



## Pro-caspase-1 patrolled to the nucleus of proatherogenic lipid LPC-activated human aortic endothelial cells induces ROS promoter CYP1B1 and strong inflammation

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### ABSTRACT

To determine the roles of nuclear localization of pro-caspase-1 in human aortic endothelial cells (HAECs) activated by proatherogenic lipid lysophosphatidylcholine (LPC), we examined cytosolic and nuclear localization of pro-caspase-1, identified nuclear export signal (NES) in pro-caspase-1 and sequenced RNAs. We made the following findings: 1) LPC increases nuclear localization of procaspase-1 in HAECs. 2) Nuclear pro-caspase-1 exports back to the cytosol, which is facilitated by a leptomycin B-inhibited mechanism. 3) Increased nuclear localization of pro-caspase-1 by a new NES peptide inhibitor upregulates inflammatory genes in oxidative stress and Th17 pathways; and SUMO activator N106 enhances nuclear localization of pro-caspase-1 and caspase-1 activation (p20) in the nucleus. 4) LPC plus caspase-1 enzymatic inhibitor upregulates inflammatory genes with hypercytokinemia/hyperchemokine and interferon pathways, suggesting a novel caspase-1 enzyme-independent inflammatory mechanism. 5) LPC in combination with NES inhibitor and caspase-1 inhibitor upregulate inflammatory gene expression that regulate Th17 activation, endothelin-1 signaling, p38-, and ERK-MAPK pathways. To examine two hallmarks of endothelial activation such as secretomes and membrane protein signaling, LPC plus NES inhibitor upregulate 57 canonical secretomic genes and 76 exosome secretomic genes, respectively, promoting four pathways including Th17, IL-17 promoted cytokines, interferon signaling and cholesterol biosynthesis. LPC with NES inhibitor also promote inflammation via upregulating ROS promoter CYP1B1 and 11 clusters of differentiation (CD) membrane protein pathways. Mechanistically, all the LPC plus NES inhibitor-induced genes are significantly downregulated in CYP1B1-deficient microarray, suggesting that nuclear caspase-1-induced CYP1B1 promotes strong inflammation. These transcriptomic results provide novel insights on the roles of nuclear caspase-1 in sensing DAMPs, inducing ROS promoter CYP1B1 and in regulating a large number of genes that mediate HAEC activation and inflammation. These findings will lead to future development of novel therapeutics for cardiovascular diseases (CVD), inflammations, infections, transplantation, autoimmune disease and cancers. (total words: 284).

### 1. Introduction

Atherosclerosis is a dominant and increasing cause of mortality and morbidity worldwide. Vascular inflammation is responsible from inception to the emergency of complications associated with atherosclerosis [1–3]. This has been further validated by the findings from the

Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS), where it was demonstrated that the inhibition of pro-inflammatory interleukin-1  $\beta$  (IL-1 $\beta$ ) reduces the atherosclerotic burden in cardiovascular disease [4,5]. Aortic arch atherosclerosis as a major atherosclerotic site [6] is an important source of embolic stroke [7]. Previously, we reported that hyperlipidemia drives aortic

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endothelial cell activation and early atherosclerosis via caspase-1-inflammasome pathway [8–12]. Additionally, we demonstrated that increased uremic toxins in chronic kidney disease (CKD) act as damage associated molecular patterns (DAMPs) and trigger vascular inflammation [13–16] by activating caspase-1-inflammasome [17] pathway. Further, secretomes in peripheral blood mononuclear cells (PBMC) may play significant roles in promoting inflammation in CKD transition to end stage renal disease [18]. Recently, we also reported that CD4<sup>+</sup>Foxp3<sup>+</sup> regulatory T cells (Treg) [19–21] may use various secretomes to maintain its “Treg-ness”, Treg suppression, modulate anti-tumor immune responses, facilitate tissue repair [22] and stem cell-like functions in tissue regeneration [23]. However, significant questions remain whether proatherogenic DAMPs-induced procaspase-1 sense nuclear danger in endothelial cells derived from *intracellular DAMPs* rather than extracellular DAMPs such as lipopolysaccharide (LPS) and oxidized low density lipoprotein (oxLDL) and promote aortic endothelial cell activation and inflammation.

Our previous paper reported [24] that, in addition to regulatory effects executed by well characterized caspase-1 substrates IL-1 $\beta$ , IL-18, Sirtuin-1 [8] and gasdermin-D, caspase-1 (enzyme ID: EC 3.4.22.36) also cleaves many other protein substrates including those listed in the Brenda Enzyme database (<https://www.brenda-enzymes.org/index.php>), several nuclear proteins such as deneddylase involved in Epstein-Barr virus replication [25], histone deacetylase Sirtuin 1<sup>8</sup>, transcription factors GATA binding protein 4 (GATA4) [26], and peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) [27]. We reported that caspase-1 regulates the transcription of many genes potentially via its known substrates, also via transcription factors and other proteins that are not yet identified [28]. Moreover, our previous findings identified 28 nuclear substrates and 7 nuclear interaction proteins [29] that are potential substrates of caspase-1, suggesting that pro-caspase-1 may have a nuclear role that needs to be further explored. The investigators in the Human Protein Atlas project found that some caspase-1 is localized in the nucleus of the cell line U-2OS (human osteosarcoma cells) detected with immunofluorescence-labeled antibody CAB002685 (Thermo Fisher) (<https://www.proteinatlas.org/ENSG00000137752-CASP1/cell#img>). Others reported that NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3), a well characterized Nod-like receptor (NLR), is a transcription regulator [30] and a key inflammasome component for caspase-1 activation, indicating that hyperlipidemia danger-sensing inflammasome components and pro-caspase-1 also can be localized to the nucleus (supported by the information in the GeneCards database <https://www.genecards.org/cgi-bin/carddisp.pl?gene=NLRP3&keywords=NLRP3>) and regulate the gene expression.

Nuclear localization of caspase-1 in nucleus in response to various stress stimuli had been reported before. Although caspase-1 is mainly localized in the cytosol in human embryonic kidney 293 cells and other mammalian cells, caspase-1 was found localized in the nucleus depending on cell types [31]. Nuclear caspase-1 activation was reported in gastric tissue sections obtained from patients with gastritis, gastric tumor and in tissues located adjacent to tumors [32]. Caspase-1 was localized in the nucleus of Hela cervical cancer cells stimulated with proinflammatory cytokine tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) [33]. Interestingly, protease-dead mutant of caspase-1 was capable of localizing into the nucleus in Hela cells [31], suggesting that caspase-1 translocation is independent of its enzymatic activity. Moreover, caspase-1 is activated in the nucleus in response to Herpes simplex virus-1 infection in human foreskin fibroblasts by interacting with nuclear DNA-sensing inflammasome interferon- $\gamma$  inducible protein 16 (IFI16) [34,35]. Furthermore, caspase-1 nuclear localization can be blocked by interleukin-1 receptor (IL-1R) antagonist [36], which suggests that caspase-1 localization to the nucleus may have inflammatory functions inhibited by IL1R antagonist.

We previously proposed a new working model to classify endogenously generated DAMPs as conditional DAMPs that bind to their intrinsic receptors rather than classical DAMP receptors such as Toll-like receptors or NOD-like receptors (NLRs) [37,38] etc. As we reported,

endothelial cells (EC) express caspase-1/inflammasomes [24], one type of PAMPs (pathogen associated molecular patterns)/DAMPs receptors (PRRs) [2], and sense cardiovascular disease risk factors such as hyperlipidemia [8,39], hyperhomocysteinemia [40], uremia toxins associated with CKD [41], and hypoxia [12], which activate EC and accelerate vascular inflammation [14,41] and atherosclerosis [28,29]. However, it remains unknown that whether activation of aortic endothelial cells has nuclear programs in addition to upregulation of secretion of cytokines, chemokines and EC adhesion molecules; and whether procaspase-1 is localized to the nucleus of human aortic vascular cells (HAECs) to sense nuclear dangers in response to conditional DAMPs derived from endogenous metabolites such as lysophosphatidylcholines (LPC) [8,12,37,38,42–45].

Due to its vast coverage and the nature of being the first cell type to encounter any PAMPs/DAMPs in the blood circulation, the endothelium (endothelial cells, EC) [46] could function as the primary intravascular sentinel system [47–49]. Thus, we compared EC to prototypic innate immune cells such as macrophages [50] in 13 innate immune features, and proposed a new paradigm that EC are innate immune cells [2,3,51], which was further supported by our new paper examining the expression changes of 1311 innate immune regulatome genes (IGs) [52,53] in ECs and reviews [54,55]. We introduced new concepts that mitochondrial reactive oxygen species (mtROS) and proton leak mediate physiological activation and pathological activation of EC [56–59]; ROS systems are a new integrated network for sensing homeostasis and alarming stresses [60]. Moreover, under the stimulation by hyperlipidemia, EC have a novel prolonged activation status, with four innate immune features including upregulation of EC adhesion molecules [61] and secretion of cytokines and chemokines (secretomes) [22,23,54,62], upregulation of additional DAMP receptors, and increased expression of co-signaling receptors and MHC class II molecules [43]. However, it remains unknown that whether proatherogenic DAMPs-induced procaspase-1 nuclear localization modulates aortic endothelial cell secretomes, membrane protein signaling and ROS regulators [60,63] in aortic endothelial cells. To address these important questions listed above, we performed subcellular fractionation, identified nuclear export signal in pro-caspase-1, and sequenced RNAs of HAEC in the presence of procaspase-1 nuclear export signal (NES) inhibitor and caspase-1 enzymatic inhibitor. Our transcriptomic results provide novel insights on the roles of nuclear caspase-1 in sensing DAMPs, inducing ROS promoter CYP1B1 [64] and in regulating a large number of genes that mediate HAEC activation and inflammation. The findings of this study will lead to future development of novel therapeutics for cardiovascular diseases (CVD), inflammations, infections, transplantation, autoimmune diseases, and cancers.

## 2. Materials and methods

### 2.1. Chemicals and antibodies

Lysophosphatidylcholine (LPC) (16:0) was purchased from Avanti Polar Lipids, AL. Nuclear export inhibitor Leptomycin B was purchased from Cell signaling technology, Danvers, MA. Cytosolic- and nuclear-pro-caspase-1 block peptide was synthesized by Lifetein LLC, Somerset, NJ. The antibodies information are as follows: Caspase-1 – Cat. No.: NBP1-45433 (Novus Biologicals), HSP 90 – Cat. No. 610418 (BD Bioscience), SP-1 - Cat. No. ab157123 (Abcam). SUMO activator N106 Cat. No. 5618 (R&D), TATA binding protein Cat. No. 63766 (Abcam).

### 2.2. Human aortic endothelial cell (HAEC) culture

HAEC (Lonza, CC2535; Walkersville, MD) were cultured in M199 (Hyclone laboratories, Logan, UT) medium, supplemented with 20% fetal bovine serum (Hyclone), endothelial cell growth supplement 50 mg/ml (BD Biosciences, San Jose, CA), heparin 50 mg/ml and penicillin streptomycin and amphotericin 1% (Invitrogen, CA) as we reported [8].

The cells were grown in 0.2% gelatin coated culture vessels. All the experiments were performed at cell passage 9. The cytoplasmic and nuclear protein fractionation of HAEC was performed by utilizing a commercially available kit (Pierce, NE-PER nuclear and cytoplasmic reagent extraction kit (Cat. No: 78833).

### 2.3. Western blot analysis

Cytosolic and nuclear protein extracts were collected from HAEC and separated by nuclear and cytoplasmic extraction kit (Thermo Fisher, Cat. NO: 78833). Protein concentration was determined by the bicinchoninic acid (BCA) assay with BSA standards. Proteins were separated on sodium dodecyl sulfate (SDS)-polyacrylamide gels and transferred onto nitrocellulose membranes. Membranes were blocked with 5% bovine serum albumin (BSA) in Tris buffered saline containing 0.01% Tween 20 [TBST, 50 mM Tris (pH 7.5), 150 mM NaCl, and 0.1% Tween 20 (v/v)]. Membranes were incubated with primary antibodies overnight at 4 °C. Membranes were then washed extensively with TBST and incubated with the appropriate horseradish peroxidase-labeled secondary antibodies for 1 h at room temperature. Afterwards, membranes were incubated with enhanced chemiluminescence (ECL) substrate for horseradish peroxidase (Pierce/Thermo, Rockford, IL) and the ECL intensity was detected by X-ray film exposure in a dark room. The X-ray films were developed by the SRX-101A medical film processor. The expression levels of proteins as indicated by the ECL intensity were measured with ImageJ software (NIH, Bethesda, MD, USA).

