



Role of Selenoprotein W in participating in the progression of non-alcoholic fatty liver disease

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease worldwide. Numerous evidence has demonstrated that metabolic reprogramming serves as a hallmark associated with an elevated risk of NAFLD progression. Selenoprotein W (SelW) is an extensively expressed hepatic selenoprotein that plays a crucial role in antioxidant function. Here, we first demonstrated that SelW is a significantly distinct factor in the liver tissue of NAFLD patients through the Gene Expression Omnibus (GEO) database. Additionally, loss of SelW alleviated hepatic steatosis induced by a high-fat diet (HFD), and was accompanied by the regulation of metabolic and inflammatory pathways as verified by transcriptomic analysis. Moreover, co-immunoprecipitation (CO-IP), liquid chromatography-tandem mass spectrometry (LC-MS), laser scanning confocal microscopy (LSCM) and molecular docking analysis were subsequently implemented to identify Pyruvate Kinase M2 (PKM2) as a potential interacting protein of SelW. Meanwhile, SelW modulated PKM2 translocation into the nucleus to trigger transactivation of the HIF-1 α , in further mediating mitochondrial apoptosis, eventually resulting in mitochondrial damage, ROS excessive production and mtDNA leakage. Additionally, mito-ROS accumulation induced the activation of the NLRP3 inflammasome-mediated pyroptosis, thereby facilitating extracellular leakage of mtDNA. The escaped mtDNA then evokes the cGAS-STING signaling pathway in macrophage, thus inducing a shift in macrophage phenotype. Together, our results suggest SelW promotes hepatocyte apoptosis and pyroptosis by regulating metabolic reprogramming to activate cGAS-STING signaling of macrophages, thereby exacerbating the progression of NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is an the immensely prevalent chronic liver condition that is characterized by abnormal aggregation of hepatic lipids in the absence of significant alcohol consumption and comprises the progression from steatosis or non-alcoholic steatotic liver (NAFL) to non-alcoholic steatohepatitis (NASH) [1,2]. The metabolic abnormality has been considered a central driver of NAFLD pathogenesis, which is often accompanied by exceeding lipid droplet accumulation [3,4]. In addition, accumulated evidence has widely publicized that reprogramming of metabolism reprogramming of metabolism in liver diseases is associated with the activity of glycolysis [5–8]. An adaptive metabolic switch has been observed in a variety of

liver diseases that preferential transition of energy production from oxidative phosphorylation to glycolysis, resulting in the partial conversion of pyruvate to lactic acid [6]. NAFLD and NASH patients have notably enhanced glycolysis, accompanied by elevated lactate levels [7, 8]. Moreover, previous study has confirmed that alternated glycolysis contributed to the further deterioration of NAFLD and eventually the progression to cirrhosis and HCC [8], elucidating the crucial role of glycolysis in the occurrence and progression of liver diseases. Furthermore, high-fat diet (HFD)-induced NAFLD mice exhibited significantly elevated plasma levels of glycolysis-associated metabolites, including glucose, lactic acid, and pyruvate, in comparison with the wild type mice [9].

As one of the well-known rate-limiting metabolic enzymes of glycolysis, Pyruvate Kinase M2 (PKM2) has been considered to be

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Abbreviations:

NAFLD	Non-alcoholic fatty liver disease
SelW	Selenoprotein W
NCD	Normal chow diet
HFD	High fat diet
GEO	Gene Expression Omnibus
HbA1C	Hemoglobin A1C
PKM2	Pyruvate Kinase M2
HK2	Hexokinase 2
LDHA	Lactate dehydrogenase A
PFKM	Phosphofructokinase
ACACA	Acetyl-CoA carboxylase alpha
FASN	Fatty acid synthase
ACLY	ATP citrate lyase

ACOX1	Acyl-Coenzyme A oxidase 1
CPT1A	Carnitine palmitoyltransferase 1A
PPAR γ	Peroxisome proliferator activated receptor gamma
HIF-1 α	Hypoxia inducible factor 1 subunit alpha
NLRP3	NLR family, pyrin domain containing 3
GSDMD	Gasdermin D
IL-1 β	Interleukin 1 beta
BAX	BCL2 associated X, apoptosis regulator
BCL2	B cell leukemia/lymphoma 2
cGAS	Cyclic GMP-AMP synthase
STING	Stimulator of interferon gene
CXCL9	C-X-C motif chemokine ligand 9
ARG1	Arginase 1
IL-10	Interleukin 10

strongly associated with obesity and NAFLD [10]. It has been demonstrated that PKM2 can convert metabolic reprogramming from glycolysis to oxidative phosphorylation in macrophages by interacting with Annexin A5, alleviating of inflammation, steatosis, and fibrosis in NASH mice [11]. Recent literature has evidenced that PKM2 performed a mitigation effect on glycolysis to markedly inhibit M1 macrophage polarization, as the key signaling pathway in the therapeutic effect of Celastrol on the NAFLD [12].

Selenium (Se), a crucial micronutrient, is indispensable for the biosynthesis of selenoproteins and closely associated with the pathogenesis of NAFLD [13–15]. The results of the National Health and Nutrition Examination Survey (NHANES) 2017–18 statistics reveal a positive correlation between elevated levels of blood Se and liver steatosis [15], contradicting with other conclusions. The meta-analysis results suggested that a negative association between Se status in the body and Se intake with cirrhosis, hepatitis, and liver cancer [16]. Therefore, the controversial aspect of Se's potential in preventing chronic liver diseases persists. Selenoprotein W (SelW) is a small molecule selenoprotein (9.32 kDa) with significant expressed in the liver, associating with antioxidant activity [17,18]. It regulates the redox tone of macrophages during inflammation, further affecting the cellular redox processes and bioenergetics [13]. However, recent investigation has indicated that SelW deficiency in the skeletal muscle of obese mice fed with HFD performs has no influence on oxidative stress and insulin sensitivity [19]. Hence, whether SelW participates in the HFD-induced NAFLD progression remains unknown.

In the present study, we found that SelW was significantly upregulated in the livers of patients with NAFLD. SelW ablation effectively alleviate hepatic steatosis and concurrently regulated metabolic and inflammatory pathways in HFD-induced NAFLD mice. In particular, we elucidated that SelW modulates PKM2 translocation and triggers HIF-1 α to induce mitochondrial apoptosis and damage, while activating the NLRP3 inflammasome-mediated pyroptosis, thereby initiating the cGAS-STING signaling pathway in macrophages and altering the macrophage phenotype. Thus, we revealed an association between SelW and PKM2 in metabolic reprogramming as well as the progression of NAFLD. This provides evidence for further studying the biological function of SelW and potential strategies for treating NAFLD.

2. Materials and methods

2.1. Animals and experimental design

SelW-knockout (KO) C57BL/6 mice were purchased from Cyagen Biosciences (Jiangsu, China), and the control wild-type (WT) mice were obtained from our laboratory. The procedure employed in this study were conducted in accordance with the Northeast Agricultural

University's Institutional Animal Care and Use Committee (certification No. NEAU- [2011]-9). All mice were kept in pairs per cage, following a 12 h light/dark cycle, at 24–27 °C. Four groups were formed by randomly assigning eight-week-old male WT and KO mice. Twenty-five WT fed normal chow diet (NCD) as the NCD WT group, or fed HFD as the HFD WT group. Twenty-five KO mice fed a NCD as the NCD KO group, or fed HFD as the HFD KO group. During the period of experiment, mice were respectively administered with NCD or HFD for 12 weeks, replacing the fresh feed and water daily, and changing bedding material weekly.

2.2. Samples collection

At the conclusion of the experiment, the blood glucose levels were detected by obtaining samples from the tail veins of mice. Liver tissue was treated with 4% paraformaldehyde (PFA) solution or fixed in 2.5% glutaraldehyde solution, preserving at 4 °C. The remaining tissue was rapidly frozen using liquid nitrogen, and subsequently preserved at –80 °C for subsequent analysis. The HbA1C contents in the blood of mice were quantified using an HbA1C ELISA kit, following the manufacturer's protocols (Huamei Biological Engineering, China).

2.3. Histological detection of liver tissue

The liver of mice was examined using transmission electron microscopy (TEM) and light microscopy to observe the pathological alterations. Additionally, H&E and ORO staining techniques were employed to evaluate the pathological changes [20]. For specific procedures, please refer to the supplementary materials.

2.4. Transcriptome sequencing

The transcriptome sequencing experiment was completed by Wuhan Bioacme biotechnology Co., Ltd. TRIzol reagent (Invitrogen, USA) were used to extract total RNA from the liver tissues. The concentration of RNA was determined using the Qubit® RNA Assay Kit in Qubit® 3.0 Fluorometer (Life Technologies, CA, U.S.A.). mRNA in total RNA were obtained by Oligo (dT) magnetic beads, and sequencing libraries were generated using the RNA Library Preparation Kit (NEB, USA). The sequencing libraries were sequenced using HiSeq 4000SBS Kit, and applying HISAT2 to map the mice genome. The data were performed using DESeq 2 combined with the difference ratio ($|\log_2(\text{Fold change})| > 2$) and significance level ($P \text{ value} < 0.01$) to determine the differentially expression genes (DEGs).

2.5. Culturing of cell and transfection

The Hepa1-6 mouse hepatoma cell line and AML12 mouse hepatoparenchymal cell line were maintained in Dulbecco modified Eagle medium (DMEM) (GIBCO, USA) medium, in which AML12 cells were supplemented with 1% ITS (100 × purchased from Sigma) [21,22]. The bone marrow-derived macrophages (BMDMs) were obtained from WT mice according to the previously reported protocol [23]. For SelW overexpression and knockdown, please refer to the supplementary materials.

