-Silva, N.O. Camara, Induction of heme oxygenase-1 can halt and even reverse renal tubule-interstitial fibrosis, *PLoS One* 5 (12) (2010) e14298.
- [37] B. Wegiel, R. Larsen, D. Gallo, B.Y. Chin, C. Harris, P. Mannam, E. Kaczmarek, P. J. Lee, B.S. Zuckerbraun, R. Flavell, M.P. Soares, L.E. Otterbein, Macrophages sense and kill bacteria through carbon monoxide-dependent inflammasome activation, *J. Clin. Invest.* 124 (11) (2014) 4926–4940.
- [38] D. Morse, The role of heme oxygenase-1 in pulmonary fibrosis, *Am. J. Respir. Cell Mol. Biol.* 29 (3 Suppl) (2003) S82–S86.
- [39] Y.A. Hong, J.H. Lim, M.Y. Kim, E.N. Kim, E.S. Koh, S.J. Shin, B.S. Choi, C.W. Park, Y.S. Chang, S. Chung, Delayed treatment with oleanolic acid attenuates tubulointerstitial fibrosis in chronic cyclosporine nephropathy through Nrf2/HO-1 signaling, *J. Transl. Med.* 12 (2014) 50.
- [40] A. Malara, C. Gruppi, V. Abbonante, D. Cattaneo, L. De Marco, M. Massa, A. Iurlo, U. Gianelli, C.L. Balduini, M.E. Tira, A.F. Muro, A.K. Chauhan, V. Rosti, G. Barosi, A. Balduini, EDA fibronectin-TLR4 axis sustains megakaryocyte expansion and inflammation in bone marrow fibrosis, *J. Exp. Med.* 216 (3) (2019) 587–604.
- [41] C. Leon, K. Evert, F. Dombrowski, F. Pertuy, A. Eckly, P. Laeuffer, C. Gachet, A. Greinacher, Romiplostim administration shows reduced megakaryocyte response-capacity and increased myelofibrosis in a mouse model of MYH9-RD, *Blood* 119 (14) (2012) 3333–3341.
- [42] Z. Xia, C. Zhang, C. Guo, B. Song, W. Hu, Y. Cui, Y. Xue, M. Xia, D. Xu, S. Zhang, J. Fang, Nanoformulation of a carbon monoxide releasing molecule protects against cyclosporin A-induced nephrotoxicity and renal fibrosis via the suppression of the NLRP3 inflammasome mediated TGF-beta/Smad pathway, *Acta Biomater.* 144 (2022) 42–53.
- [43] A. Wree, A. Eguchi, M.D. McGeough, C.A. Pena, C.D. Johnson, A. Canbay, H. M. Hoffman, A.E. Feldstein, NLRP3 inflammasome activation results in hepatocyte pyroptosis, liver inflammation, and fibrosis in mice, *Hepatology* 59 (3) (2014) 898–910.
- [44] N. Hosseini, Y. Cho, R.F. Lockey, N. Koliputi, The role of the NLRP3 inflammasome in pulmonary diseases, *Ther. Adv. Respir. Dis.* 9 (4) (2015) 188–197.
- [45] T. Oyake, K. Itoh, H. Motohashi, N. Hayashi, H. Hoshino, M. Nishizawa, M. Yamamoto, K. Igarashi, Bach proteins belong to a novel family of BTB-basic leucine zipper transcription factors that interact with MafK and regulate transcription through the NF-E2 site, *Mol. Cell Biol.* 16 (11) (1996) 6083–6095.
- [46] J. Sun, H. Hoshino, K. Takaku, O. Nakajima, A. Muto, H. Suzuki, S. Tashiro, S. Takahashi, S. Shibahara, J. Alam, M.M. Taketo, M. Yamamoto, K. Igarashi, Hemoprotein Bach1 regulates enhancer availability of heme oxygenase-1 gene, *EMBO J.* 21 (19) (2002) 5216–5224.
- [47] M. Ishikawa, S. Numazawa, T. Yoshida, Redox regulation of the transcriptional repressor Bach1, *Free Radic. Biol. Med.* 38 (10) (2005) 1344–1352.
- [48] S. Davudian, B. Mansoori, N. Shajari, A. Mohammadi, B. Baradaran, BACH1, the master regulator gene: a novel candidate target for cancer therapy, *Gene* 588 (1) (2016) 30–37.
- [49] C. Marty, C. Lacout, N. Droin, J.P. Le Couedic, V. Ribrag, E. Solary, W. Vainchenker, J.L. Villeval, I. Plo, A role for reactive oxygen species in JAK2 V617F myeloproliferative neoplasm progression, *Leukemia* 27 (11) (2013) 2187–2195.
- [50] H.C. Hasselbalch, M. Thomassen, C.H. Riley, L. Kjaer, T.S. Larsen, M.K. Jensen, O. W. Bjerrum, T.A. Kruse, V. Skov, Whole blood transcriptional profiling reveals deregulation of oxidative and antioxidative defence genes in myelofibrosis and related neoplasms. Potential implications of downregulation of Nrf2 for genomic instability and disease progression, *PLoS One* 9 (11) (2014) e112786.
- [51] J.F. Reichard, G.T. Motz, A. Puga, Heme oxygenase-1 induction by Nrf2 requires inactivation of the transcriptional repressor BACH1, *Nucleic Acids Res.* 35 (21) (2007) 7074–7086.
- [52] R. Liao, E.H. Bresnick, Heme as a differentiation-regulatory transcriptional cofactor, *Int. J. Hematol.* 116 (2) (2022) 174–181.
- [53] T. Wang, Y. Dong, Z. Huang, G. Zhang, Y. Zhao, H. Yao, J. Hu, E. Tuksammel, H. Cai, N. Liang, X. Xu, X. Yang, S. Schmidt, X. Qiao, S. Schlisio, S. Stromblad, H. Qian, C. Jiang, E. Treuter, M.O. Berge, Antioxidants stimulate BACH1-dependent tumor angiogenesis, *J. Clin. Invest.* 133 (20) (2023).
- [54] L.J. Wang, P.F. Guo, Y.Z. Chen, H.W. Yan, X.L. Zhang, Tangeretin in the treatment of pulmonary fibrosis: current advances and future perspectives, *Curr. Mol. Pharmacol.* 17 (2024) e18761429385520.
- [55] A. Lin, M. Xiong, A. Jiang, L. Huang, H.Z.H. Wong, S. Feng, C. Zhang, Y. Li, L. Chen, H. Chi, P. Zhang, B. Ye, H. Zhang, N. Zhang, L. Zhu, W. Mou, J. Shen, K. Li, W. Xu, H. Ying, C. Zhang, D. Zeng, J. Xie, X. Deng, Q. Wang, J. Xu, W. Shi, C. Qi, C. Qu, X. Huang, A. Hajdu, C. Li, C. Peng, X. Cao, G. Pei, L. Zhang, Y. Huo, J. Xu, A. Glaviano, A.G. Szollosi, S. Bian, Z. Li, H. Tang, B. Tang, Z. Liu, J. Zhang, K. Miao, Q. Cheng, T. Wei, S. Yuan, P. Luo, The microbiome in cancer, *iMeta* 4 (5) (2025) e70070.