### 2.4. RNA sequencing (RNA-Seq)

All the RNA-Sequencing experiments followed by library preparation were conducted by BGI (Shenzhen, China). Initially, HAECs were treated with the peptide at a concentration of 100 mg overnight and treated with LPC (40  $\mu$ M) for 6 h on the following day. Total RNAs were extracted from samples, and then mRNA was extracted with a commercially available kit (Qiagen). Using the fragmentation buffer, the mRNAs were fragmented into short fragments (about 200–500 nucleotides), then the first-strand cDNA was synthesized by random hexamer primer using the fragments as templates, and dTTP was substituted by dUTP during the synthesis of the second strand. Short fragments were purified and resolved with elution buffer for end repair and single nucleotide A (adenine) addition. After that, the short fragments were connected with adaptors, and then the second strand was degraded finally using uracil *N*-glycosylase (2). After agarose gel electrophoresis, the suitable fragments were selected for PCR amplification as templates. During the quality control steps, an Agilent 2100 Bioanalyzer and ABI StepOnePlus Real-Time PCR System (Thermo Fisher) were used for quantification and qualification of the sample library. At last, the library was sequenced with an Illumina HiSeq4000 using the PE100 strategy. Primary sequencing data produced by the Illumina HiSeq4000, called raw reads, were filtered into clean reads by removing adaptor containing and low-quality reads by BGI in-house software. A reference annotation-based assembly method was used to reconstruct the transcripts by TopHat (v2.0.10) and Cufflinks (v2.1.1), and background noise was reduced using fragments per kilobase million and coverage threshold.

### 2.5. Sequencing data analysis

Data analysis was carried out using the statistical computing environment, R, the Bioconductor suite of packages for R, and RStudio (<https://www.rstudio.com/>) [65]. Like our previously description, raw data were background subtracted, variance stabilized, and normalized by robust spline normalization. Differentially expressed genes were identified by linear modeling and Bayesian statistics using the Limma package. Pathway analysis was performed using Gene Set Enrichment Analysis (GSEA, <http://software.broadinstitute.org/gsea/index.jsp>) as we reported [66]. GSEA is a computational method that determines

whether a priori defined set of genes shows statistically significant, concordant differences between two biological states. GSEA does not focus on only significantly changed genes, but examines all the genes that belongs to a certain biological process instead.

### 2.6. Ingenuity pathway analysis

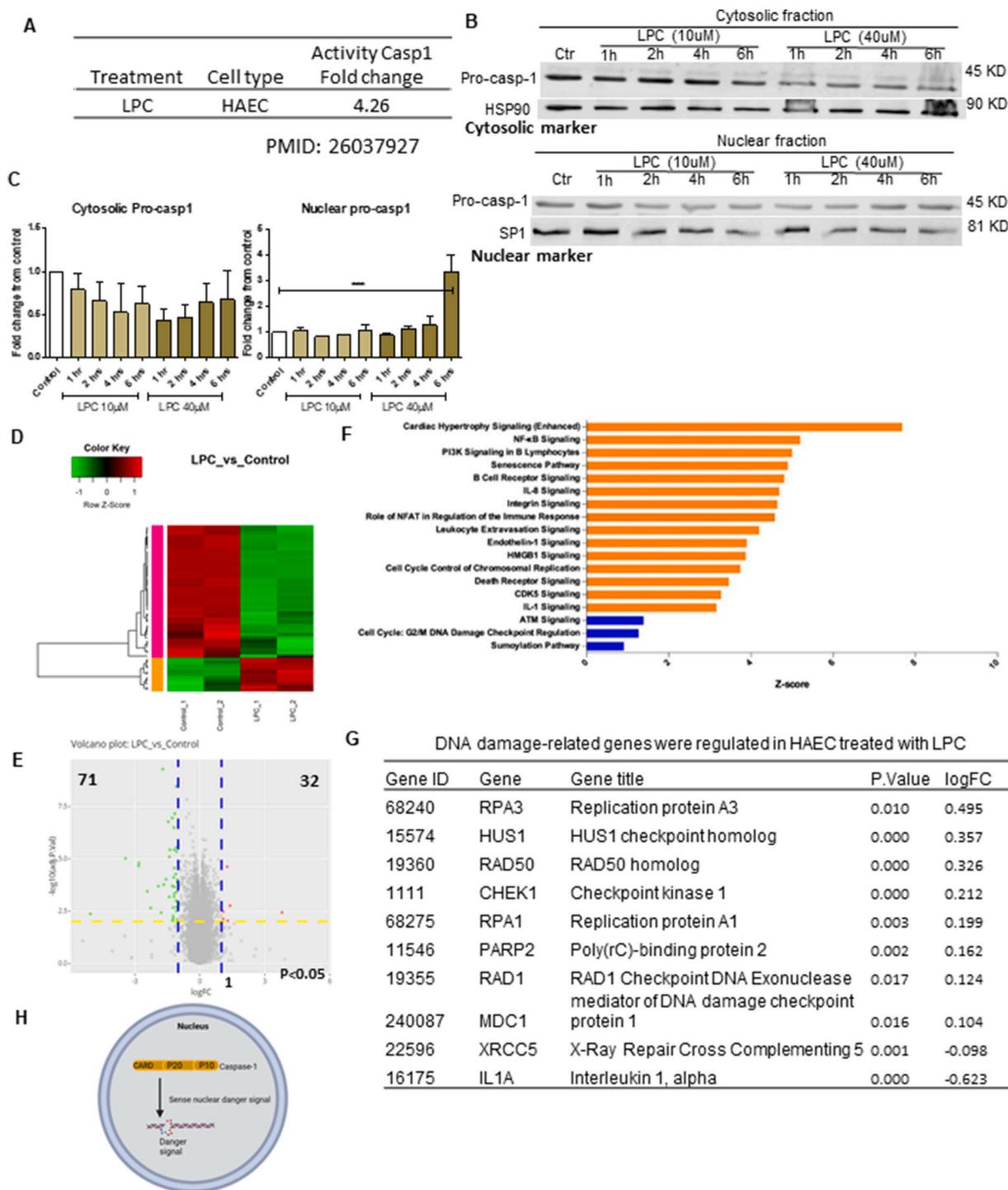
The Ingenuity Pathway Analysis (IPA, <https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-ipa/>) using genes with P value < 0.05 as expression value cutoff. Gene set enrichment analysis were performed by using Hallmark gene sets from Molecular Signatures Database (MSigDB) [67, 68].

## 3. Results

### 3.1. LPC increase nuclear localization of procaspase-1 in HAECs, which mediates transcriptomic changes and modulate inflammatory and immune response pathways

Previously, we reported that proatherogenic conditional DAMP lysophosphatidylcholine (LPC, lysoPC) promotes caspase-1 activation in human aortic endothelial cells (HAECs) [8,12] (Fig. 1A). Moreover, we identified 28 nuclear proteins and 7 nuclear interaction proteins that are potential caspase-1 substrates [29]. Therefore, we hypothesized that LPC may induce procaspase-1 translocation to the nucleus in HAECs. To examine this hypothesis, we treated HAEC with LPC (10  $\mu$ M and 40  $\mu$ M) followed by cell fractionation into cytosol and nuclear fractions. Interestingly, we indeed observed that pro-caspase-1 was present in the nuclear fraction of HAECs (Fig. 1B). Further, 40  $\mu$ M of LPC treatment increased presence of pro-caspase-1 in nuclear fraction after 4–6 h treatment in HAEC, while significant changes in pro-caspase-1 level in cytoplasmic fraction was not observed (Fig. 1B & C). To determine whether LPC mediated increase in nuclear translocation of pro-caspase-1 is associated with transcriptomic changes in HAECs, we performed RNA sequencing (RNA-Seq) on HAECs treated with 40  $\mu$ M for 6 h. As shown in Fig. 1D and E, LPC-treated HAECs upregulated 32 genes and downregulated 71 genes significantly (fold change, FC,  $\log_2FC \geq 1$  or  $\leq 1$ ). The Ingenuity Pathway Analysis (IPA) results on LPC modulated genes (both upregulated and downregulated) showed that LPC treated HAECs significantly upregulated top 15 inflammatory and immune response pathways including cardiac hypertrophy, nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) signaling, phosphoinositide 3-kinase (PI3K) signaling in B cells, senescence pathway, B cell receptor signaling, IL-8 signaling, integrin signaling, role of nuclear factor of activated T cells (NFAT) regulation in immune response, leukocyte extravasation signaling, endothelin-1 signaling, high-mobility group box 1 (HMGB1) signaling, cell cycle control of chromosomal replication, death receptor signaling, cyclin-dependent kinase 5 (CDK5) signaling, and IL-1 signaling and three down-regulated pathways such as ATM signaling (gene mutated in ataxia telangiectasia encodes a serine/threonine kinase recruited to sites of DNA double-strand breaks), cell cycle: G2/M DNA damage checkpoint regulation, and small ubiquitin-like modifier (SUMO) posttranslational modification (SUMOylation) pathway. In addition, as shown in Fig. 1G, eight DNA damage-related genes were upregulated and two genes were downregulated in LPC-treated HAECs in comparison to that of controls although the fold change numbers were low ( $\log_2FC > 1$ ,  $p < 0.05$ ). The results were well correlated with the IPA results of downregulated pathway: cell cycle: G2/M DNA damage checkpoint regulation.

Taken together, our results have demonstrated that pro-caspase-1 is localized in the nucleus of HAECs in the absence of LPC stimulation, suggesting that as a key danger sensing molecule, pro-caspase-1 patrols around in the cytosol and nucleus of human aortic endothelial cells even in homeostatic conditions. LPC treatment can increase pro-caspase-1 translocation to the nucleus and may potentiate transcriptomic



**Fig. 1.** LPC increase nuclear localization of procaspase-1 in HAECs, which mediates transcriptomic changes and modulate inflammatory and immune response pathways. **A.** Proatherogenic oxidized lipids increase active-caspase-1 in HAEC. **B.** HAECs are treated with LPC at concentrations of 10uM and 40uM, respectively, and at different time points including 1 h, 2 h, 4 h and 6 h. The expression of pro-caspase-1 is examined in cytosolic and nuclear fraction by Western blot, respectively. **C.** The cytosolic and nuclear pro-caspase-1 expression are quantified. \*\*\*P value less than 0.01. **D and E.** HAECs are treated with LPC at concentrations of 40uM for 6 h, and RNA are collected for RNA-seq analysis. Gene regulation is showed in heat map and volcano plot. **F.** The pathway regulation of LPC is analyzed by IPA. **G.** DNA damage-related genes are regulated by LPC treatment. **H.** Working model.

changes that modulate potent immune and inflammatory responses Fig. 1H. This conclusion is supported by the IPA analysis conducted on genes that were significantly modulated by LPC treatment in HAECs. According to the RNA-seq and IPA results, HMGB1 alarmin signaling [69] pathway was significantly upregulated with a list of genes that regulate DNA damage response while DNA double-strand breaks-sensing kinase ATM and G2/M DNA damage checkpoint signaling pathways

were downregulated. Of note, alarmins as a group of proteins can either exert beneficial cell housekeeping functions, leading to tissue repair, or provoke deleterious uncontrolled inflammation [69]. The results have demonstrated for the first time that aortic endothelial cells activated by LPC have a new functional nuclear program in addition to cytosolic and membrane programs such as increased secretion of cytokines and chemokines and upregulation of EC adhesion molecules as we reported [8,

42,70]; and that inflammatory cell death (pyroptosis) has a new functional nuclear program in addition to cytosolic and membrane programs such as secretion of IL-1 $\beta$ , IL-18, cleavage of cytosolic/nuclear protein/histone deacetylase sirtuin 1<sup>8</sup> and formation of N-terminal gasdermin D protein pore on the plasma membrane [71]. At the moment, it is not clear whether LPC mediated nuclear accumulation of pro-caspase-1 is due to a response to nuclear DAMP or cytosolic DAMP. These results of upregulating proinflammatory signaling are also well correlated with our previous report on LPC treatment of HAECs at 10  $\mu$ M for 18 h [72].