2.6. Co-immunoprecipitation (CO-IP) and liquid chromatography tandem mass spectrometry (LC-MS)

We used FLAG® Immunoprecipitation Kit provided by Sigma Aldrich (St. Louis, USA) to detect potential interaction proteins. Hepa1-6 cells were respectively transfected with pCDNA-W plasmid and pCDNA 3.1 (+) vector (Negative control) for 72 h. The collected cells were lysed with cell lysate contained a protease inhibitor cocktail on ice for 30 min, following the manufacturer's protocol for subsequent operations. Finally, the immunoprecipitated proteins was detected by western blot and LC-MS analysis by Sangon Biotech (Shanghai, China).

2.7. Molecular docking

The possible binding pattern of SelW and PKM2 protein was predicted by protein-protein molecular docking. Swiss-Model (<https://swissmodel.expasy.org/>) were utilized for performing homology modeling based on the protein sequences of SelW and PKM2. Then protein-protein docking was conducted using AutoDocktools 1.5.6 software for measurement purposes. Finally, the most optimal interaction mode of SelW and PKM2 was analyzed using PyMol 2.3.0 software.

2.8. Nuclear and cytoplasmic fractionation

We used Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, China) to separated cellular cytoplasmic and nuclear fractions based on previous studies [24]. Subsequently, western blot analysis was performed separately on the nuclear and cytoplasmic proteins.

2.9. Disuccinimidyl suberate (DSS) cross-linking assay

We used DSS crosslinkers provided by MedChemExpress (New Jersey, USA) to determine the structural morphology of PKM2. AML 12 cells were transfected by pCDNA-W, Si-W and pCDNA 3.1 vector (+) (as Vehicle group) plasmids for 72 h, and respectively collected cells to rinsed with cold PBS. The samples were reacted with 500 μM DSS solution for 30 min at room temperature, and added 10 mM Tris to incubated for 15 min. Lysates of the sample were analysis by western blot.

2.10. Apoptosis analysis

The TdT mediated dUTP nick-end labeling (TUNEL) were employed to illustrate the apoptosis in liver of mouse according to the method described by Sun et al. [25]. The paraffin sections were permeabilized and incubated with dUTP buffer at 37 °C for 2 h. DAPI (Beyotime, China) were implemented to stain and localize the nuclei of cells.

For Annexin V-FITC/PI staining (KENGGEN, China), the post-transfection cells were labelled with Annexin V-FITC and PI base on the established instructions [26]. The amount of apoptosis cells was detected by flow cytometry.

For Hoechst staining (Beyotime, China), cells labelled with Hoechst working solution (10 μg/mL) for 5 min after transfected with plasmids, and the images of fluorescence signal were captured by fluorescence microscope (Thermo Fisher, USA).

2.11. Mitochondrial function assessment and ATP detection

Both 10 μg/ml JC-1 fluorescent probe (Beyotime, China) and 20 nm MitoTracker Green probe (Beyotime, China) were performed in present study to accurately determine the mitochondrial membrane potential ($\Delta\Psi_m$) and mitochondrial morphology. In addition, 10 μM 2,7-dichloro-fluorescein diacetate (DCFH-DA) (Nanjing Jianchang, China) fluorescent probe were used to assess intracellular ROS levels by fluorescence microscopy (Thermo Fisher Scientific, USA). While mitochondrial ROS (mito-ROS) levels were loaded with 5 μM MitoSOX Red (ABclonal Technology, China) and visualized by Laser confocal microscopy (Olympus Optical, Japan) and fluorescence microplate reader [27]. For ATP detection, the cellular ATP content and Na^+K^+ -ATPase activity were assessed by ATP assay kit (Product No.A095-1-1, Nanjing Jianchang, China). The measurement of mitochondrial ATP (mito-ATP), mitochondria were isolated using a mitochondria isolation kit (Beyotime, China) according to the instruction manual and then detected using an ATP assay kit (Product No.A095-1-1, Nanjing Jianchang, China) [28].

2.12. mtDNA analysis

Base on the described previously [29], the TIANamp Genomic DNA Kit (Tiangen, China) were extracted and purified the DNA from both whole cells and the culture supernatant of cells. For cytosolic DNA, we used 0.1% NP-40 (Beyotime, China) to cytoplasmically lysis for 20 min on ice. the supernatant was also utilized TIANamp Genomic DNA Kit to extract and purify cytosolic DNA after centrifuged at 15000 rpm at 4 °C for 20 min. Subsequently, the mtDNA primers (Dloop1-3) and nuclear DNA primers (Tert) were used to quantify the levels of mtDNA by qRT-PCR, following the sequence described by Ma et al. [30]. The primers for Tert and mtDNA (Dloop1-3) were synthesized as shown in Table S2. The $2^{-\Delta\Delta Ct}$ method was used to calculation. The mtDNA of whole cells were used as internal references.

Additionally, we also used 20 nM MitoTracker Red CMXRos (Beyotime, China) and Picogreen dsDNA Quantitation Reagent kit (Yeasen Biotech, China) to colocalize dsDNA and mitochondria. In accordance with the established protocols described, and the image of samples were visualized by laser confocal microscopy (Olympus Optical, Japan).

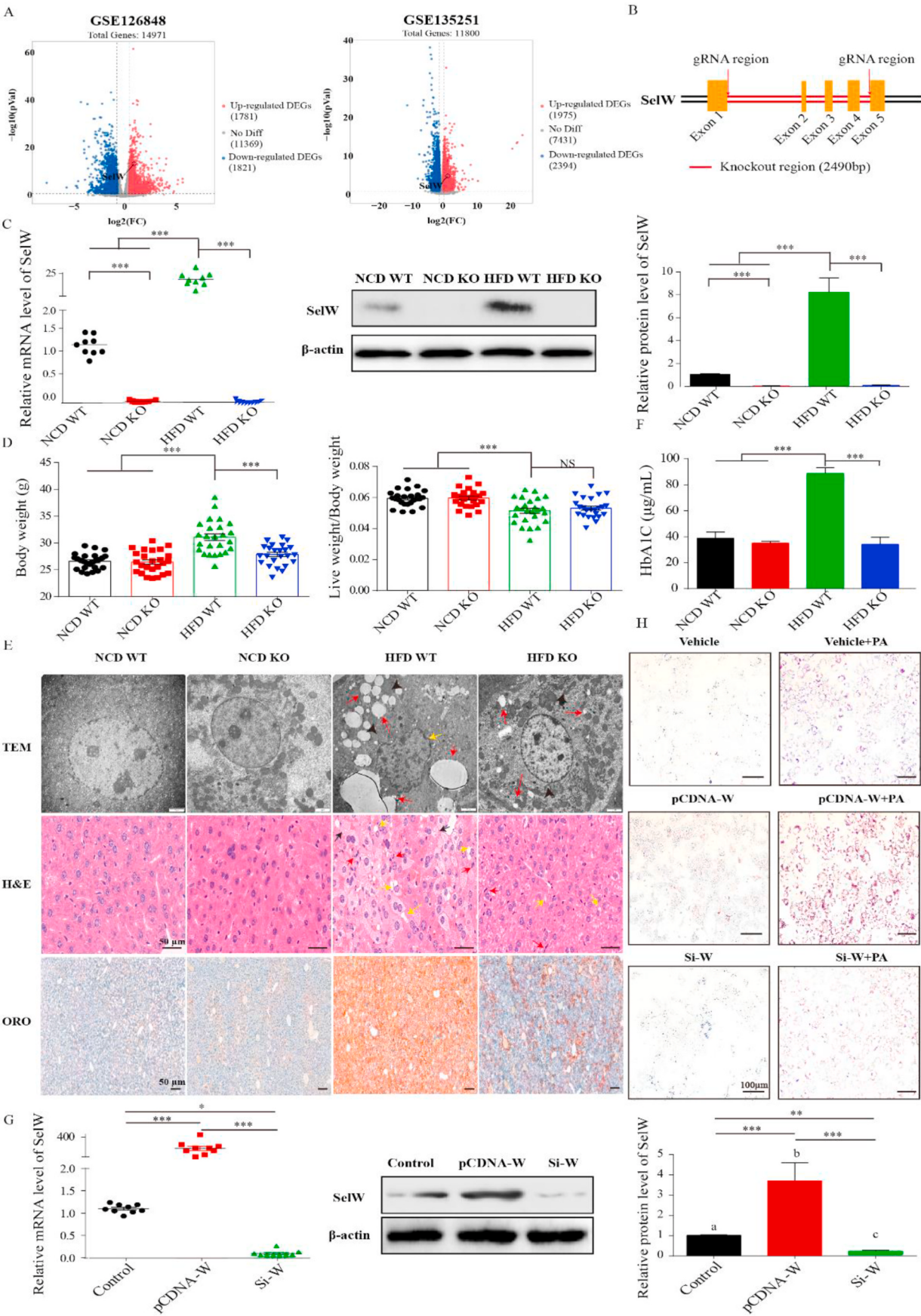
2.13. Statistical analysis

All data were expressed as the mean ± standard error of the mean (SEM). The data calculation was performed using GraphPad Prism (version 8), and used One-Way and tow-Way ANOVA, Tukey's multiple comparisons test to assess differences between different groups. And p-value < 0.05 was considered statistically significant (*p < 0.05, **p < 0.005, ***p < 0.001).