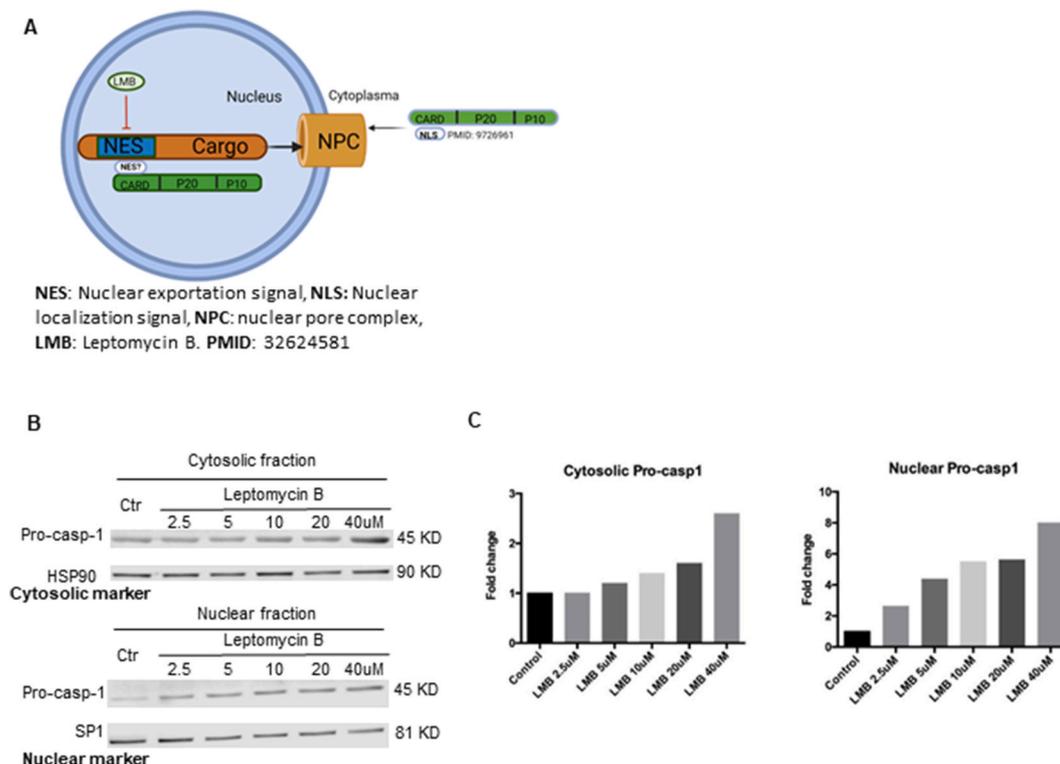
### 3.2. Nuclear pro-caspase-1 patrols back to the cytosol; and pro-caspase-1 nuclear exportation is mediated by exportin 1-nuclear pore complex (NPC)-mediated mechanism

Nuclear pore complex (NPC) facilitates transport of macromolecules such as proteins and ribonucleotide protein complexes to transport back and forth through the double membrane nucleus and the cytoplasm [73]. There are mobile receptors called karyopherins that bind with specific amino acid sequences present in proteins and determine the direction of the protein transport through NPC. A group of karyopherins termed importins recognize nuclear localization signals (NLS) in proteins and facilitate transport of such proteins from cytoplasm to nucleus. Procaspase-1 translocation to the nucleus is mediated by an NLS mapped to the amino acids 4 to 11 at the N-terminal caspase-1 recruitment domain (CARD) [33]. In addition, mutation of L265S (leucine changed to serine at amino acid 265) impairs nuclear localization of pro-caspase-1 [74], suggesting that the amino acid 265 at p20 of active caspase-1 also plays a role in localizing caspase-1 to the nucleus. However, the molecular mechanisms that are responsible for nuclear accumulation of pro-caspase-1 had not been identified yet; and the question of whether pro-caspase-1 has the ability to translocate from nucleus to the cytosol through NPC had not been explored so far [75,76] (Fig. 2A).

The karyopherins that facilitate transport of macromolecules from the nucleus to cytoplasm are called as exportins. Exportin 1 (also known as chromosome maintenance region 1 (CRM1)), is the major mammalian exportin and it binds to specific amino acid sequences called nuclear export signals (NES) to promote nucleocytoplasmic transport of a large variety of proteins and ribonucleotide protein (RNP) complexes [77]. Therefore, we hypothesized that the exportin 1 may mediate pro-caspase-1 export from the nucleus to cytosol. To test this hypothesis, we used specific exportin 1 inhibitor leptomycin B (LMB) [77] to block the nuclear export of pro-caspase-1 to the cytosol (Fig. 2B and C). LMB treatment of HAECs at concentrations of 5, 10, 20 and 40  $\mu$ M, respectively, for 24 h led to increased accumulation of nuclear pro-caspase-1. Of note, LMB treatment also increased cytosolic pro-caspase-1 in HAECs treated with 40  $\mu$ M LMB presumably via a feed-back mechanism of decreased nucleocytoplasmic transport. These results have demonstrated that nuclear pro-caspase-1 patrols back to the cytosol; and that pro-caspase-1 nuclear exportation is mediated by exportin 1-NPC mediated mechanism.

### 3.3. Increased nuclear localization of pro-caspase-1 in HAECs by pro-caspase-1 NES peptide inhibitor upregulates inflammatory genes in oxidative stress and Th17 activation signaling pathways

We then hypothesized that pro-caspase-1 has a nuclear exporting signal, which mediates pro-caspase-1 export from the nucleus to cytosol. To examine this hypothesis, we used an online nuclear exporting signal (NES) prediction database (ValidNES, <http://validness.ym.edu.tw/>). We identified a leucine-rich NES sequence motif "LAGTLGL" located at amino acids 83 to 89 in the N-terminal caspase recruitment domain (CARD) domain of pro-caspase-1. Of note, the NES of pro-caspase-1 was similar to the consensus sequence of the reported leucine-rich NES consensus sequence L<sub>AAA</sub>L<sub>AA</sub>L<sub>A</sub>L (L: leucine; A: any amino acid). We synthesized a peptide analogous to pro-caspase-1 NES expecting that

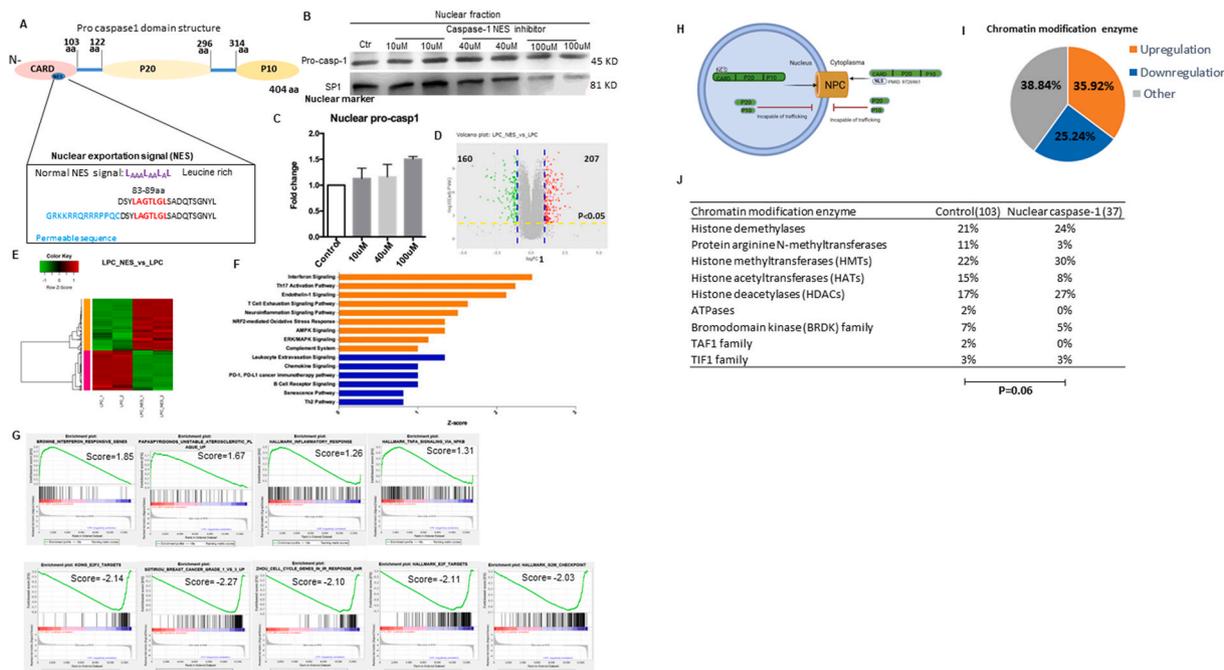


**Fig. 2. Nuclear pro-caspase-1 patrols back to the cytosol; and pro-caspase-1 nuclear exportation is mediated by exportin 1-nuclear pore complex (NPC)-mediated mechanism.** A. Nuclear exportation signal (NES) are required for nuclear membrane machinery; Leptomycin B can inhibit the exportin cargo formation. B. HAECs are treated with Leptomycin B by different doses, and pro-caspase-1 in cytosol and nucleus are examined by Western blot. C. The quantification of cytosolic and nuclear pro-caspase-1 expression in C.

pro-caspase-1 NES has to compete for exportin in the presence of custom made NES peptide. Herein, we will refer this custom made peptide as NES inhibitor. As shown in Fig. 3A, a 14 amino acid cell permeable sequence (Tat protein sequence (NCBI protein database ID: QGT15341.1) of human immunodeficiency virus, HIV) [78,79] was introduced to a portion (20 amino acid long) of N-terminus of pro-caspase-1 containing the potential NES sequence. We have previously used this design strategy to synthesize a peptide that can inhibit the cleavage of Sirtuin 1 by active caspase-1 [8]. As shown in Fig. 3B–C, treatment HAECs with NES inhibitor for 6 h resulted in increased accumulation of pro-caspase-1 in the nucleus (reaching 1.5 folds). This data suggested that our new NES inhibitor can inhibit pro-caspase-1 export from nucleus to cytosol.

To determine the roles of accumulated nuclear procaspase-1, we performed RNA-Seq again. As shown in Fig. 3D–E, in comparison to that of LPC treated HAECs, LPC + pro-caspase-1 NES peptide inhibitor upregulated 207 genes and downregulated 160 genes significantly. The transcriptomic modulation of LPC + pro-caspase-1 NES peptide inhibitor led to upregulated nine pathways including interferon signaling, T helper cell 17 (Th17) activation, endothelin-1 signaling, T cell exhaustion signaling, neuroinflammation signaling, NRF2-mediated oxidative stress response, AMP-activated protein kinase (AMPK) signaling, extracellular signal regulated kinase (ERK)/mitogen activated protein kinase (MAPK) signaling and complement system, and downregulated three pathways leukocyte extravasation signaling, chemokine signaling and programmed death-1 (PD-1), programmed death ligand-1 (PD-L1) cancer immunotherapy pathway Fig. 3F. In addition, we performed the gene set enrichment analysis (GSEA) ([https://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Using\\_RNA-seq\\_Datasets\\_with\\_GSEA](https://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Using_RNA-seq_Datasets_with_GSEA)). The GSEA results indicated that the transcriptomic modulation

of LPC + pro-caspase-1 NES peptide inhibitor led to significantly upregulated four pathways (scores >1.25) including unstable atherosclerosis, inflammatory response, TNF- $\alpha$  signaling, and interferon signaling Fig. 3G. The GSEA results were well correlated with the IPA pathway results. Taken together, the results have demonstrated that first, pro-caspase-1 is capable for patrolling from nucleus back to cytosol via its N-terminal CARD domain. A previous report showed that the N-terminal CARD domain of pro-caspase-1 is also responsible for its nuclear localization [33]. Therefore, pro-caspase-1 but not activated caspase-1 patrols around the cytosol and nucleus mediated by its N-terminal CARD domain (Fig. 3H). Additionally, we hypothesized that increased nuclear procaspase-1 may modulate gene expression via regulating the expression of chromatin modification enzymes. To examine this hypothesis, we listed 103 chromatin modification enzymes [80] and 14 pioneering transcription factors [81] and measured the expression changes of these genes in our datasets. As shown in Fig. 3I, we found that expression of 61.16% chromatin modification enzyme genes were modulated (35.92% upregulated) in HAEC treated with LPC in combination with pro-caspase-1 NES peptide inhibitor. The percentages of nine functional groups of total 103 chromatin modification enzymes and LPC mediated increased procaspase-1 upregulated 37 chromatin modification enzymes were shown in Fig. 3J. The increased nuclear procaspase-1 may disproportionately increase the gene expression of histone deacetylases ( $p = 0.06$ ). Since LPC promote inflammatory cell death (pyroptosis) [8,12], our finding here indicate that pro-caspase-1 increases its nuclear localization during pyroptosis. Of note, our results were well correlated with the previous reports that prodomain of pro-caspase-2 mediates its nuclear localization [82]; and that caspase-2, -3, -8, and -9 accumulate in the nucleus during apoptosis [83]; and second, nuclear pro-caspase-1 accumulated in the nucleus of HAECs by pro-caspase-1 NES peptide



**Fig. 3.** Increased nuclear localization of pro-caspase-1 in HAECs by pro-caspase-1 NES peptide inhibitor upregulates inflammatory genes in oxidative stress and Th17 activation signaling pathways. **A.** Pro-caspase-1 has 404 amino acids including CARD domain, P20 and P10; Competitive caspase1 nuclear exportation signal inhibitor are designed, including a cell permeable sequence. NES prediction source: ValidNES, <http://validness.yu.edu.tw/> (PMID: 26186223) **B and C.** HAECs are treated with pro-caspase-1 specific- Nuclear Exporting Signal (NES) peptide at different concentrations, and nuclear pro-caspase-1 are examined by Western blot. NES significantly keeps pro-caspase-1 in nucleus. \*P. value less than 0.05. **D and E.** HAECs are treated with LPC (40uM) with or without NES (100uM), and RNA are collected for RNA-seq analysis. Gene regulation is showed in heat map and volcano plot. **F.** The pathway regulation of LPC\_NES vs LPC is analyzed by IPA. **G.** RNA-seq data is analyzed in Gene Enrichment Analysis (GSEA). **H.** Pro-caspase-1 can traffic between nucleus and cytosol, active caspase1 (p20 and p10) cannot. **I.** 103 chromatin modification enzyme list are generated from <https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=865>. 61.16% chromatin modification enzyme are modulated by nuclear caspase-1. **J.** The functional distribution of 9 groups totally 103 chromatin modification enzymes and upregulated 37 chromatin modification enzymes in LPC NES vs LPC.

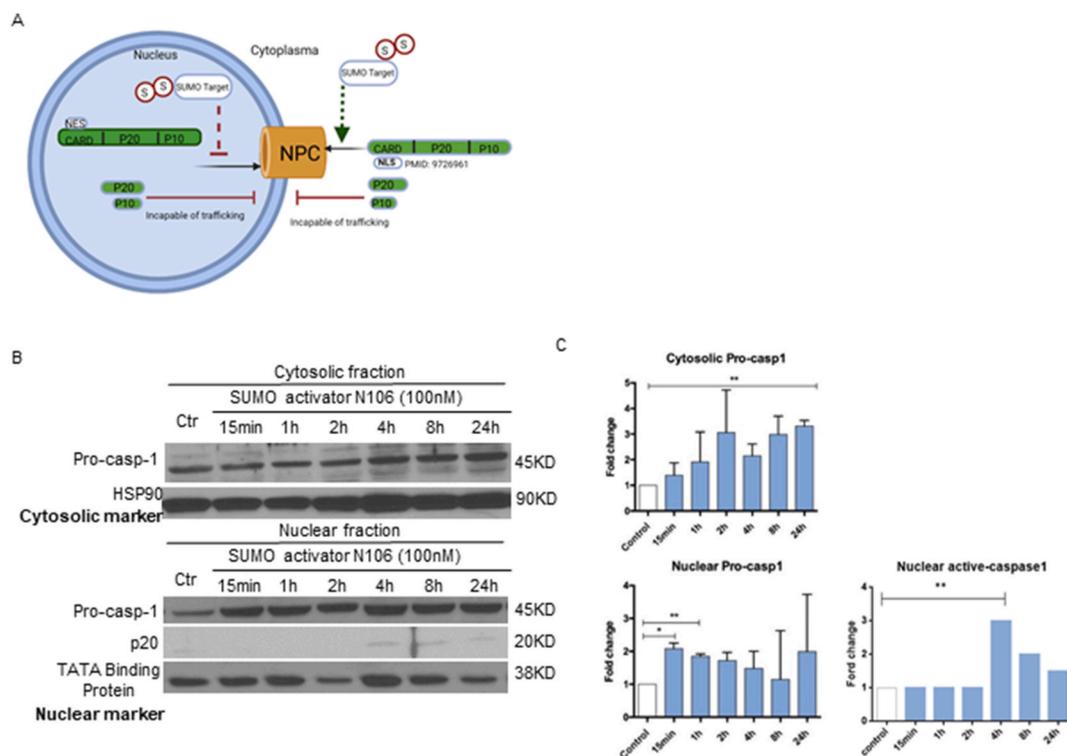
inhibitor increase inflammation, oxidative stress and Th17 activation signaling pathways in comparison to that of LPC treatment alone controls.

**3.4. LPC + SUMO activator treatment of HAECs increase procaspase-1 nuclear translocation; increased nuclear localization of pro-caspase-1 is associated with increased caspase-1 activation in the nucleus; and LPC + caspase-1 inhibitor increases inflammatory gene upregulation with role of hypercytokinemia/hyperchemokinema and interferon pathways**

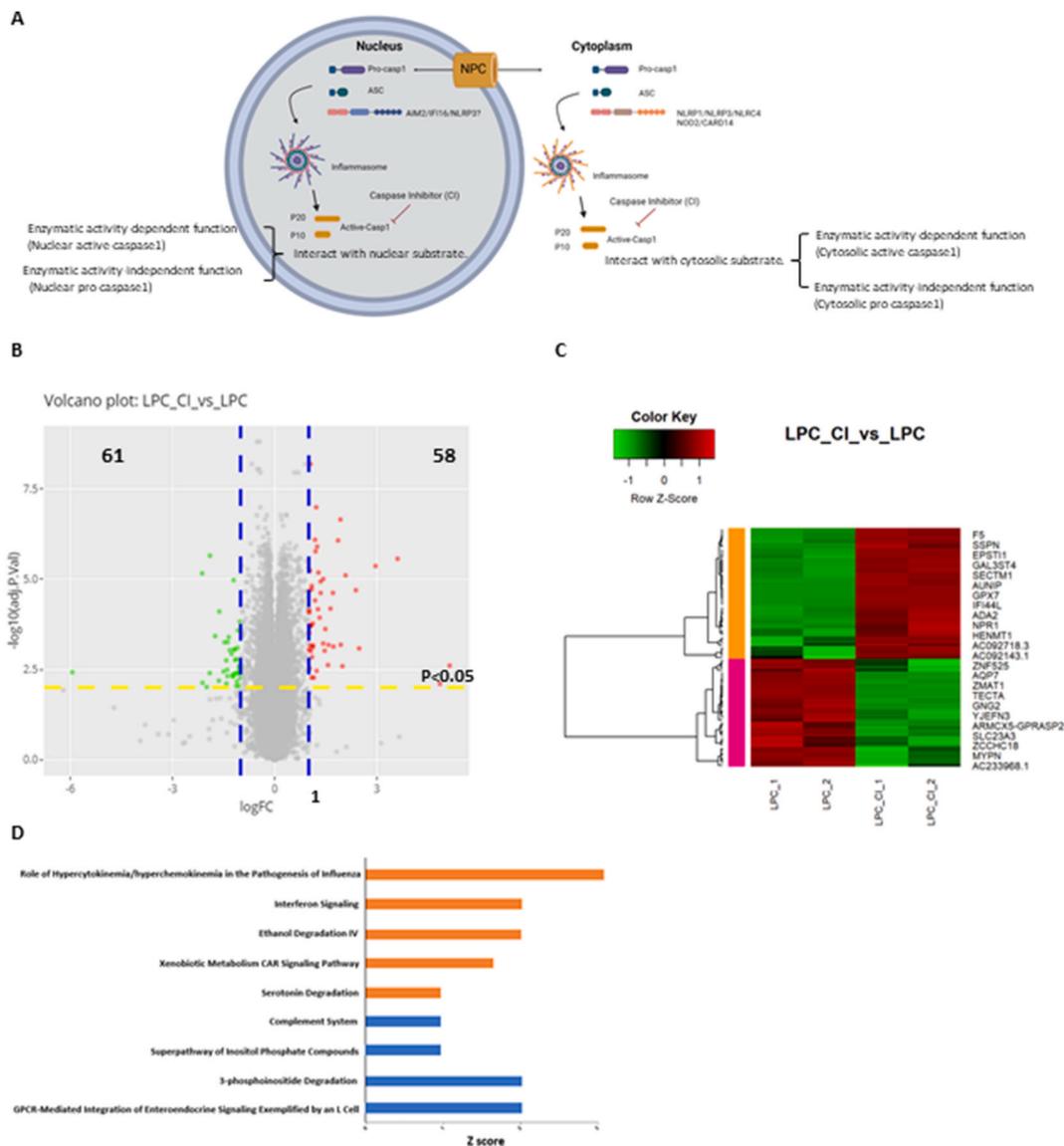
Previous report showed that: caspase-8 SUMOylation (small ubiquitin-like modifier proteins covalently attached) at lysine 156 is associated with its nuclear localization but not affects its function [84]; and SUMO-1 modification of the CARD domain of procaspase-2 is essential for its nuclear localization [85,86]. We reported that caspase-1 have transcriptomic modulation functions independent of caspase-1 substrates such as IL-1 $\beta$ , IL-18 and sirtuin-1 [28]. The important questions remained: i) whether promotion of SUMOylation on other proteins increases nuclear localization of procaspase-1; ii) whether pro-caspase-1 is activated in the nucleus; and iii) whether pro-caspase-1 have non-enzymatic functions in promoting inflammation in HAECs after caspase-1 enzymatic function gets inhibited. To examine these issues, we performed cell fractionation after LPC activation in the presence of absence of SUMO activator N-106 (100 nM) for 15 min, 1 h (h), 2h, 4h, 8h, and 24h, respectively, and Western blots with anti-caspase-1 antibody. As shown in Fig. 4B–C, procaspase-1 was increased in the cytosol fraction potentially via a feedback mechanism, which were well correlated with that LMB treatment of LPC-treated HAECs (Fig. 2C). In addition, SUMO activator N106 also increased LPC-induced procaspase-1 nuclear translocation. However, we did not find that the molecular weight of procaspase-1 became larger in the presence of SUMO activator N106 than that in the absence of SUMO activator N106, suggesting that SUMO activator N106 promotes procaspase-1 nuclear

localization via potentially SUMOylated indirect nuclear protein export inhibitors and/or potentially SUMOylated cytosolic protein nuclear localization facilitators (Fig. 4A). Moreover, activated caspase-1 p20 was also increased although in low levels, suggesting that at least increased nuclear pro-caspase-1 is partially activated. Of note, previous reports showed that 1) inflammasome component NLRP3 [10] can be a transcription factor (nuclear localization) for Th2 cell differentiation [30]; 2) inflammasome component apoptotic speck-like protein containing a caspase recruitment domain (ASC) is localized in both nucleus and cytosol [87]; 3) pro-caspase-1 is activated in response to inflammatory cytokine TNF- $\alpha$  stimulation in Hela cells [33]; 4) caspase-1 is activated in the nucleus in response to Herpes simplex virus-1 infection in human foreskin fibroblasts by interacting with nuclear DNA-sensing inflammasome interferon- $\gamma$  inducible protein 16 (IFI16) [34,35]; and 5) caspase-1 nuclear localization can be blocked by interleukin-1 receptor (IL-1R) antagonist [36]; pro-caspase-1 can be activated in both cytosol inflammasomes and nuclear inflammasomes and activated caspase-1 does not need to travel between cytosol and nucleus.

Previous reports showed that 1) protease-dead mutant of pro-caspase-1 can still be translocated to the nucleus [31], suggesting that pro-caspase-1 may travel to nucleus for its enzymatic activities-independent functions such as promotion of gene transcription as we reported [28]; and 2) enzymatically inactive pro-caspase-1 stabilizes the apoptosis-associated speck-like protein containing a CARD (ASC, PYCARD) pyroptosome (a supramolecular assembly of ASC dimers mediating inflammatory cell death via caspase-1 activation [88]) and supports pyroptosome spreading during cell division [89]. We then hypothesized that LPC modulate RNA transcriptomes in HAECs in the presence and absence of caspase-1 inhibitor (Fig. 5A). As shown in Fig. 5B–C, LPC stimulation with caspase-1 inhibitor upregulated 58 genes significantly ( $\log_2FC > 1$ ,  $p < 0.05$ ) and downregulated 61 genes. To our surprise, the IPA results showed that LPC plus caspase-1 inhibitor upregulated five pathways such as role of



**Fig. 4.** LPC + SUMO activator treatment of HAECs increase procaspase-1 nuclear translocation; increased nuclear localization of pro-caspase-1 is associated with increased caspase-1 activation in the nucleus. **A.** SOMO activator can increase procaspase1 nuclear localization and promote nuclear caspase1 activation. **B.** HAECs are treated with sumoylation activator-N106 at different time points. The expression of pro-caspase-1 is examined in cytosolic and nuclear fraction by Western blot. **C.** Quantification of Fig. 4B. \* $P < 0.05$ .