3. Results

3.1. SelW ablation alleviated HFD-induced NAFLD

To determine the involvement of SelW in the pathogenesis of NAFLD, we conducted a comprehensive analysis of the publicly available from NAFLD patients in the GEO datasets. First, SelW was identified as the differentially expressed genes (DEGs) in both GEO datasets, exhibiting a remarkable upregulation in the hepatic tissue of NAFLD patients (Fig. 1A). Thus, we developed SelW knockout (KO) mice to further elucidate the role of SelW in the progression of NAFLD (Fig. 1B). As anticipated, the expression of SelW at both mRNA and protein levels were notably higher in HFD WT mice than NCD WT mice, while it was silenced in KO mice (Fig. 1C). There was a notable disparity in body weight gain between HFD WT mice and HFD KO mice, but no significant difference observed in the liver weight to body weight ratio (Fig. 1D), suggesting that SelW silencing alleviates HFD-induced both body and



(caption on next page)

Fig. 1. SelW is associated with the progression of NAFLD. **(A)** Volcano Plot of DEGs in the liver of health and NAFLD patients in the GEO datasets. **(B)** A schematic illustration of the knockout of SelW by CRISPR/Cas-mediated genome engineering. **(C)** The mRNA and protein expressions of SelW in the liver tissue. **(D)** The body weight and liver weight to body weight ratio of adult wild-type mice (WT) and SelW-knockout (KO) mice fed with NCD or HFD (n = 25). **(E)** The TEM observation, H&E and Oil Red O staining of the liver tissues. (Scale bar = 2/50/50 μm in upper/medium/lower panel). For TEM observation, red arrows represented LD accumulation, yellow arrows demonstrated nuclear shrinkage, black arrows indicated mitochondrial morphological change. For H&E staining, red arrows represented inflammatory cell infiltration, yellow arrows demonstrated increased macrovesicular fat level, and black arrows indicated macrophage aggregation. **(F)** The detection of HbA1C in serum (n = 5). **(G)** The mRNA and protein expression of SelW *in vitro*. **(H)** The Oil Red O staining of AML12 cells. The results are presented as mean $\pm$ SEM. NS $P > 0.05$, $^*P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.001$.

liver weight gain. In addition, the TEM observed arrestive changes in the liver tissue of HFD WT group, including prominent lipid droplet (LD) formation in hepatocyte (red arrows), nuclear shrinkage (yellow arrows), swollen mitochondria, and cristae disappeared (black arrows)

(Fig. 1E). However, the size and number of LDs in hepatocyte of HFD KO mice were decreased, and only partial mitochondrial swelling (black arrows). H&E staining indicated that HFD WT livers exhibits notable inflammatory cell infiltration (red arrows), increased macrovesicular fat

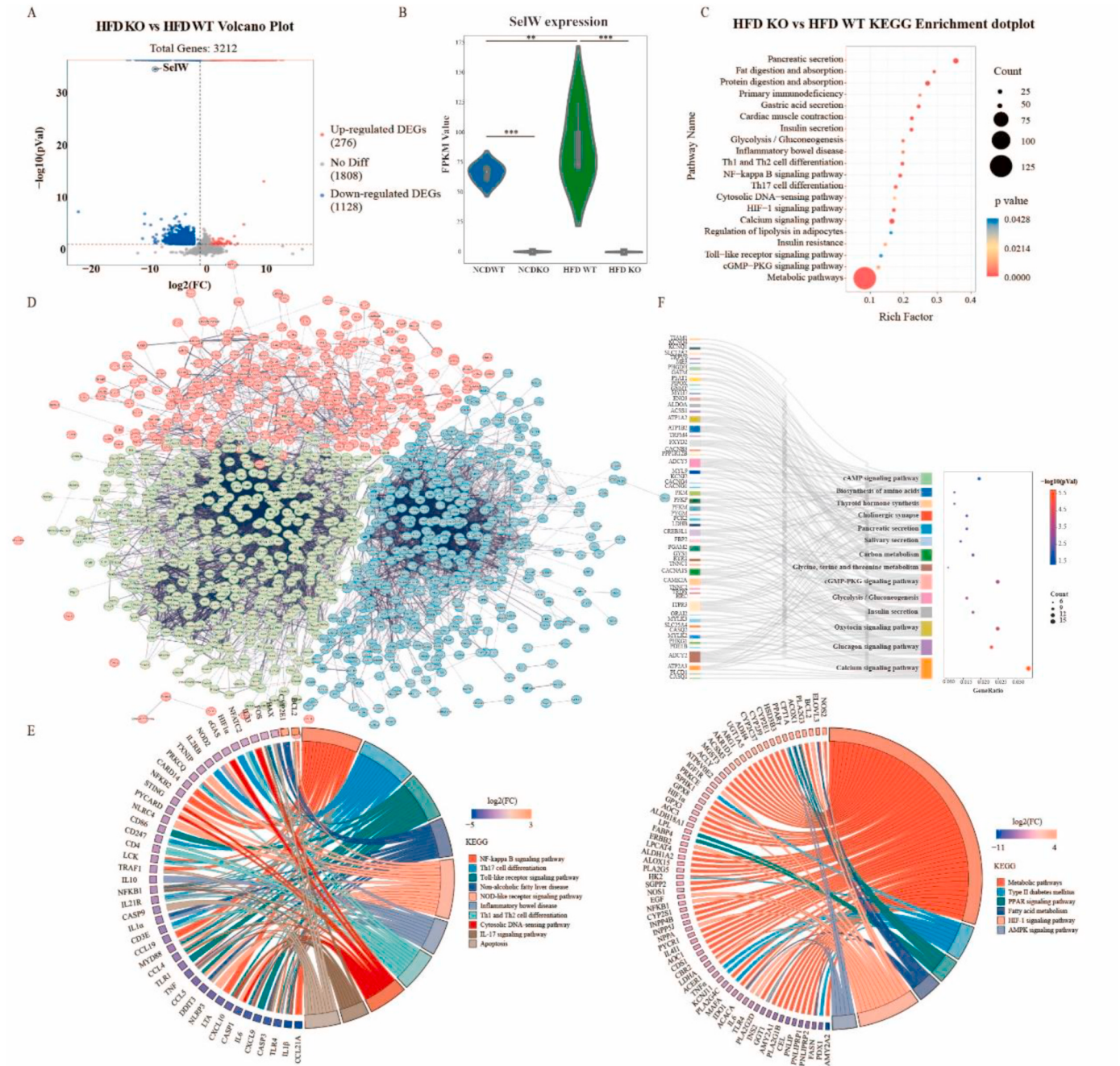


Fig. 2. SelW regulates metabolism and inflammation. **(A)** The transcriptome analysis of the liver tissues which obtained from WT and KO mice fed HFD. **(B)** Violin Plot of SelW expression among groups. **(C)** KEGG pathways enrichment dotplot. **(D)** PPI network of DEGs. **(E)** Chord diagrams revealed KEGG analysis of metabolic and inflammatory related genes in cluster 2. **(F)** Pathway enrichment analysis of metabolic related genes in cluster 3 based on the KEGG database.

level (yellow arrows), and crown-like structure formation due to macrophage aggregation near ballooned hepatocytes (black arrows), compared with HFD KO livers. Subsequently, KO mice revealed arresting protection against the accumulation of LD and HbA1C induced by HFD, in comparison with WT mice (Fig. 1E and F). Meanwhile, the mRNA and protein expression of SelW were indicated satisfactory overexpression and knockdown efficiency using pCNA-W and Si-W plasmid in AML12 cells, as illustrated in Fig. 1G. Compared to the Vehicle group, fat deposition was obviously aggravated by SelW overexpression supplemented with palmitic acid (PA, 0.5 mM), whilst alleviated by SelW knockdown in AML12 cells (Fig. 1H). Together, our findings revealed that SelW is a key regulator of HFD-induced hepatic steatosis.

3.2. Metabolism and inflammation were transcriptionally regulated by SelW

To further investigate the potential mechanism by which SelW mediating the progression of NAFLD, transcriptome analysis displayed 1404 genes differentially expressed between WT and KO mice fed with HFD. In particular, SelW were prominently downregulated in HFD KO versus HFD WT (Fig. 2A). Violin Plot depicted the expression of SelW consistent with the result of qPCR and Western blotting (Figs. 1C and 2B). The Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis illuminated that DEGs was associated with metabolic pathways, HIF-1 signaling pathway, Glycolysis/Gluconeogenesis, cytosolic DNA-sensing pathway, as well as fat digestion and absorption etc. (Fig. 2C). Subsequently, we visualized the intricate interactions among DEGs. As shown in Fig. 2D, PPI network of DEGs was found to consist of 3 gene clusters through K-means clustering, which genes present in each cluster is listed in Table S1. Remarkably, metabolic and inflammatory related genes were predominantly concentrated in cluster 2 and 3, thus, those as key cluster to further analysis. The genes in cluster 2 were primarily associated with inflammatory pathways included cytosolic DNA-sensing pathway, NOD-like receptor signaling pathway and apoptosis pathway, while metabolic pathways included PPAR signaling pathway, fatty acid metabolism and HIF-1 signaling pathway (Fig. 2E). Glycolysis/Gluconeogenesis was mainly enriched in the cluster 3 (Fig. 2F). Overall, these results demonstrated that SelW ablation exerts a negative regulation on HFD-induced metabolic and inflammatory effects.

3.3. SelW mediated PKM2 translocation and altered glycolysis, fatty acid oxidation (FAO) and fatty acid synthesis (FAS)

Transcriptome analysis and KEGG enrichment analysis of potential proteins interacting with SelW showed that SelW is associated with glycolysis (Fig. 2C and S1A). Therefore, we selected potential proteins PKM2 to further confirm the mechanism. The CO-IP complex was immunoblotted by anti-FLAG and anti-PKM2, indicating a strong interaction between SelW and PKM2 (Fig. 3A). Moreover, the binding mode prediction illustrated that SelW binds with PKM2 by hydrogen bonds at residues GLY40, LYS16 and TYR15 (two binding sites), and the binding distance were 2.8 Å, 3.1 Å, 2.2 Å and 3.1 Å respectively, and occurring in both cytoplasm and nucleus by co-localization analysis (Fig. 3B and C).