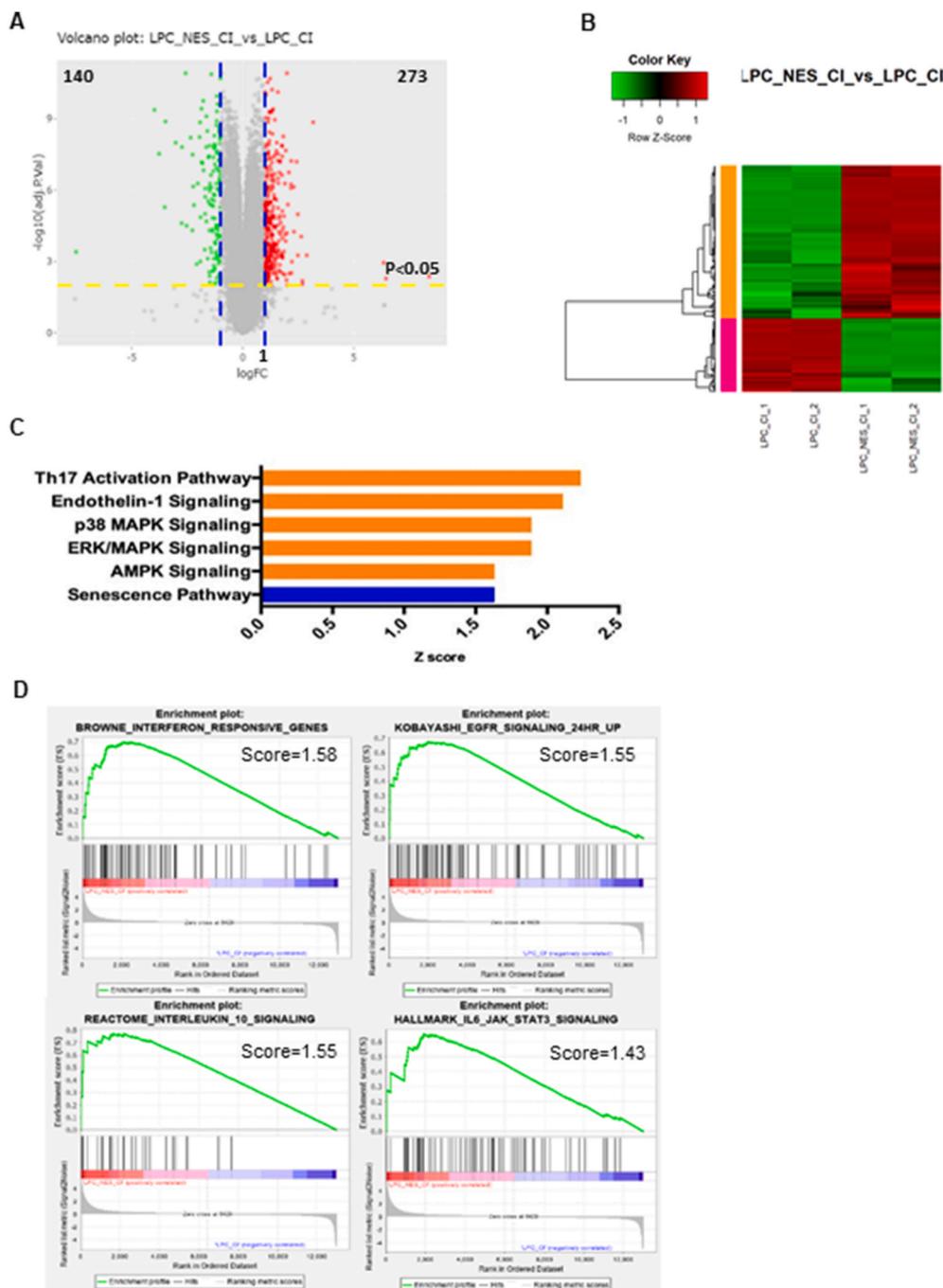


**Fig. 5.** LPC + caspase-1 inhibitor increase inflammatory gene upregulation with role of hypercytokinemia/hyperchemokinaemia and interferon pathways. **A.** Caspase1 inhibitor (CI) can inhibit caspase1 enzymatic activity in cytoplasm and nucleus. **B and C.** HAECs are treated with LPC with or without caspase1 inhibitor (CI) and RNA are collected for RNA-seq analysis. Gene regulation is showed in heat map (C) and volcano plot (B). **D.** The pathway regulation of LPC\_CI\_vs\_LPC is analyzed by IPA.

hypercytokinemia/hyperchemokinaemia, interferon signaling, ethanol degradation IV, xenobiotic metabolism CAR signaling and serotonin degradation and downregulated four pathways including complement system, superpathway of inositol phosphate compounds, 3-phosphoinositide degradation, and GPCR-mediated integration of enteroendocrine signaling (Fig. 5D). Taken together, our results have demonstrated that i) LPC plus SUMO activator treatment of HAECs increase procaspase-1 nuclear translocation potentially via indirect mechanisms (Fig. 4A); ii) increased pro-caspase-1 into the nucleus of HAECs is associated with increased caspase-1 activation with identification of active caspase-1 p20 subunit. The results have demonstrated for the first time that i) nuclear caspase-1 can be activated during pyroptosis in human aortic endothelial cell by conditional DAMP LPC; ii) activated caspase-1 may have enzymatic functions in cleaving nuclear substrates as we reported [29]; and iii) LPC plus caspase-1 inhibitor increase inflammatory pathways such as role of hypercytokinemia/hyperchemokinaemia and interferon signaling, suggesting that caspase-1 enzyme-independent functions in the nucleus also promote inflammation potentially via ASC pyroptosome [88,89].

### 3.5. Increased nuclear localization of procaspase-1 in the presence of caspase-1 inhibitor significantly upregulates inflammatory gene expression in Th17 activation, endothelin-1 signaling, p38-, and ERK- MAPK pathways

To examine whether inhibition of nuclear pro-caspase-1 export into cytosol in the presence of caspase-1 inhibitor upregulates inflammatory genes and pathways, we compared RNA-Seq data in the group of LPC + NES peptide inhibitor + caspase-1 inhibitor treated HAECs with LPC + caspase-1 inhibitor treated HAECs. As shown in Fig. 6A–B, inhibition of nuclear procaspase-1 export into cytosol in the presence of caspase-1 inhibitor upregulated 273 genes and downregulated 140 genes. The IPA results in Fig. 6C showed that inhibition of nuclear procaspase-1 export into cytosol in the presence of caspase-1 inhibitor upregulated five pathways including Th17 activation pathway, endothelin-1 signaling, p38 MAPK signaling, ERK/MAPK signaling and AMPK signaling and downregulated senescence pathway. In addition, as shown in Fig. 6D, the GSEA results showed similar upregulation of interferon response genes, EGFR signaling, IL-10 signaling (an inflammation-



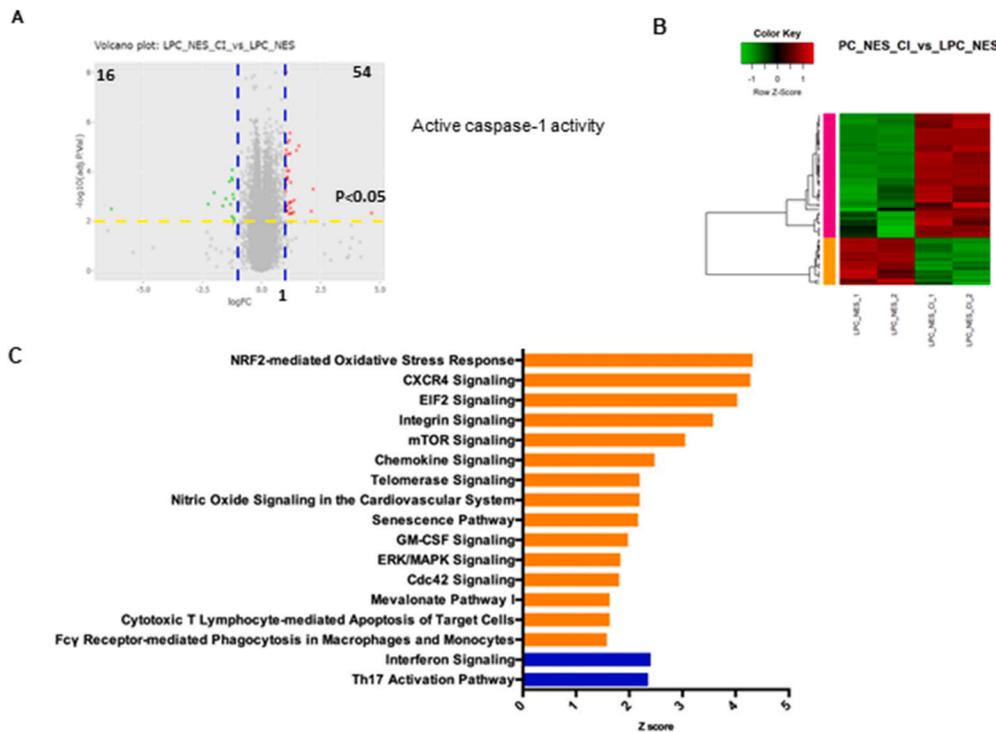
**Fig. 6.** Increased nuclear localization of procaspase-1 in the presence of caspase-1 inhibitor significantly upregulates inflammatory gene expression in Th17 activation, endothelin-1 signaling, p38-, and ERK- MAPK pathways. A and B. HAECs are treated with LPC (40uM) and caspase1 inhibitor with or without NES (100uM), and RNA are collected for RNA-seq analysis. Gene regulation is showed in volcano plot (A) and) heat map (B). C. The pathway regulation of LPC\_NES\_CI vs LPC CI is analyzed by IPA indicate the function of nuclear pro-caspase1. D. RNA-seq data is analyzed in Gene Enrichment Analysis (GSEA).

induced cytokine as we reported for IL-35 [63,90–94]) and IL-6-JAK-STAT3 signaling. Taken together, these results have demonstrated that increased nuclear localization of pro-caspase-1 by inhibition of nuclear procaspase-1 export into cytosol in the presence of caspase-1 inhibitor significantly increase inflammatory pathways including Th17 activation, endotheli-1 signaling, p38 MAPK signaling, and ERK/MAPK signaling, suggesting that nuclear pro-caspase-1 and activated caspase-1 in the nucleus have caspase-1 enzymatic activity-independent functions of promoting inflammation.

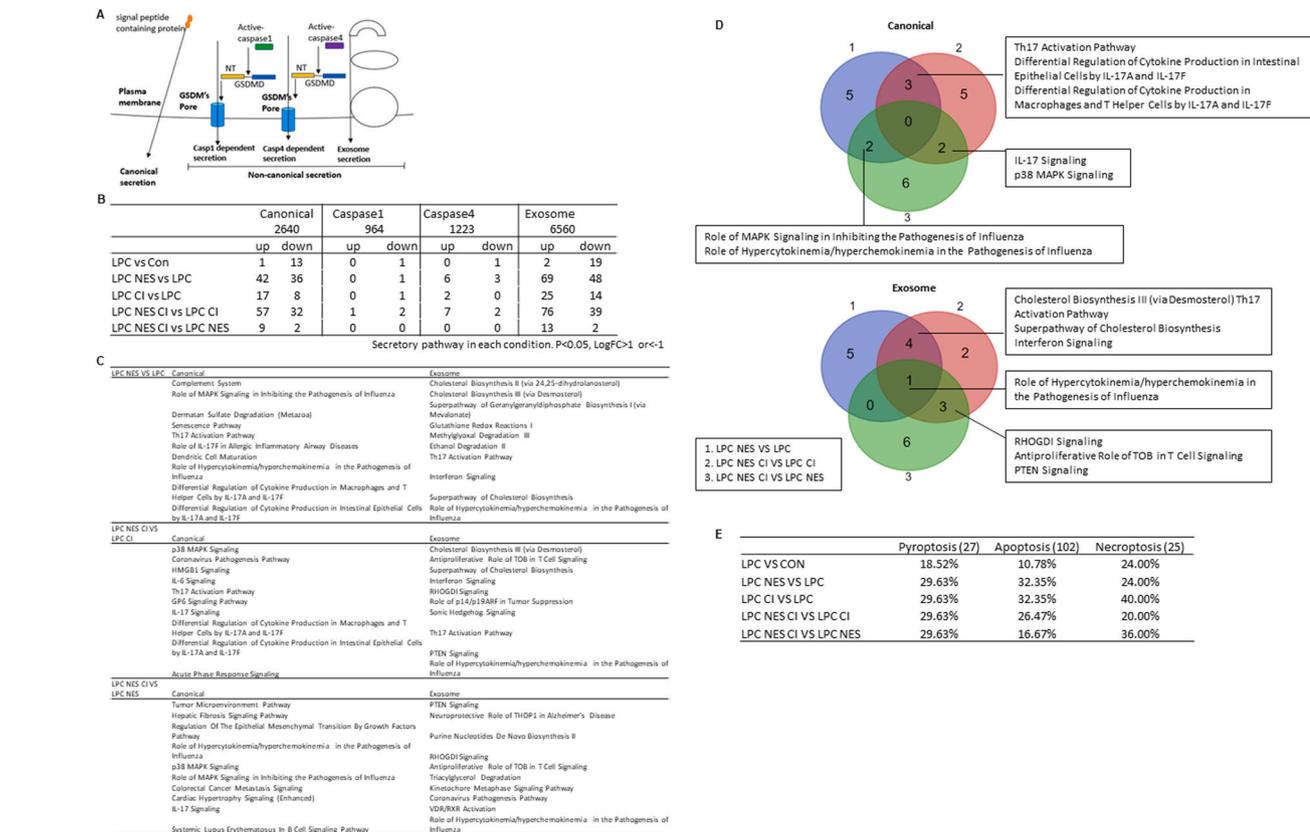
**3.6. Fifteen functions of nuclear caspase-1 such as NRF2 oxidative stress response, GM-CSF and chemokine signaling are caspase-1 enzymatic activities-independent**

To examine whether inhibition of nuclear caspase-1 enzymatic

activities in the presence of procaspase-1 NES peptide inhibitor upregulates inflammatory genes and pathways, we compared RNA-Seq data in the group of LPC + NES peptide inhibitor + caspase-1 inhibitor treated HAECs with LPC + NES peptide inhibitor treated HAECs. As shown in Fig. 7A–B, inhibition of nuclear caspase-1 enzymatic activities in the presence of procaspase-1 NES peptide inhibitor upregulated 42 genes and downregulated 16 genes. The IPA results in Fig. 7C showed that caspase-1 enzymatic activities-independent functions lead to upregulation of 15 pathways including nuclear factor erythroid-derived 2-like 2 (NRF2)-mediated oxidative stress response, C-X-C motif chemokine receptor 4 (CXCR4) signaling, eukaryotic Initiation Factor 2 (EIF2) signaling, integrin signaling, mammalian target of rapamycin (mTOR) signaling, chemokine signaling, telomerase signaling, nitric oxide signaling in cardiovascular system, senescence pathway, granulocyte-macrophage colony stimulation factor (GM-CSF) signaling,



**Fig. 7.** Fifteen functions of nuclear caspase-1 such as NRF2 oxidative stress response, GM-CSF and chemokine signaling are caspase-1 enzymatic activities-independent. A and B. HAECs are treated with LPC (40uM) and NES (100uM) with or without caspase1 inhibitor, and RNA are collected for RNA-seq analysis. Gene regulation is shown in heat map and volcano plot. C. The pathway regulation of LPC\_NES\_CI vs LPC\_NES is analyzed by IPA indicate the function of nuclear active-caspase1.