According to the structural characteristics of PKM2 [11], we evaluated whether SelW regulates the PKM2 formation and translocation from cytoplasm to nucleus. The protein expression of PKM2 were obviously increased in both cytoplasm and nucleus of HFD WT group compared to the NCD WT group, while HFD-fed KO mice displayed the opposite trend (Fig. 3D). Subsequently, the formation of PKM2 tetramer and dimer were aggravated by overexpression of SelW, in particular, the increase of dimer was significantly more than that of tetramer by DSS cross-linking (Fig. 3E). The blots revealed that SelW time-dependently aggravated Y105 phosphorylation of PKM2 (Fig. 3F), revealing SelW interacted with PKM2 to enhance dimer formation that is translocated

into the nucleus to promote transcription.

In addition, the glycolysis, FAO and FAS were detected to verify SelW interacted with PKM2 and thus evoking glucose and lipid metabolic reprogramming. Pearson correlation network demonstrated that SelW positively correlated with glycolysis relative genes (PKM, ENO3, HK2, LDHA, ACSS1, PCK2 and PFKM) and FAS relative genes (ACACA, ACLY, and FASN), negatively correlated with FAO relative genes (ACOX1, CPT1A and PPAR γ) (Fig. 3G), in accordance with these gene expression in both mRNA and protein manners (Fig. 3H and S1B). Compared with HFD WT mice, lactic acid production and glucose consumption in liver tissues of HFD KO mice were reduced (Fig. 3I). Meanwhile, SelW overexpression exacerbated PA-induced glycolysis (LDHA, HK2, and PFKM) and FAS-related genes expression in mRNA and protein manners, as well as lactic acid production and glucose consumption, and preventing FAO-related genes, while the SelW knockdown observed the opposite effect (Fig. 3I–J and Fig. S1C), which would be declined by shikonin (an PKM2 inhibitor, 3 μ M) exposure (Fig. S1D–E). Thoroughly, these findings demonstrated that SelW targeting PKM2 facilitates dimer translocation into the nucleus, accompanied by aggravated glycolysis and FAS, and attenuated FAO, ultimately causing hepatic fat accumulation.

3.4. SelW regulate PKM2 initiated transactivation of the HIF-1 α , mediates mitochondrial apoptosis

PKM2 is a transcriptional co-activator for HIF-1 α binding after translocation into the nucleus [31]. Thus, we assessed the expression of HIF-1 α transcription. The immunofluorescence staining of PKM2 and HIF-1 α in the hepatic tissue of HFD WT mice were observed plentiful overlap in DAPI-stained nuclei, whereas this phenomenon would be declined in HFD KO mice, which is consistent with the protein and mRNA expression of HIF-1 α , demonstrating that SelW ablation ameliorates PKM2 initiating HIF-1 α transcription (Fig. 4A and B). Meanwhile, in comparison with the vehicle group, overexpression of SelW expectedly increased fluorescence intensity of immunostained HIF-1 α , whereas HIF-1 α expression seriously rescued by shikonin addition, which coincided with HIF-1 α expression in mRNA and protein manners, hence SelW regulated PKM2 triggers transactivation of the HIF-1 α (Fig. 4C–E).

In view of the role of HIF-1 α in apoptosis [31], we subsequently assessed SelW/PKM2-mediate HIF-1 α functions in apoptosis. As depicted in Fig. 4F, circos plot clearly confirmed that mitochondrial apoptosis-related genes (caspase 3, caspase 9 and BAX) were positive correlated with SelW, PKM and HIF-1 α by Pearson correlation analysis of transcriptome results, while in contrast, BCL2 was negative correlated. Furthermore, TUNEL staining results revealed that hepatocyte apoptosis in HFD KO mice was expressively less than that in HFD WT mice, manifesting that SelW ablation play a resistance role under HFD-induced apoptosis, in accordance with apoptosis relative gene expression in mRNA and protein manners (Fig. 4G–H and Fig. S2A). Whereas, BAY87-2243 (10 μ M, HIF-1 α inhibitor) treatment significantly diminished SelW induced apoptosis assessed by Annexin V-FITC/PI and Hoechst staining, along with declined expression of mitochondrial apoptosis-related genes, and BCL2 exhibited contrary expression (Fig. 4I–J and Fig. S2B). Take together, these results strongly suggest that SelW can mediate mitochondrial apoptosis by targeting PKM2 to trigger the transactivation of the HIF-1 α .

3.5. SelW exacerbates mitochondrial damage, generates excessive ROS and promotes mtDNA release

Next, we monitored the levels of ROS and mitochondrial injury to evaluate the impact of apoptosis induced by the SelW/PKM2/HIF-1 α axis on the liver. In contrast to the vehicle groups, ROS production was dramatically exacerbated in the SelW overexpression, conversely, SelW knockdown mitigated PA that induced ROS production (Fig. 5A and B). Interestingly, the supply of mito-TEMPO (10 nM, a mitochondria-targeted antioxidant) not only reduced intracellular ROS activation

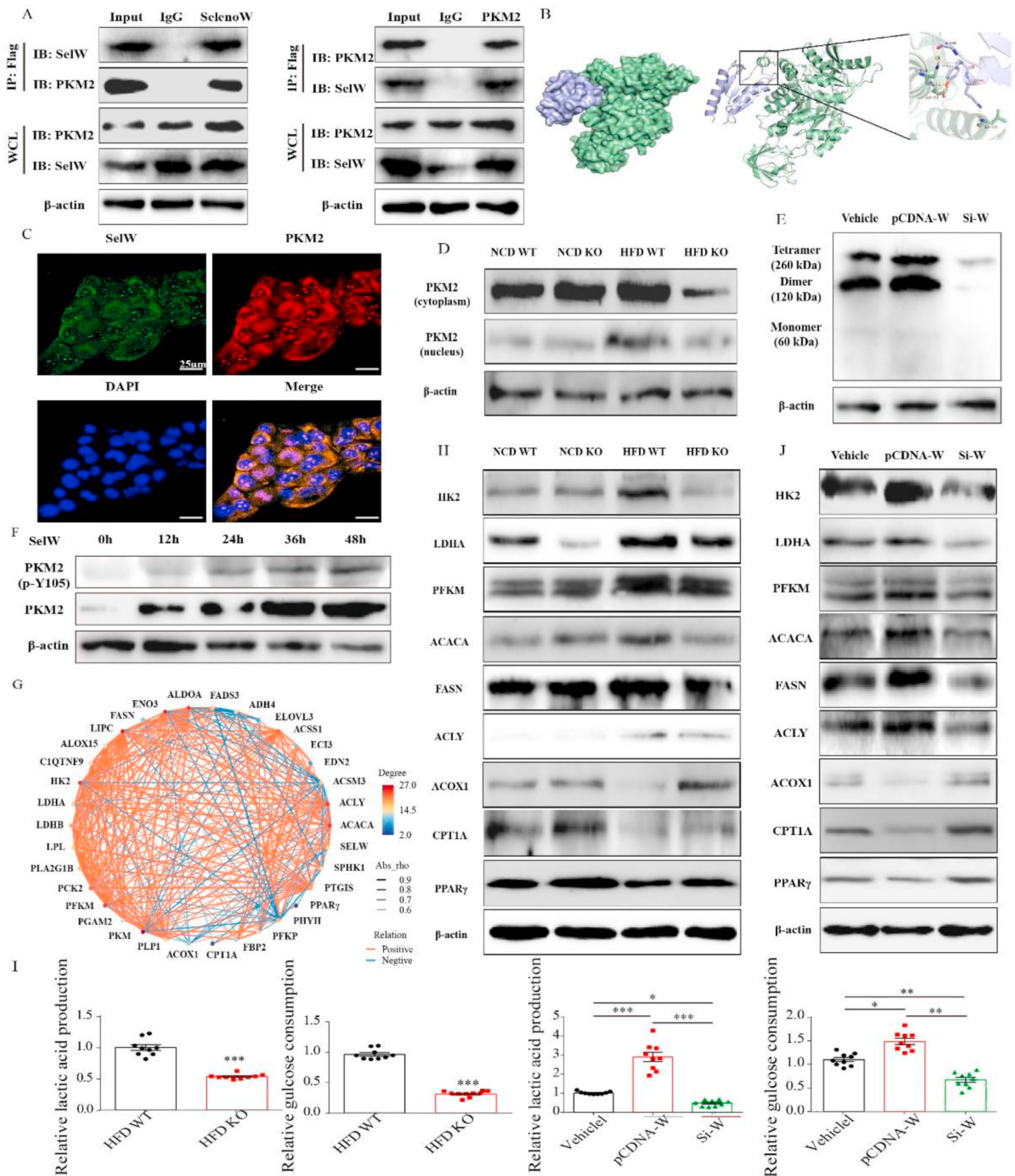
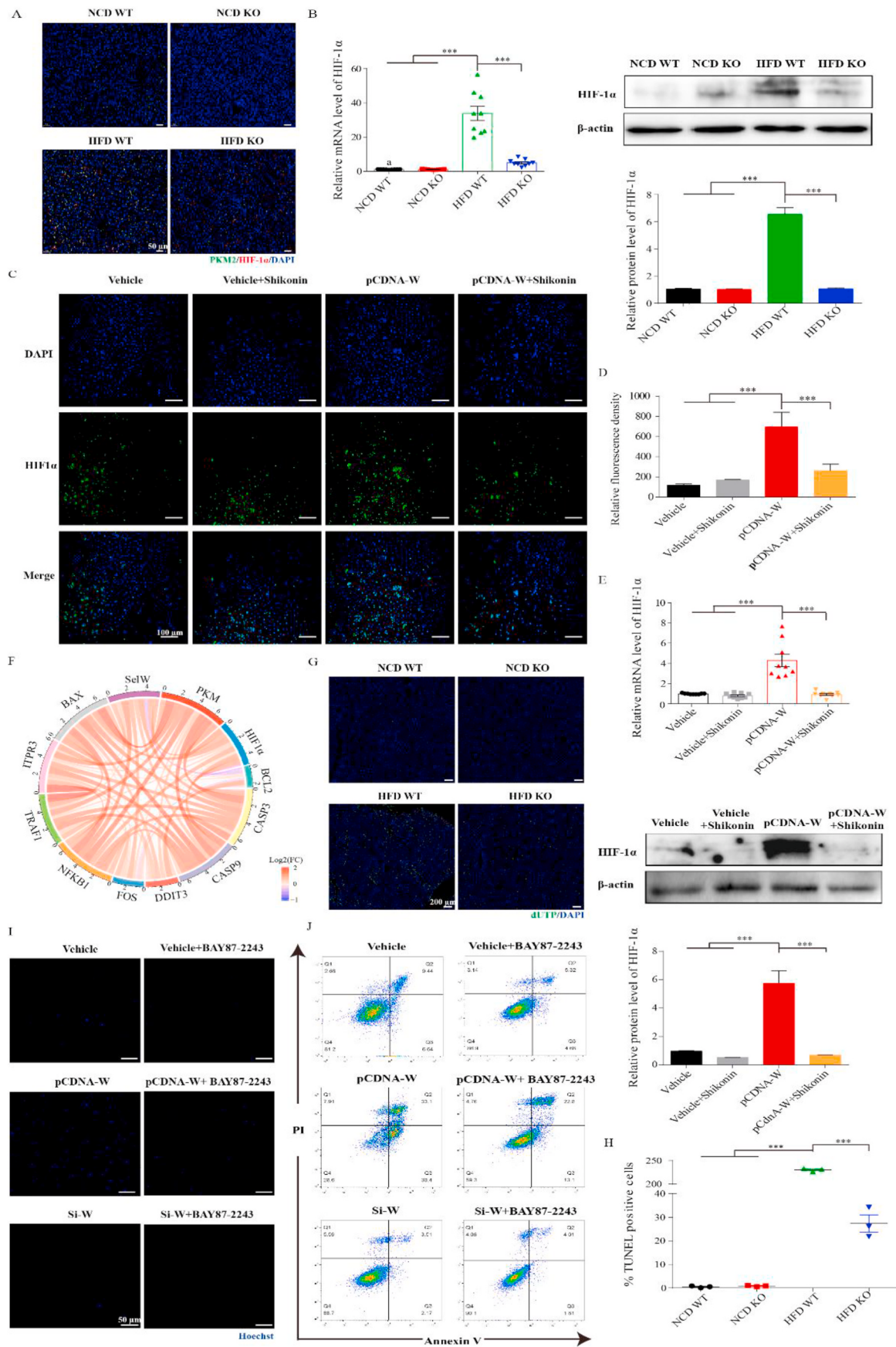


Fig. 3. SelW interacts with PKM2 and evokes a reprogramming of liver metabolism. (A) The interaction of SelW and PKM2 was determined by co-immunoprecipitation. (B) The binding domains of SelW and PKM2 were predicted by molecular docking. (C) Co-localization analysis of SelW and PKM2 in AML12 cells (Scale bar = 25 μ m). (D) The expression of PKM2 in both the nucleus and cytoplasm of AML12 cells. (E) Results of cross-linking of AML12 cells transfected with different plasmids by DSS. (F) The expressions of phosphorylated PKM2 (Y105) and PKM2 in time-dependent manner in AML12 cells. (G) Pearson correlation network of the glycolysis, fatty acid biosynthetic and fatty acid oxidative process by transcriptome analysis. (H and J) The protein expression of glycolysis, fatty acid biosynthetic and fatty acid oxidative relative genes in liver tissues and cell models. (I) The lactic acid productions and glucose consumptions in liver tissues and cell models. The results are presented as mean $\pm$ SEM. * P < 0.05, ** P < 0.005, *** P < 0.001.



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Fig. 4. SelW regulates mitochondrial apoptosis by PKM2/HIF-1 α axis. **(A)** The immunofluorescence staining of PKM2 and HIF-1 α in the liver tissue (Scale bar = 50 μ m). **(B)** The mRNA and protein expressions of HIF-1 α in the liver tissue among the groups. **(C–D)** The immunofluorescence staining of HIF-1 α in AML12 cells of the Vehicle and pCDNA-W groups with or without shikonin (3 μ M) (Scale bar = 100 μ m). **(E)** The mRNA and protein expressions of HIF-1 α in AML12 cells of the Vehicle and pCDNA-W groups with or without shikonin. **(F)** Circos plot demonstrated the correlation between apoptosis relative genes and SelW, PKM and HIF-1 α based on the Pearson comparison analysis. **(G)** TUNEL stained sections demonstrated hepatocyte apoptosis in the liver tissue among the groups (Scale bar = 100 μ m). **(H)** Quantification of the percentage of TUNEL cells per captured field. **(I–J)** Hoechst and Annexin V-FITC/PI staining shown apoptosis in AML12 cells of the Vehicle, pCDNA-W and Si-W groups with or without BAY87-243 (10 μ M) (Scale bar = 50 μ m). The results are presented as mean $\pm$ SEM. *** P < 0.001.

induced by SelW overexpression, but also alleviated $\Delta\Psi_m$ (Fig. 5C). The level of mito-ROS illustrated that SelW overexpression and the vehicle group was notably exacerbated by contrast with the SelW knockdown, and then eliminated with mito-TEMPO by MitoSOX Red (a mitochondria specific superoxide indicator) staining and commercial kit (Fig. 5D and E). Moreover, mito-ATP content showed the opposite trend with mito-ROS (Fig. 5F). The cellular ATP content and Na^+-K^+ -ATPase activity showed a significant decline in the SelW overexpression group, while the extracellular ATP levels of cell supernatant were not statistically significant (Fig. S2C–D). As depicted in Fig. 5G, MitoTracker Green probe staining revealed an observable decrease in fluorescence of the SelW overexpressed and the vehicle groups, indicating that the mitochondrial morphology is disrupted by SelW expression.

The mtDNA liberation from mitochondria is the crucial feature of mitochondrial apoptosis. Interestingly, SelW overexpression observably enhanced the accumulation of cytosolic free dsDNA, revealing mtDNA leakage from mitochondria into the cytoplasm and extracellular matrix, compared with the SelW ablation (Fig. 5H). The qRT-PCR analysis showed consistent results, and Tert expression was not detected in the cytoplasmic DNA, implying that the presence of dsDNA in the cytoplasm did not derive from the nucleus (Fig. 5I). Therefore, SelW expression aggravated PA-induced accumulation of cytosolic dsDNA derived from mitochondria, while this effect would be attenuated by mito-TEMPO or BAY87-2243. Hence, SelW/PKM2/HIF-1 α axis induced apoptosis mediates mitochondrial damage, resulting in mtDNA leakage.

3.6. SelW triggers the activation of NLRP3 inflammasome-mediated pyroptosis and facilitate the extracellular leakage of mtDNA

Previous study has reported that excess ROS is a pivotal stimulus for the initiation of NLRP3 inflammasome [32]. Thus, we next evaluated whether SelW can induced NLRP3 inflammasome-mediated pyroptosis to participate in the progression of NAFLD. As shown in Fig. 6A, liver sections from HFD WT mice exhibited a higher presence of NLRP3⁺Caspase1⁺ cells compared to the HFD KO mice. NOD-like receptor signaling pathway were significant differences (Fig. 2C), with elevated NLRP3, IL-1 β and Caspase1 levels in HFD WT mice compared with HFD KO mice, and consistent with the pyroptosis-related markers expression in both mRNA and protein levels (Fig. 6B and C). Annexin V-FITC/PI/Hoechst staining revealed that SelW overexpression remarkably increased the characteristic pyroptotic cell swelling that appeared green membrane blebbing (red arrows) as compared with the vehicle and SelW knockdown group, which can be mitigated by mito-TEMPO (Fig. 6D). Immunofluorescence was conducted and revealed that NLRP3 and Caspase1 expression increased in the SelW overexpression group, indicating the activation of NLRP3 inflammasome pathway in response to SelW expression. Whereas the mito-TEMPO supplementation would diminish this effect and the elevated expression of pyroptosis-related markers caused by SelW overexpression (Fig. 6E and F). Additionally, N-acetylcysteine (NAC, 5 mM, a ROS scavenger) could inhibit the activation of NLRP3 inflammasome under SelW overexpression, indicating the SelW overexpression-induced NLRP3 inflammasome activation is a ROS-dependent manner (Fig. S2E). A wide range of studies have provided evidence that mtDNA plays a significant role in the activation of NLRP3 inflammasome. We also observed a decrease in the expression of NLRP3 inflammatory-related genes in AML12 cell model with the supplement of dideoxycytidine (ddC, 5 μ M, mtDNA inhibitor) (Fig. S2F).