**Fig. 8.** Up to 76 canonical and exosome secretomic genes are upregulated, respectively, which upregulate four pathways of Th17, IL-17 promoted cytokines, interferon signaling and cholesterol biosynthesis in the presence of nuclear export inhibitor of procaspase-1. A. Four types of secretomes with total 11,387 proteins including canonical secretome (<https://www.proteinatlas.org/>), caspase-1-GSDMD dependent non-canonical secretome (PMID 18329368), caspase-4-GSDMD dependent non-canonical secretome (PMID28196878), exosome secretome are analyzed (<http://exocarta.org/download>). B. Upregulated gene number in each experiment.  $P < 0.05$ ,  $\text{LogFC} > 1$  or  $< -1$ . C. IPA analysis of modulated gene in B. D. Venn diagram of upregulated pathway in each group of C. E. We generated a gene list of inflammatory cell death, including pyroptosis (27 genes), apoptosis (102 genes) and necroptosis (25 genes) (PMIDs:33242752, 31355136). The percentage of upregulated gene number in each condition.

ERK/MAPK signaling, Cell Division Cycle 42 (CDC42) signaling, mevalonate pathway I, cytotoxic T cell-mediated apoptosis of target cells, antibody Fc fragment Fc $\gamma$  receptor-mediated phagocytosis in macrophages and monocytes, and downregulation of two pathways such as interferon signaling and Th17 activation pathway. Of note, NRF2 is required for NLRP3 and Interferon-inducible protein AIM2 (absent in melanoma 2) inflammasome activation [95]. These results have demonstrated that many functions of nuclear caspase-1 such as NRF2 oxidative stress response, GM-CSF and chemokine signaling are caspase-1 enzymatic activities-independent, and presumably mediated via caspase-1 nuclear interaction proteins as we reported previously [29].

### 3.7. Up to 76 canonical and exosome secretomic genes are upregulated, respectively, which upregulate four pathways of Th17, IL-17 promoted cytokines, interferon signaling and cholesterol biosynthesis in the presence of nuclear export inhibitor of procaspase-1

As we and others reported, increased secretion of cytokines and chemokines are one hallmark of aortic endothelial cell activation [3,8,9,42,51,55,66,70,92,94]. To determine whether LPC induced nuclear localization of procaspase-1 leads to upregulation of cytokines and chemokines, we collected several sets of large secretome genes such as canonical secretome (secretory proteins with signal peptide) [18], caspase-1-GSDMD (GSDMD) secretome, caspase-4-GSDMD secretome, and exosome secretomes [20] as we reported [18,22,23]. We hypothesized that the expressions of aortic vascular cell canonical secretome, three types of non-canonical secretomes such as caspase-1-GSDMD, caspase-4-GSDMD, and exosomes are modulated in LPC-induced nuclear localization of pro-caspase-1 in HAECs (Fig. 8A). To examine this hypothesis, as shown in Fig. 8A–B, we collected four types of secretomes with total 11,387 proteins including canonical secretome (signal peptide-mediated exocytic secretory pathway, 2640 proteins) [96], caspase-1-GSDMD dependent non-canonical secretome (non-signal peptide-mediated; 964 proteins) [97], caspase-4-GSDMD dependent non-canonical secretome (1223 proteins) [98], exosome secretome (6560 proteins, downloaded from a comprehensive exosome database <http://exocarta.org/download>) [20]. Of note, most updated human genome-encoded protein-coding genes are 21,306 [99], suggesting that 53.4% of human proteins (11,387 secretory protein genes in the four secretomes out of total 21,306 human protein-encoding genes) examined here potentially carry out secretory functions physiologically and/or pathophysiologically in HAECs. As shown in Fig. 8B, increased pro-caspase-1 nuclear localization in LPC treated HAECs in comparison to that of LPC treated HAECs upregulated 42 canonical secretomic genes, zero caspase-1 secretomic gene, six caspase-4 secretomic genes, and 69 exosome secretomic genes, respectively. In addition, increased pro-caspase-1 nuclear localization and inhibition of caspase-1 enzymatic activities in LPC treated HAECs in comparison to that of caspase-1 enzymatic activities-inhibited LPC treated HAECs upregulated 57 canonical secretomic genes, one caspase-1-GSDMD secretomic gene, seven caspase-4-GSDMD secretomic genes, and 76 exosome secretomic genes, respectively.

We then performed the IPA analysis for the functions of modulated secretomic genes in Fig. 8C. Since the numbers of modulated secretomic genes were too small, three comparison groups were examined with the IPA: including 1) LPC + procaspase-1 NES peptide inhibitor -treated HAECs versus (vs) LPC-treated HAECs; 2) LPC + procaspase-1 NES peptide inhibitor + caspase-1 enzymatic inhibitor -treated HAECs versus (vs) LPC + caspase-1 enzymatic inhibitor-treated HAECs versus LPC + caspase-1 enzymatic inhibitor-treated HAECs; and 3) LPC + procaspase-1 NES peptide inhibitor + caspase-1 enzymatic inhibitor -treated HAECs versus (vs) LPC + procaspase-1 nuclear export inhibitor-treated HAECs. Since the numbers of modulated secretomic genes in caspase-1-GSDMD secretome and caspase-4-GSDMD secretome were too small, canonical secretomic genes and exosome secretomic genes were examined with

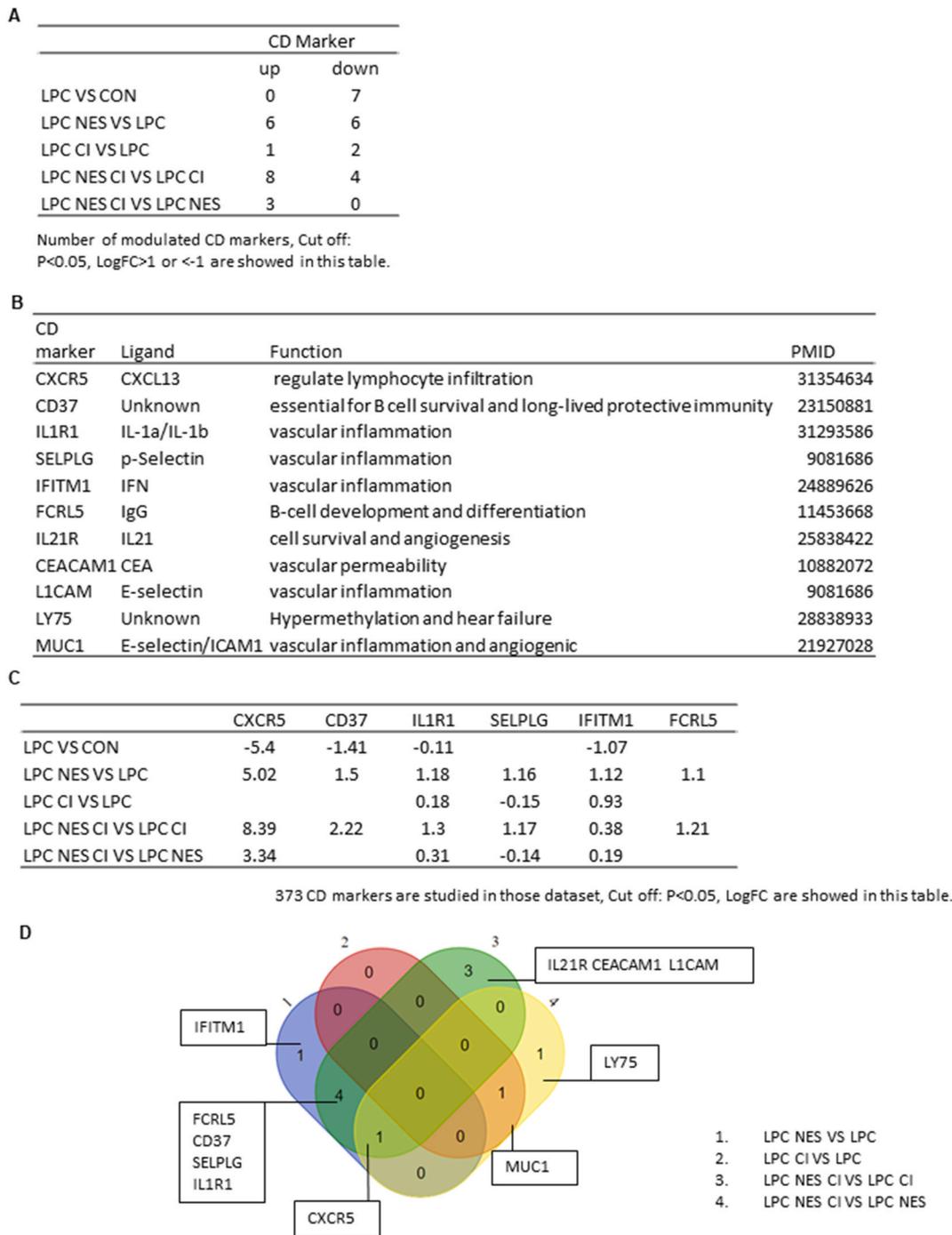
the IPA. The canonical secretomic genes in LPC + NES inhibitor -treated HAECs in comparison to LPC-treated HAEC controls upregulated ten functions including complement system, role of MAPK signaling in inhibiting pathogenesis of influenza, dermatan sulfate degradation, senescence pathway, Th17 activation, role of IL-17F in allergic inflammatory airway disease, dendritic cell maturation, role of hypercytokinemia/hyperchemokinememia in pathogenesis of influenza, differential regulation of cytokine production in macrophages and T helper cells by IL-17A and IL-17F, and differential regulation of cytokine production in intestinal epithelial cells by IL-17A and IL-17F. The exosome secretomic genes in the LPC + NES inhibitor -treated HAECs in comparison to LPC-treated HAEC controls upregulated ten functions including cholesterol biosynthesis II, cholesterol biosynthesis III, superpathway of geranylgeranyldiphosphate biosynthesis I, glutathione redox reaction I, methylglyoxal degradation III, ethanol degradation II, Th17 activation, interferon signaling, superpathway of cholesterol biosynthesis, and role of hypercytokinemia/hyperchemokinememia in pathogenesis of influenza. In addition, the canonical secretomic genes in LPC + NES inhibitor + caspase-1 enzymatic inhibitor -treated HAECs in comparison to LPC + caspase-1 enzymatic inhibitor-treated HAEC controls upregulated ten functions including p38 MAPK signaling, coronavirus pathogenesis pathway, HMGB1 signaling, IL-6 signaling, TH17 activation, GP6 signaling, IL-17 signaling, differential regulation of cytokine production in macrophages and T helper cells by IL-17A and IL-17F, differential regulation of cytokine production in intestinal epithelial cells by IL-17A and IL-17F, and acute phase response signaling. The exosome secretomic genes LPC + NES inhibitor + caspase-1 enzymatic inhibitor -treated HAECs in comparison to LPC + caspase-1 enzymatic inhibitor-treated HAEC controls upregulated cholesterol biosynthesis III, antiproliferative role of transducer of ERBB2-1 (TOB) in T cell signaling, superpathway of cholesterol biosynthesis, interferon signaling, Rho GDP-Dissociation Inhibitors (RHOGDI) signaling, role of p14/p19ARF (p14ARF in human and p19ARF in mouse) in tumor suppression, sonic hedgehog signaling, Th17 activation, Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) signaling and role of hypercytokinemia/hyperchemokinememia in pathogenesis of influenza. Moreover, the canonical secretomic genes in LPC + NES inhibitor + caspase-1 enzymatic inhibitor -treated HAECs in comparison to LPC + NES inhibitor-treated HAEC controls upregulated ten functions including tumor microenvironment pathway, hepatic fibrosis signaling, regulation of epithelial mesenchymal transition by growth factors, role of hypercytokinemia/hyperchemokinememia in pathogenesis of influenza, p38 MAPK signaling, role of MAPK signaling in inhibiting pathogenesis of influenza, colorectal cancer metastasis signaling, cardiac hypertrophy signaling, IL-17 signaling and systemic lupus erythematosus in B cell signaling. The exosome secretomic genes in LPC + NES inhibitor + caspase-1 enzymatic inhibitor -treated HAECs in comparison to LPC + NES inhibitor-treated HAEC controls upregulated ten functions including PTEN signaling, neuroprotective role of Thimet oligopeptidase (THOP1) in Alzheimer's disease, purine nucleotide de novo biosynthesis II, RHOGDI signaling, antiproliferative role of TOB in T cell signaling, triacylglycerol degradation, kinetochore metaphase signaling, coronavirus pathogenesis pathway, vitamin D receptor/retinoic acid receptor (VDR/RXR) activation, and role of hypercytokinemia/hyperchemokinememia in pathogenesis of influenza.