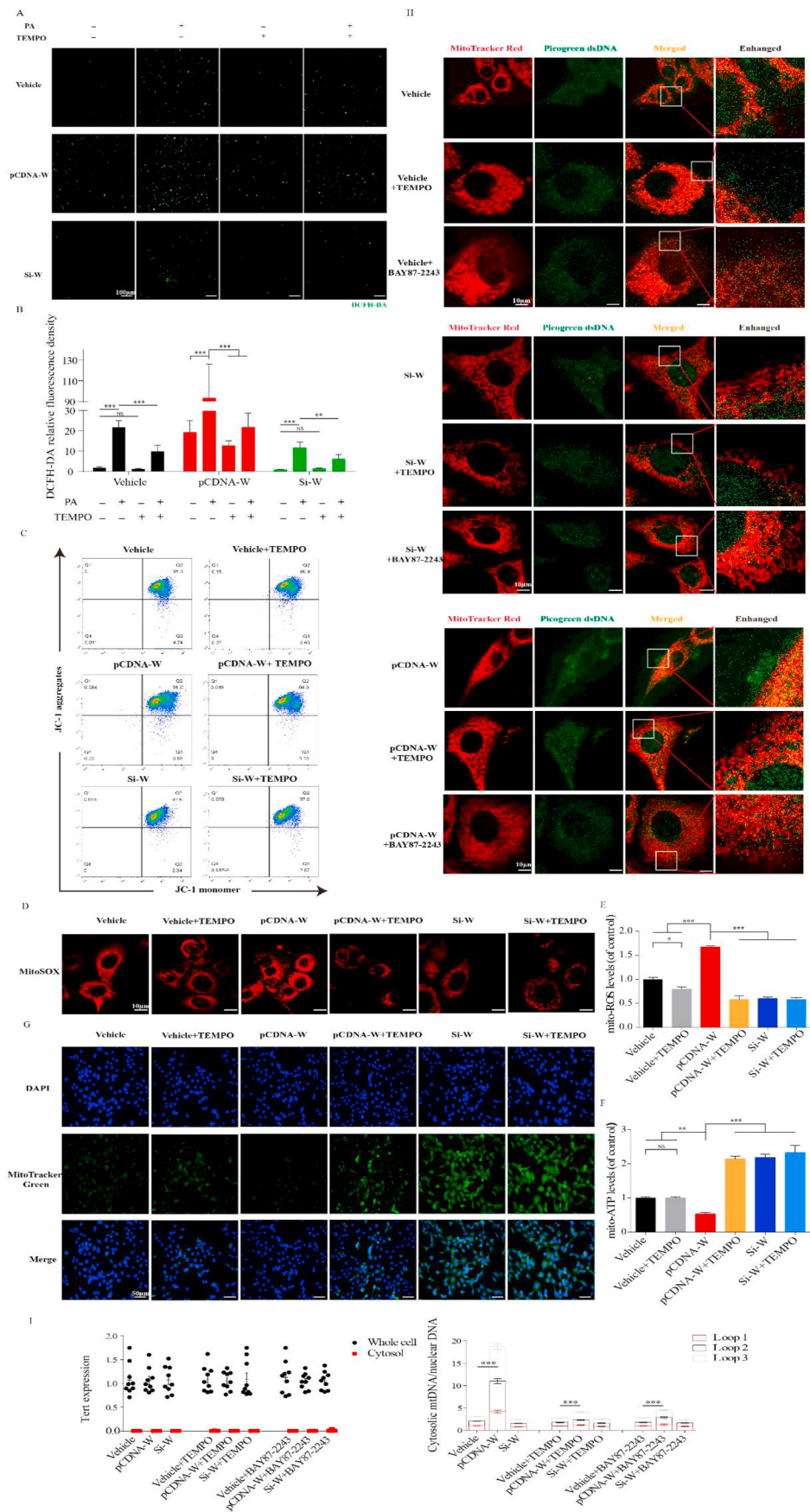
These results suggested that NLRP3 inflammasome-mediated pyroptosis in SelW-overexpressed cells is attributed to the production of mtROS and mtDNA leakage into the cytoplasm.

Pyroptosis is a form of lytic programmed cell death, which resulting in the rupture of membrane integrity [29]. Thus, we next investigated whether SelW-induced hepatocyte pyroptosis could promoting the extracellular leakage of mtDNA. The supernatant of SelW overexpression group exhibited a significantly elevated level of mtDNA compared to both the vehicle and knockdown groups, which could be reversed by treating pyroptosis inhibitor MCC950 (5 μ M) (Fig. 6G). Thus, these results suggested that SelW promotes mtDNA cytosolic accumulation to induce pyroptosis in hepatocytes, which also aggravates the extracellular release of mtDNA.

3.7. SelW promote the activation of the cGAS-STING signaling pathway in macrophage, inducing a shift in macrophage phenotype

Based on macrophages are involved in the progression of NAFLD [33], and we found that SelW ablation obviously reduces macrophage aggregation near ballooning degeneration hepatocytes (Fig. 1E). Hence, we speculated that SelW-mediated mtDNA extracellular leakage from hepatocyte may cause impact on macrophages. The cGAS is recognized as a cytoplasmic DNA biosensor [29]. Thus, we subsequently investigated whether the activation of the cGAS-STING pathway in macrophages would attribute to extracellular leakage of mtDNA. Immunofluorescence staining was implemented to assess the expression of STING⁺ and F4/80 (macrophages/Kupffer cells)⁺ cells in liver tissue. As shown in Fig. 7A, overlapping fluorescence signals (White arrows) in the HFD-fed mice were dramatically increased as compared to those on the NCD diet. Meanwhile, in comparison to HFD WT mice, the share of STING⁺F4/80⁺ and STING⁺F4/80⁺ population was significantly reduced in HFD KO mice, while that of STING⁺F4/80⁺ was no difference (Fig. 7B). The quantitative analysis revealed remarkably highest levels of the cGAS and STING in HFD WT mice (Fig. 7C and D). Furthermore, the SelW overexpression and knockdown AML12 cells with bone marrow-derived macrophages (BMDMs) were cocultured in *trans*-well plates containing DMEM medium supplemented with PA for 24 h (Fig. 7E). SelW overexpression significantly augmented expression levels of cGAS and STING in macrophages, in comparison with the vehicle group, conversely, the knockdown group exhibited a declining trend. The protein and mRNA levels of cGAS and STING exhibited congruent outcomes (Fig. 7F). Furthermore, after transfect extracted mtDNA from the supernatants of SelW overexpressed hepatocytes into macrophages, a notable increase in cGAS and STING expression were detected as compared to transfect mtDNA which extracted from the both vehicle and knockdown groups (Fig. S3A). Collectively, these findings suggested that SelW triggers extracellular leakage of mtDNA, resulting in the activation of the cGAS-STING signaling pathway in macrophages.

Subsequently, we investigated whether the initiation of the cGAS-STING signaling pathway in macrophage can impact macrophage polarization. As illustrated in Fig. 7G and H, the CD86⁺M1 macrophages and CD206⁺M2 macrophages were significantly increased in HFD WT mice, and markedly decreased after SelW silencing, with CD86 the most, and the heatmap of macrophage polarization-related genes expressions revealed the corresponding results. Furthermore, SelW ablation effectively attenuated HFD-induced polarization of both M1 and M2 macrophages surface markers expression and related cytokines release (Fig. 7I–J and S3B). These findings indicated that SelW ablation exerts



(caption on next page)

Fig. 5. SelW causes accumulation of damaged mitochondria and promotion mtDNA release. (A–B) DCFH-DA staining shown ROS generation in AML12 cells of the Vehicle, pCDNA-W and Si-W groups with or without mito-TEMPO (10 nM) (Scale bar = 100 μ m). (C) JC-1 staining demonstrated mitochondrial membrane potential in AML12 cells. (D) Mito-SOX detection of the levels of mito-ROS in AML12 cells (Scale bar = 10 μ m). (E) Quantification of mito-ROS levels. (F) The levels of mito-ATP in AML12 cells. (G) MitoTracker Green probe staining observation (Scale bar = 50 μ m). (H) The fluorescence confocal analysis with MitoTracker Red CMXRos and Picogreen dsDNA probes in AML12 cells (Scale bar = 10 μ m). (I) Quantification of nuclear gene (Tert) expression and cytoplasmic mtDNA levels (n = 9). The results are presented as mean $\pm$ SEM. NS $P > 0.05$, * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$.