To further dissect the roles of increased procaspase-1 nuclear localization in LPC-treated HAECs with or without caspase-1 inhibitor, we performed Venn Diagram. As shown in Fig. 8D, the roles of procaspase-1 nuclear export inhibitor in LPC-treated HAECs with or without caspase-1 inhibitor (groups 1 and 2) lead to upregulation of canonical secretomic genes with three functions such as Th17 activation, differential regulation of cytokine production in intestinal epithelial cells by IL-17A and IL-17F, and differential regulation of cytokine production in macrophages and T helper cells by IL-17A and IL-17F. In addition, the roles of procaspase-1 nuclear export inhibitor in LPC-treated HAECs with or without caspase-1 inhibitor (groups 1 and 2) lead to upregulation of

exosome secretomic genes with four functions including cholesterol biosynthesis III, Th17 activation, superpathway of cholesterol biosynthesis, and interferon signaling. Moreover, the roles of caspase-1 enzymatic inhibitor in LPC-treated HAECs with or without procaspase-1 nuclear export inhibitor lead to upregulation of six specific canonical secretomic pathways and six specific exosome secretomic pathways, respectively. Since pro-caspase-1 is essential for a newly reported cell death type termed PANoptosis, which includes three previously classified cell death forms such as pyroptosis, apoptosis and necroptosis, we generated a gene list of inflammatory cell death, including pyroptosis (27 genes), apoptosis (102 genes) and necroptosis (25 genes) [100,101].

We found that the expression of pyroptosis genes were increased from 18.52% to 29.63%; and the expression of apoptosis genes were increased from 10.78% to 32.35% in LPC + caspase-1 NES inhibitor group as shown in Fig. 8E.

Taken together, these results have demonstrated that *first*, LPC stimulation of HAECs significantly modulates the expressions of canonical secretomic genes and exosome secretomic genes among all four secretomes examined in HAECs; *second*, increased procaspase-1 nuclear localization significantly upregulates in the presence and absence of caspase-1 enzymatic inhibitor upregulates canonical secretomic genes and exosome secretomic genes much more than that of caspase-1-



**Fig. 9. Increased nuclear localization of procaspase-1 promotes inflammation via upregulating 11 CD markers-mediated signaling pathways. A.** Number of modulated CD marker (CD marker list from PMID: 32179051) in each experiment, P < 0.05, LogFC>1 or < -1. **B.** 11 modulated CD marker and function. **C.** The expression of six modulated CD marker when increased nuclear localization of procaspase-1. **D.** Venn diagram of modulated CD marker in each experiment condition.

GSDMD secretomic genes and caspase-4-GSDMD secretomic genes; and *third*, significant upregulation of canonical and exosome secretomic genes after blocking procaspase-1 in the nucleus in LPC-activated HAECs indicates stronger EC activation with four pathways of Th17, IL-17 promoted cytokines, interferon signaling and cholesterol biosynthesis in the presence of nuclear export inhibitor of procaspase-1 than that controls in the absence of this inhibitor.

### 3.8. Increased nuclear localization of procaspase-1 promotes inflammation via upregulating 11 CD markers-mediated signaling pathways

As we and others reported, upregulations of membrane adhesion molecules potentially involved in EC adhesion and signaling are another hallmark of aortic endothelial cell activation [3,8,9,42,51,55,66,70,92,94]. To determine whether LPC induced nuclear localization of procaspase-1 leads to upregulation of membrane CD markers potentially involved in EC adhesion and signaling, we collected 373 clusters of differentiation (CD) markers as we reported [23]. As shown in Fig. 9A, inhibition of pro-caspase-1 nuclear export into cytosol in LPC treated HAECs in comparison to that of LPC treated HAECs upregulated six CD marker genes; inhibition of caspase-1 in LPC-activated HAECs in comparison to LPC activated HAECs upregulated one CD marker gene; inhibition of procaspase-1 nuclear export into cytosol and inhibition of caspase-1 activities in comparison to inhibition of caspase-1 activities in LPC-activated HAECs upregulated 8 CD marker genes; and inhibition of procaspase-1 nuclear export into cytosol and inhibition of caspase-1 activities in comparison to inhibition of pro-caspase-1 nuclear export into cytosol upregulated three CD marker genes. As shown in Fig. 9B, C and 9D, analysis of CD marker gene expression in the five groups resulted in the upregulation of 11 CD marker genes including C-X-C motif chemokine receptor 5 (CXCR5), CD37, IL-1R1, SELPLG, IFITM1, Fc receptor Like 5 (FCRL5), IL21R, CEA cell adhesion molecule 1 (CEACAM1), L1 cell adhesion molecule (L1CAM), LY75, and Mucin 1, cell surface associated (MUC1). Of note, CXCR5 and its ligand CXCL13 play fundamental roles in inflammatory, infectious, and immune responses and cancer [102]. Tetraspanin CD37 is a membrane organizing protein that controls C-type lectin-like receptor 2-dependent prototypic innate immune cell dendritic cell migration [103]. Interleukin-1 receptor 1 (IL-1R1) signaling roles in promoting inflammation have been well documented by CANTOS trial with monoclonal antibody Canakinumab to IL-1 $\beta$  [5,104]. SELPLG/PSGL-1 (P-selectin ligand) is proinflammatory molecule, and elevated soluble P-selectin is associated with coronavirus disease-2019 (COVID-19) disease severity [105]. Interferon inducible transmembrane protein 1 (IFITM1) is involved in adaptive immunity and regulate CD4<sup>+</sup> T helper cell differentiation [106]. FCRL5 plays important roles in autoimmunity with anti-phosphatidylserine antibodies produced by FCRL5<sup>+</sup>T-Bet<sup>+</sup> atypical memory B cells [107]. IL-21 receptor (IL21R) promotes inflammation in myocardial infarction [108] and respiratory disease [109]. CEACAM1 is a coronavirus receptor on endothelial cells as we reported [54] and promotes vascular aging process [110]. L1CAM regulates microRNAs associated with inflammation and progression of Alzheimer's disease and Parkinson's disease [111]. Lymphocyte antigen 75 (LY75) polymorphisms are associated with susceptibility and phenotype of inflammatory bowel disease [112]. MUC1 promotes chronic inflammation and cancer [113]. Taken together, our results have demonstrated that increase of procaspase-1 nuclear localization promotes inflammation via upregulating 11 CD markers-mediated signaling pathways.

### 3.9. LPC-increased nuclear localization of procaspase-1 induces ROS promoter, CYP1B1, which counteracts the functions of LPC-induced antioxidant genes and promotes strong inflammation

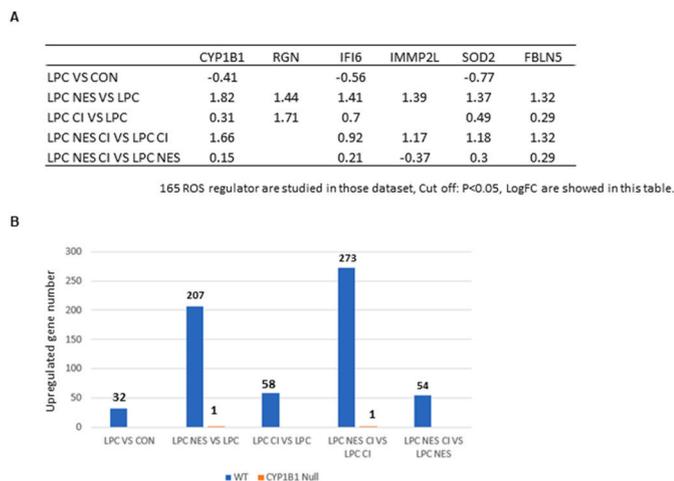
We reported that reactive oxygen species (ROS) systems are a new integrated network for sensing homeostasis and alarming stresses in

organelle metabolic process including nucleus [60]. Our recent report showed that the expressions of 165 ROS regulators are significantly modulated in inflammatory angiogenesis [63]. We hypothesized that increased nuclear localization of procaspase-1 modulates the expression of ROS regulators. We collected 165 ROS regulators from the GSEA database as we reported [63]. As shown in Fig. 10A, six ROS regulators including cytochrome P450 family 1 subfamily B member 1 (CYP1B1, localized in mitochondrion, endoplasmic reticulum, and nucleus (the GeneCards database at <https://www.genecards.org/cgi-bin/carddisp.pl?gene=CYP1B1&keywords=CYP1B1>), is also termed Aryl Hydrocarbon Hydroxylase, Flavoprotein-Linked Monooxygenase), Regucalcin (Senescence Marker Protein-30) (RGN), interferon alpha inducible protein 6 (IFI6), inner mitochondrial membrane peptidase subunit 2 (IMMP2L), superoxide dismutase 2 (SOD2) and fibulin 5 (FBLN5) were significantly modulated in LPC activated HAECs. It has been reported that CYP1B1 plays a detrimental role in both cardiovascular diseases and cancer, via perturbed metabolism of endogenous compounds, production of carcinogenic metabolites, DNA adduct formation, and generation of ROS [114]. RGN has been shown to have antioxidant properties by its activity reducing the production of reactive oxygen species and increasing the antioxidant defenses [115]. IFI6 depletion suppresses proliferation and induces apoptosis by increasing ROS accumulation, suggesting that IFI6 inhibits ROS accumulation [116]. Mitochondrial peptidase IMMP2L mutation causes early onset of age-associated disorders and impairs adult stem cell self-renewal [117], suggesting that IMMP2L inhibits ROS generation. Reduced antioxidant response mediated by NRF2 and downregulation of SOD2 contributes to the establishment of chronic oxidative stress in aged vessels [118], suggesting that SOD2 is anti-oxidant. FBLN5 blocks microenvironmental ROS [119]. These results have demonstrated that CYP1B1 promotes ROS generation and plays a detrimental role in cardiovascular disease but other five ROS regulators are antioxidant and play compensatory effects in counteracting CYP1B1 roles in promoting ROS generation.

We then hypothesized that CYP1B1, upregulated in LPC-activated HAECs with increased nuclear localization of procaspase-1, plays significant roles in promoting LPC-upregulated genes in HAECs. To examine this hypothesis, we collected a dataset GSE53910 in the NIH-NCBI GeoDatasets database (<https://www.ncbi.nlm.nih.gov/gds>). The dataset was the transcriptomic analysis with high fat diet fed (HF) CYP1B1 knock-out (KO) mice versus wild-type mouse controls, in which the experimental setting was similar to HF-increased LPC identified in apolipoprotein E deficient (ApoE KO) mouse aortas using metabolomic approach as we reported [42]. As shown in Fig. 10B, in the presence or absence of procaspase-1 nuclear export inhibitor and/or caspase-1 inhibitor, the five groups of LPC-induced genes in HAECs were all downregulated in the CYP1B1 KO dataset. The results have demonstrated that LPC-increased nuclear localization of procaspase-1 induces ROS promoter, CYP1B1, which counteracts the functions of LPC-induced antioxidant genes and promotes strong inflammation than that localized in cytosol.

## 4. Discussion

Our and others' previous reports showed that human aortic endothelial cells (EC) are innate immune cells that have inflammasome/caspase-1 functions in sensing PAMPs and DAMPs [3,9,51,54,55,120]. However, several important questions remained: i) whether procaspase-1 or activated caspase-1 are localized in the nucleus in sensing nuclear DAMPs in HAECs during pyroptosis; ii) what the transcriptomic regulation functions of procaspase-1 in modulating inflammation if localized in the nucleus; iii) whether procaspase-1 has nuclear export signal; iv) whether procaspase-1 is activated in the nucleus after sensing conditional DAMPs; and v) whether procaspase-1 and/or activated caspase-1 have enzymatic activity-independent functions in the nucleus of HAECs. To answer these questions, we performed extensive transcriptomic analyses of HAECs activated with proatherogenic lipid



**Fig. 10. LPC-increased nuclear localization of procaspase-1 induces ROS promoter, CYP1B1, which counteracts the functions of LPC-induced antioxidant genes and promotes strong inflammation. A.** 165 ROS regulator are studied in 5 experiment condition.  $P < 0.05$ , LogFC are showed in figure A. **B.** Most of upregulated gene are downregulated in CYP1B1 null dataset (GSE53910),  $P < 0.05$  and  $\text{LogFC} > 1$ .