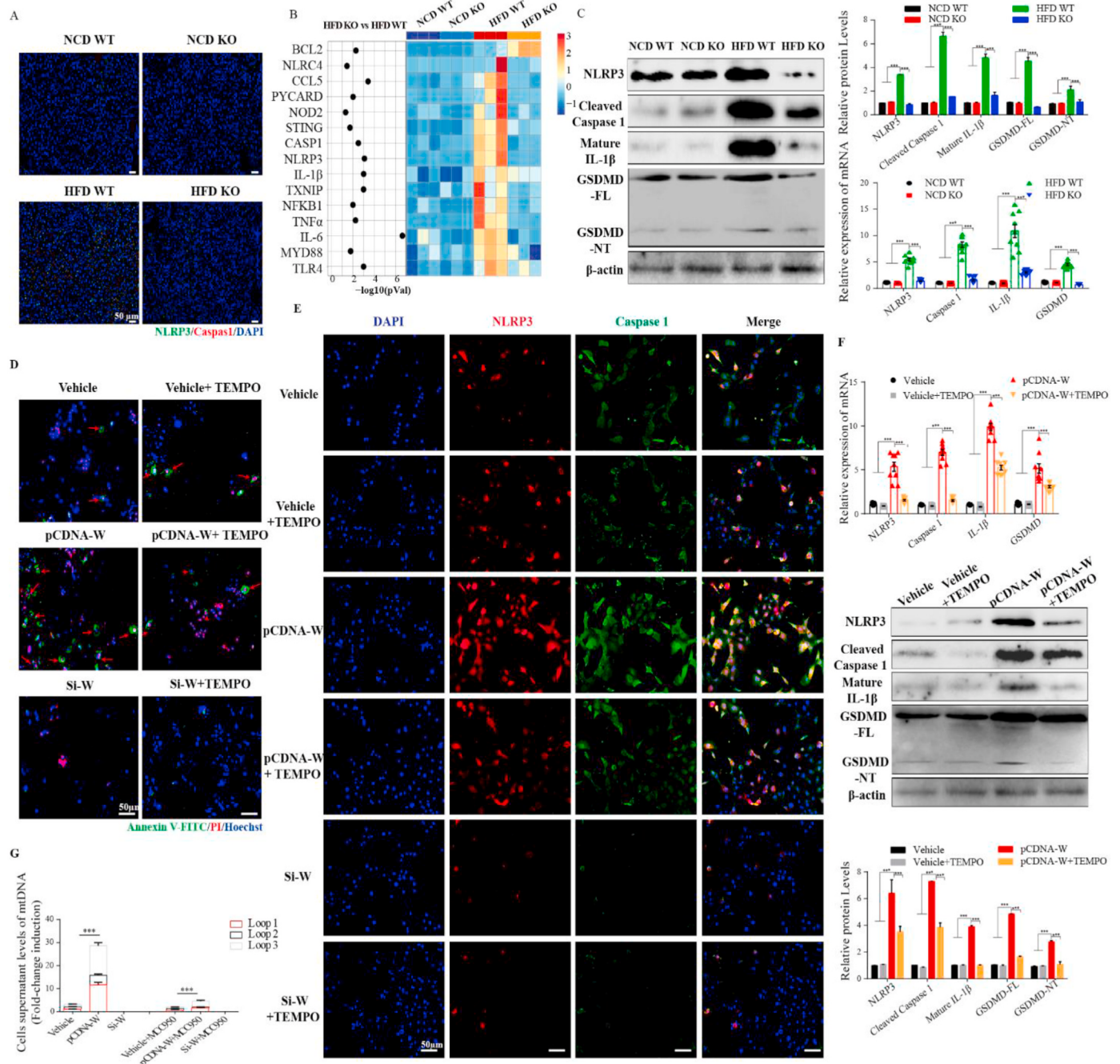


Fig. 6. SelW triggers NLRP3 inflammasome-mediated pyroptosis and facilitates the extracellular leakage of mtDNA. (A) The immunofluorescence staining of NLRP3 and Caspase 1 in the liver tissue (Scale bar = 50 μ m). (B) The transcriptomic analysis results of NOD-like receptor signaling pathway. (C) The mRNA and protein expressions of pyroptosis-related genes in the liver tissues. (D) Annexin V-FITC/PI/Hoechst staining demonstrated pyroptosis in AML12 cells (Scale bar = 50 μ m). (E) The immunofluorescence staining of NLRP3 and Caspase 1 in AML12 cells (Scale bar = 50 μ m). (F) The mRNA and protein expressions of pyroptosis-related genes were examined in AML12 cells. The results are presented as mean $\pm$ SEM. *** $P < 0.001$.

inhibitory effects on the activation of M1 and M2 macrophages. Moreover, BMDMs in bottom chamber were detected by flow cytometry after cocultured with SelW overexpression or knockdown AML12 cells under

PA treatment (Fig. 7K). Compared with the vehicle group, SelW expression increased the percentage of CD86⁺ and CD206⁺ macrophage, while SelW knockdown was reversed that. Meanwhile, pre-treated ddC

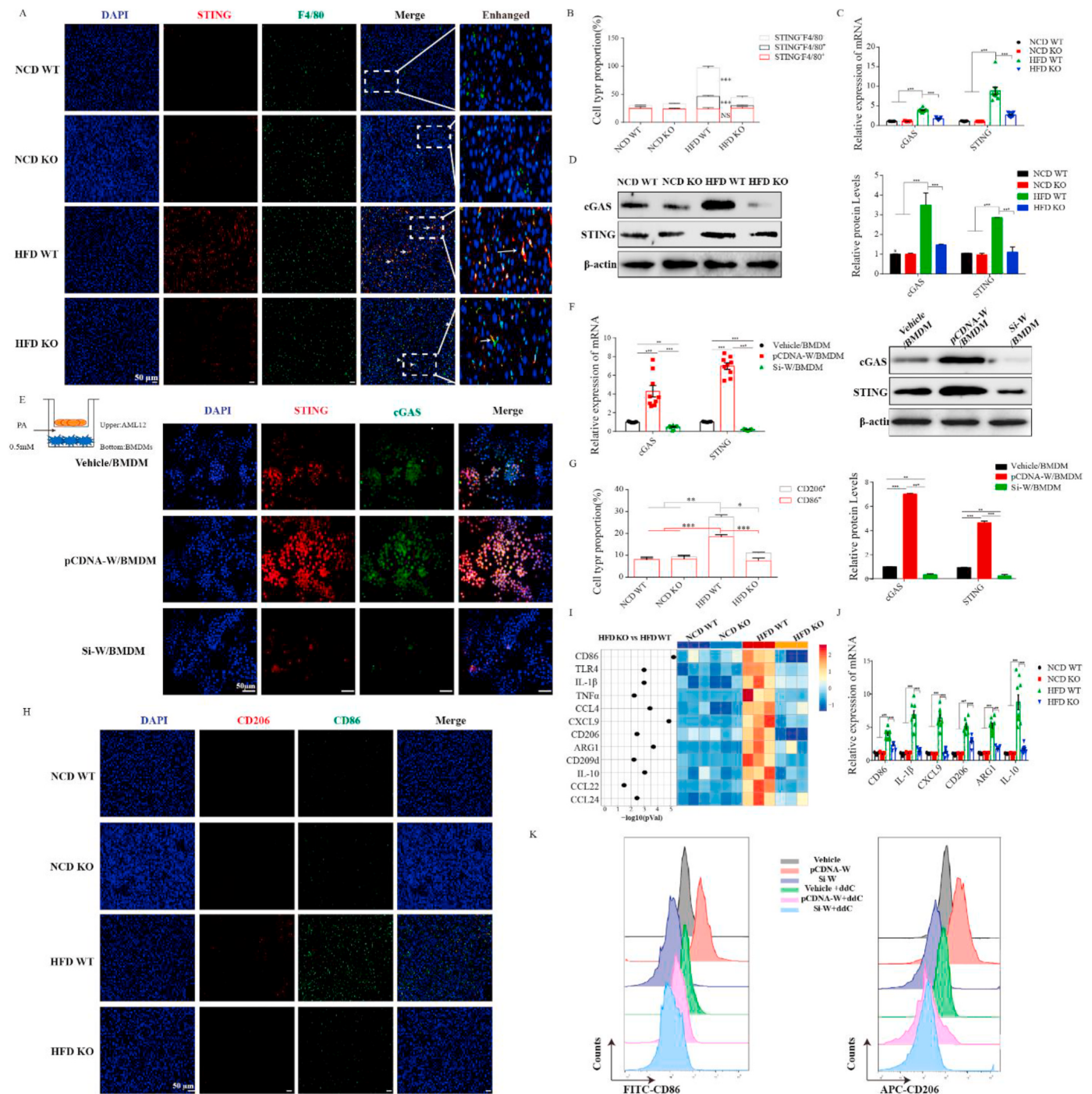


Fig. 7. SelW promotes the activation of the cGAS-STING signaling pathway and macrophage polarization in macrophage. **(A)** The immunofluorescence staining of STING and F4/80 in the liver tissue (Scale bar = 50 μ m). **(B)** Quantification of the percentage of STING⁺F4/80⁺ and STING⁺F4/80⁻ cells per captured field. **(C–D)** The mRNA and protein expressions of cGAS and STING in the liver tissues. **(E)** The dual immunofluorescence staining of STING and cGAS in the *trans*-well cell models (Scale bar = 50 μ m). **(F)** The mRNA and protein expressions of STING and cGAS in the *trans*-well cell models. **(G)** Quantification of the percentage of CD86⁺ and CD206⁺ cells per captured field. **(H)** The immunofluorescence staining of CD86 and CD206 in the liver tissues (Scale bar = 50 μ m). **(I)** The transcriptomic analysis results of M1 and M2-related genes in the liver tissues. **(K)** The surface marker CD86 and CD206 of the *trans*-well cell models were determined by flow cytometry. The results are presented as mean $\pm$ SEM. NS $P > 0.05$, $*P < 0.05$, $**P < 0.005$, $***P < 0.001$.

in the SelW overexpression (upper chamber) of *trans*-well medium illustrated that the CD86 and CD206 were declined, accompanied by receding the M1 and M2 macrophage polarization-related marker genes expressions, indicating that mtDNA-mediated cGAS-STING signaling pathway activation caused by SelW were contribution to promote macrophage polarization (Fig. 7K and S3). Cumulatively, the SelW-mediated leakage of mtDNA activated the initiation of the cGAS-

STING signaling pathway in macrophage, promoting macrophage polarization.

4. Discussion

The prevalence of NAFLD as the most common cause of chronic liver disease is rising globally. Nevertheless, the influence of Se on NAFLD

remains controversial. Wang et al. confirmed that there were non-linear associations between high serum Se content and NAFLD prevalence [14], while Squar et al. subsequently elucidated that blood Se level was positively associated with NAFLD among the US population [15]. However, the effects of SelW on HFD-induced NAFLD remains unclear. Meanwhile, an increasing body of evidence suggests that metabolic disorder, hepatocyte death, and the activation of macrophage innate immune responses are closely associated with NAFLD. The reprogramming of hepatic metabolism in NAFLD has been demonstrated to be a crucial factor in the aberrant accumulation of lipids into hepatocytes, and increasing the risk of hepatocellular carcinoma (HCC) [8]. Additionally, liver chronic inflammation is a frequent feature of NAFLD and that fuels the development of NAFL to NASH. The pyroptosis of hepatocytes induced the release of inflammatory cytokines, thereby triggering the activation of innate immune response involving macrophages and ultimately exacerbating the prevalence of NAFLD [11,33]. In the present investigation, the results demonstrated that SelW plays a crucial role in the advancement of NAFLD. We revealed that SelW interacts with PKM2 to regulate glycolysis, FAS and FAO, mediating HIF-1 α function in apoptosis, resulting in excess mito-ROS generation and mtDNA leakage. Abnormally high increase in ROS triggered NLRP3 inflammasome-mediated pyroptosis to facilitate the release of mtDNA into the extracellular, subsequently triggered the cGAS-STING signaling pathway and phenotype shift in macrophage, aggravating the progression of NAFLD.

Increasing evidence has substantiated the crucial roles of selenoproteins in diverse biological processes, including the maintenance of intracellular homeostasis, enhancement of immune function [13,34], and participation in glucose and lipid metabolism. Previous studies have underscored the close association between selenoprotein deficiency and diabetes as well as metabolic syndrome [35,36]. Moreover, elevated levels of Se can increase the prevalence of diabetes, importantly, serum Se levels are positively correlated with fasting blood glucose [37]. Our findings indicated a positive association between the SelW expression of liver tissue and the development of NAFLD. SelW was expressed in HFD-fed WT mice and showed an increasing trend compared with NCD-fed mice. Meanwhile, SelW ablation significantly alleviated HFD-induced LDs accumulation, HbA1C upregulation and hepatic steatosis, revealing a regulator role of SelW in the progression of NAFLD. Subsequently, we determined an interaction between SelW and PKM2, whereby the expression of both tetrameric and dimeric forms of PKM2 is significantly enhanced by SelW, suggesting that SelW not only augments pyruvate kinase activity but also translocates into the nucleus to promote transcription.

In addition, numerous studies have reported that glycolysis is a crucial mechanism of metabolic reprogramming-induced liver inflammation and eventual progression to cirrhosis and HCC [8]. Fan et al. illuminated that PKM2 reprograms metabolic pathways to ameliorate hepatic fibrosis in NAFLD mice models [12]. In the present study, our results demonstrated that SelW exhibits a significant correlation with metabolic and inflammatory responses, as evidenced by transcriptome analysis of the liver tissues. Meanwhile, SelW effectively regulates glucose and lipid metabolic reprogramming by interacting with PKM2.

Recently, accumulating evidence has shown that metabolic alterations caused by the accumulation of lipids are considered as a significant contributor to hepatocyte cell death [3,38]. Furthermore, multiple mechanisms of hepatocyte cell death can trigger immune cell recruitment to drive the development of various liver diseases [39]. In this study, we have identified that SelW facilitates the translocation of PKM2 dimer into the nucleus, thus initiating HIF-1 α transcription. HIF-1 α processes pro-apoptotic properties, and can mediate the mitochondrial damage, resulting in excessive mito-ROS generation and increased mtDNA fragmentation, thereby escaping into the cytoplasm [31]. Our findings were consistent with prior researches, immunofluorescence analysis elucidated a significant decrease in the levels of PKM2 and HIF-1 α in the livers of KO mice compared with WT mice fed HFD. We

demonstrated that SelW mediates mitochondrial apoptosis through the PKM2/HIF-1 α axis, resulting in excessive production of mito-ROS, decreased $\Delta\Psi_m$, disrupted mitochondrial morphology, and released of mtDNA. PKM2-dependent glycolysis can promote the activation of NLRP3 and AIM2 inflammasome, thereby promoting the release of pro-inflammatory cytokines in macrophages and participating in the development of sepsis [40]. Moreover, when energy metabolism is blocked, extracellular ATP can stimulate the efflux of potassium ions (K^+), resulting in the diversion of NLRP3 inflammasome activation-dependent pyroptosis to NLRP3-independent necrosis [41], highlights a strong correlation between energy metabolism and NLRP3 inflammasome activation. While our results showed that overexpression of SelW significantly decreased the cellular ATP content, Na^+K^+ -ATPase activity, and mitochondrial ATP content, but there was no notable alteration observed in extracellular ATP levels. Meanwhile, the production of mtROS and mtDNA accumulation in the cytoplasm resulting from mitochondrial damage, are recognized as crucial factors in the activation of NLRP3 inflammasome. Mito-ROS was proposed to promote deubiquitylation of NLRP3 and then activate the NLRP3 inflammasome [32,42]. Mitochondrial damage induced the release of fragmented mtDNA and the increased production of ROS that convert mtDNA into an oxidized form (ox-mtDNA) in the cytosol. This ox-mtDNA then binds to and activates the NLRP3 inflammasome [43,44]. Recent studies have provided evidence that NLRP3 inflammasome-mediated pyroptosis is a contributing factor in the progression of NAFLD [45]. Therefore, we examined whether the overexpression of SelW has an effect on the activation of the NLRP3 inflammasome. Our data demonstrated that SelW can significantly induce pyroptosis, mitochondrial targeted antioxidant mito-TEMPO reduced NLRP3 inflammasome-mediated pyroptosis by inhibiting the generation of mito-ROS in SelW overexpression. Treatment with NAC or ddC also effectively attenuated the activation of NLRP3 inflammasome induced by SelW overexpression, suggesting that mito-ROS and mtDNA accumulation induced by the SelW/PKM2/HIF-1 α axis activates the NLRP3 inflammasome, thereby mediating pyroptosis. Furthermore, the pores in the plasma membrane caused by pyroptosis aggravated the extracellular leakage of mtDNA.

In addition, innate immunity plays an indispensable role in the pathogenesis of hepatic steatosis and NAFLD [46]. As one of the crucial inflammatory cell types in innate immunity, macrophages have been demonstrated to be highly contributes to the progression of NAFLD and development to NASH [33]. STING is a crucial regulator of innate immunity, and its activation in macrophages drives the occurrence of various liver diseases [1]. Recently, DNA lesions have been demonstrated to be associated with NAFLD progression [1]. Accumulation of cytoplasmic DNA fragments caused by nuclear DNA damage recognized by cGAS, positioned upstream of STING, thus eliciting the activation of STING [29,47]. We provide evidenced that the loss of SelW remarkably alleviates HFD-induced recruitment and activation of macrophages and STING expression in macrophages was significantly reduced. In line with this observation, overexpression of SelW in hepatocytes potentiated the activation of macrophage cGAS-STING pathway in *trans*-well assay, conversely, SelW ablation exhibited a declining trend, and mtDNA extractions cocultured assay obtained consistent results. These findings indicated that the SelW/PKM2/HIF-1 α axis mediated extracellular leakage of mtDNA in hepatocytes drives the cGAS-STING signaling pathway activation. Macrophage polarization is considered a hallmark of hepatic steatosis and inflammation [46]. In general, the polarization of M1 macrophages exacerbates the severity of NAFLD, as it predominantly produces pro-inflammatory cytokines that contribute to the pathogenesis of hepatic steatosis and inflammation [48], whereas the M2 polarized macrophages inhibited inflammation to ameliorates hepatic steatosis [49]. However, A significantly increased expression of M2 polarized macrophage markers were found in NASH patients, speculating that elevated M2 polarized macrophages might participate in liver remodeling and repair, but could also enhance the development of

fibrosis [50]. A recent study revealed a significant reduction in the expression of the M2 macrophage polarization marker enzyme ARG1 and the ability to enhance glycolytic metabolism in SelW knockout murine-derived BMDMs, under the stimulation of LPS [13]. Our findings demonstrated that SelW ablation attenuates the expression of the M1 and M2 macrophage polarization under HFD-induced. In vitro, over-expression of SelW in hepatocytes induces increased polarization of M1 and M2 in macrophages, while SelW knockout can significantly reduce the polarization of M1 and M2. Moreover, mtDNA inhibitor ddC reduced the M1 and M2 macrophage polarization, revealing that the macrophage polarization induced by cGAS-STING signaling pathway activation after SelW mediated extracellular leakage of mtDNA in hepatocytes, promoting the pro-inflammatory cytokines production, thereby exacerbating the progression of NAFLD. Nevertheless, the increased M2 polarization by SelW may be caused by protective mechanisms of the liver, such as the elimination of M1 macrophages and liver remodeling, but can aggravate the risk of liver fibrosis and even the development of HCC.

In conclusion, SelW plays a novel role in the progression of NAFLD. We elucidated that regulated glycolysis, FAS and FAO by interacting with PKM2, initiated HIF-1 α -mediated apoptosis, promoted the production of mito-ROS, and further activated NLRP3 inflammasome-mediated pyroptosis, which was accompanied by extracellular leakage of mtDNA and induced macrophage phenotype transformation through cGAS-STING signaling pathway, eventually promoting the progression of NAFLD, liver fibrosis and HCC. Our study provides significant evidence for further biological function study of SelW and potentially provide new avenues for the treatment of NAFLD.

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CRediT authorship contribution statement

Zhiruo Miao: Writing – original draft, Investigation, Data curation, Conceptualization. **Wei Wang:** Data curation. **Zhiying Miao:** Investigation, Data curation. **Qiyuan Cao:** Investigation. **Shiwen Xu:** Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.redox.2024.103114>.

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