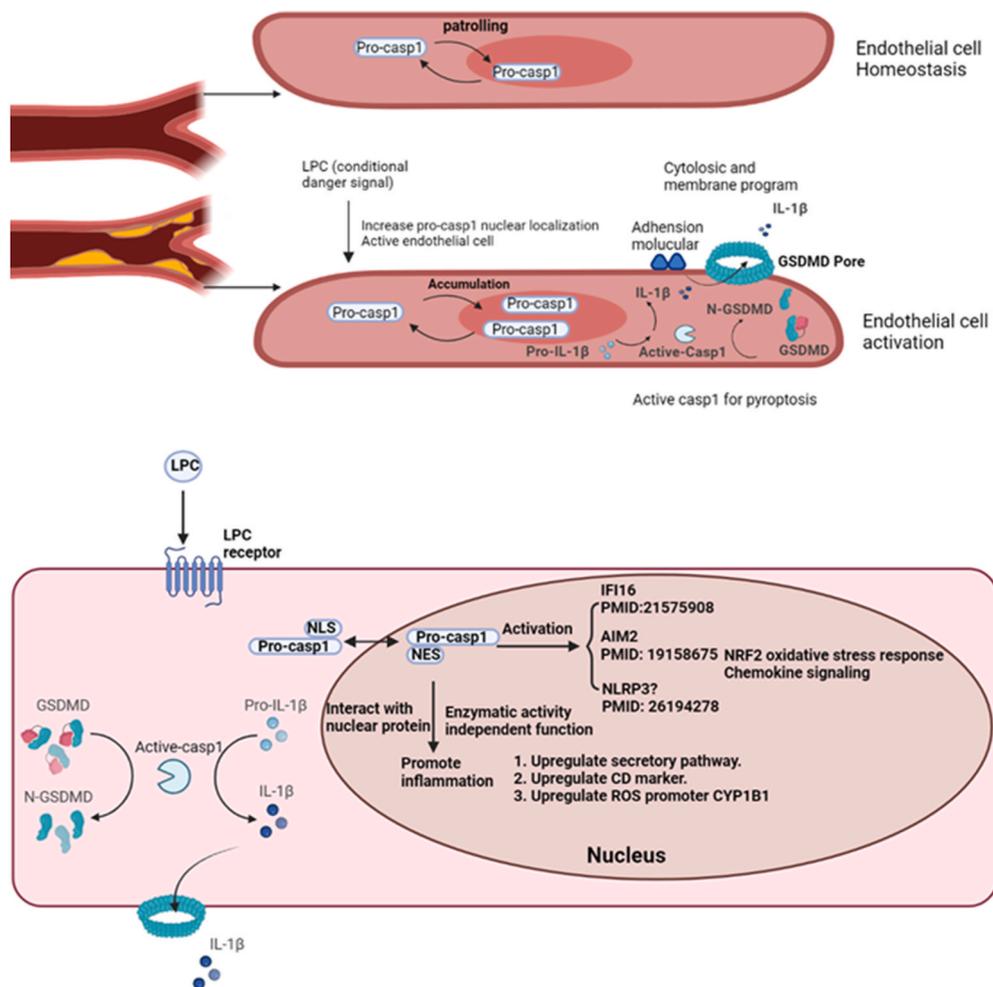
LPC with subcellular fractionation, characterization of procaspase-1 nuclear export signal, RNA-Seq and extensive pathway analyses. We made the following findings: LPC increase nuclear localization of procaspase-1 in HAECs, which is associated with transcriptomic changes for increased inflammatory and immune response pathways. Nuclear pro-caspase-1 patrols back to the cytosol; and pro-caspase-1 nuclear exportation is mediated by exportin 1-nuclear pore complex (NPC)-mediated mechanism. Increased nuclear localization of pro-caspase-1 in HAECs by pro-caspase-1 NES peptide inhibitor upregulates inflammatory genes in oxidative stress and Th17 activation signaling pathways. LPC + SUMO activator treatment of HAECs increase procaspase-1 nuclear translocation; increased nuclear localization of pro-caspase-1 is associated with increased caspase-1 activation in the nucleus; and LPC + caspase-1 inhibitor increased inflammatory gene upregulation with role of hypercytokinemia/hyperchemokinememia and interferon pathways. Increased nuclear localization of procaspase-1 in the presence of caspase-1 inhibitor significantly upregulates inflammatory gene expressions in Th17 activation, endotheli-1 signaling, p38-, and ERK- MAPK pathways. Fifteen functions of nuclear caspase-1 such as NRF2 oxidative stress response, GM-CSF and chemokine signaling are caspase-1 enzymatic activities-independent. Up to 76 canonical and exosome secretomic genes are upregulated, respectively, which upregulate four pathways of Th17, IL-17 promoted cytokines, interferon signaling and cholesterol biosynthesis in the presence of nuclear export inhibitor of procaspase-1. Increased nuclear localization of procaspase-1 promotes inflammation via upregulating 11 CD markers-mediated signaling pathways. LPC-increased nuclear localization of procaspase-1 induces ROS promoter, CYP1B1, for strong inflammation.

We observed that both cytosolic and nuclear pro-caspase-1 levels were increased in response to LPC 40 mM stimulation for 6 h in HAEC (Fig. 1B and C). We acknowledge that future experiments will be required to determine the relationship between procaspase-1 protein translation dynamics and procaspase-1 trafficking levels to the nucleus in response to LPC stimuli in HAEC. We hypothesize one of the following scenarios may contribute to this observation: 1) The distribution of procaspase-1 between the nucleus and the cytosol may depend on the dose and the duration of treatment of LPC; 2) LPC dose and duration of treatment may affect the expression of procaspase-1 including the processes such as increased protein translation, mRNA transcription and decreased mRNA degradation; and 3) LPC dose and duration of treatment may affect independent degradation of procaspase-1 in the

cytosolic and nuclear compartments.

Current models on the DAMPs-sensing and inflammatory functions of caspase-1 inflammasomes focus on activation of inflammasomes and caspase-1 mediated maturation of IL-1 $\beta$ , IL-18 in the cytosol, cleavage of sirtuin-1 [8,9] and generation of N-terminal gasdermin D (GSDMD) protein channel/pore on the plasma membrane in response to DAMP stimulations. Previous reports showed that procaspase-1 can be localized in the nucleus of TNF- $\alpha$ -treated Hela (cancer) cells mediated by a nuclear localization signal (NLS) in the N-terminus of procaspase-1 [33]. In addition, in green fluorescence protein (GFP) fusion protein tagged experiments, procaspase-1 was found mainly in the cytosol with low levels of nuclear localization depending on the cell types [31]. Moreover, it has been reported that procaspase-1 can be activated in the nucleus in TNF- $\alpha$ -treated Hela cells; and in the nucleus of Kaposi's sarcoma-associated herpesvirus (KSHV) infected endothelial and B cells mediated by interferon- $\gamma$  inducible protein 16 (IFI16) inflammasome [121], herpes simplex virus-1 infected foreskin fibroblasts mediated by IFI16, NLRP3 inflammasomes [34] and NLRP12 inflammasome [122]. However, our results here have demonstrated for the first time that endogenously generated conditional DAMP [37,38] proatherogenic lipids LPC [42] induces nuclear localization of procaspase-1 during pyroptosis [9] and endothelial cell activation [8], nuclear activation of caspase-1 and procaspase-1 trafficking back to cytosol mediated by a nuclear export signal located in the N-terminal amino acids 83–89 of procaspase-1 CARD domain. Increase of nuclear localization of procaspase-1 induce ROS promoter CYP1B1 and upregulate proinflammatory gene expressions.

To summarize our findings here, we propose a new working model (Fig. 11). *First*, pro-caspase-1 is localized in the nucleus of human aortic endothelial cells in the absence of LPC stimulation, suggesting that as a key danger sensing molecule, pro-caspase-1 patrols around in the cytosol and nucleus of human aortic endothelial cells even in homeostatic conditions. These new findings are well correlated with our model on nuclear sensors of DNA checkpoint and repair factors reported in 2018 [123]. *Second*, our results have demonstrated for the first time that proatherogenic lipid LPC as a prototypic endogenous metabolite-derived conditional DAMP [37,38] increases nuclear localization of procaspase-1 during endothelial cell activation and pyroptosis [101]. Pyroptosis has well-characterized cytosolic program and membrane program represented by maturation of pro-IL-1 $\beta$  and pro-IL-18 into mature cytokines IL-1 $\beta$  and IL-18, respectively [28], and secretion extracellularly via caspase-1-caspase4/11 cleaved N-terminal gasdermin D protein pore on the plasma membrane [71]. Our results presented here have demonstrated a new nuclear program of pyroptosis. *Third*, our and others' previous findings emphasized that endothelial cell activation have several key features including increased secretion of cytokines and chemokines, upregulation of endothelial cell adhesion molecules, upregulation of additional DAMP receptors and upregulation of major histocompatibility complex (MHC) molecules for antigen presentation, which represent cytosolic and membrane programs of endothelial cell activation [51,66]. Our findings here have demonstrated the new nuclear program of endothelial cell activation. *Fourth*, previous report showed that procaspase-1 has a nuclear localization signal in the N-terminal amino acids 7–11 (CARD) mediating procaspase-1 nuclear localization [33], whereas our results showed that procaspase-1 has a new nuclear export signal in the N-terminal amino acids 83–89 of procaspase-1 (CARD domain) mediating procaspase-1 nuclear export into cytosol. These results suggest that procaspase-1 as the only form, but not the activated caspase-1 with p20-p10 heterodimer, patrols back and forth between the cytosol and nucleus. The subcellular localizations of cytosolic inflammasomes such as NLRP1, NLRP3, NLR Family CARD domain containing 4 (NLRC4), nucleotide binding oligomerization domain containing 2 (NOD2) and CARD14, and nuclear DNA-sensing inflammasomes IFI16 are subcellular location-specific except versatile DNA sensing inflammasomes AIM2, NLRP3 and inflammasome adaptor molecule ASC may patrol back and forth between cytosol and nucleus.



**Fig. 11. The new working model:** LPC increases pro-caspase1 nuclear localization, suggesting a new nuclear program for pyroptosis and endothelial cell activation. (See details in the last paragraph of discussion).

*Fifth*, our results have demonstrated that LPC induced procaspase-1 nuclear localization results from upregulations of DNA damage-related genes, HMGB1 nuclear alarmin [69] and ROS promoter CYP1B1, and oxidative stress pathways, suggesting that increased nuclear ROS may serve as nuclear dangers that increase procaspase-1 nuclear localization and caspase-1 nuclear activation when LPC and SUMO activator N106 are in high concentrations. *Sixth*, our results have demonstrated for the first time that alterations in the nucleocytoplasmic transport in pyroptosis (inflammatory cell death) similar to that reported for apoptosis (non-inflammatory cell death) [86]. Our results have also demonstrated for the first time that in response to endogenous intracellular dangers in the nucleus, procaspase-1 nuclear localization in aortic endothelial cells results in strong upregulation of inflammatory genes, four novel secretomic pathways of canonical secretome, exosome secretome, caspase-1-Gasdermin D (GSDMD) secretome and caspase-4-GSDMD secretome, novel CD markers-membrane protein signaling pathway and master ROS promoter CYP1B1. *Seventh*, these transcriptomic results provide novel insights on the roles of nuclear caspase-1 in sensing DAMPs, inducing ROS promoter CYP1B1 and in regulating a large number of genes that mediate human aortic endothelial cell activation and inflammation. These findings will lead to future development of novel therapeutics for cardiovascular diseases (CVD), inflammations, infections, transplantation, autoimmune disease and cancers.

#### Author contributions

YL, GN, YS, LL carried out data collections, data analyses and drafted

the manuscript. KX, CDIV, YS, FS, ETC, XJ, and HW provided material input. XY supervised experimental design and data analyses. XY edited the manuscript.

#### Disclosures

None.

#### Declaration of competing interest

We do not have any conflict of interest in this paper.

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#### References

- [1] O. Soehnlein, P. Libby, Targeting inflammation in atherosclerosis - from experimental insights to the clinic, *Nat. Rev. Drug Discov.* 20 (2021) 589–610.
- [2] X.F. Yang, Y. Yin, H. Wang, Vascular inflammation and atherogenesis are activated via receptors for pamps and suppressed by regulatory T cells, *Drug Discov. Today Ther. Strat.* 5 (2008) 125–142.
- [3] J. Mai, A. Virtue, J. Shen, H. Wang, X.F. Yang, An evolving new paradigm: endothelial cells—conditional innate immune cells, *J. Hematol. Oncol.* 6 (2013) 61.
- [4] T.P. Fidler, C. Xue, M. Yalcinkaya, B. Hardaway, S. Abramowicz, T. Xiao, W. Liu, D.G. Thomas, M.A. Hajjehbrahimi, J. Pircher, C. Silvestre-Roig, A.G. Kotini, L.

- L. Luchsinger, Y. Wei, M. Westerterp, H.W. Snoeck, E.P. Papapetrou, C. Schulz, S. Massberg, O. Soehnlein, B. Ebert, R.L. Levine, M.P. Reilly, P. Libby, N. Wang, A.R. Tall, The AIM2 inflammasome exacerbates atherosclerosis in clonal haematopoiesis, *Nature* 592 (2021) 296–301.
- [5] P.M. Ridker, B.M. Everett, T. Thuren, J.G. MacFadyen, W.H. Chang, C. Ballantyne, F. Fonseca, J. Nicolau, W. Koenig, S.D. Anker, J.J.P. Kastelein, J. H. Cornel, P. Pais, D. Pella, J. Genest, R. Cifkova, A. Lorenzatti, T. Forster, Z. Kobalava, L. Vida-Simiti, M. Flather, H. Shimokawa, H. Ogawa, M. Dellborg, P. R.F. Rossi, R.P.T. Troquay, P. Libby, R.J. Glynn, C.T. Group, Antiinflammatory therapy with Canakinumab for atherosclerotic disease, *N. Engl. J. Med.* 377 (2017) 1119–1131.
- [6] P.A. VanderLaan, C.A. Reardon, G.S. Getz, Site specificity of atherosclerosis: site-selective responses to atherosclerotic modulators, *Arterioscler. Thromb. Vasc. Biol.* 24 (2004) 12–22.
- [7] M. Suzuki, K. Furuya, M. Ozawa, K. Miura, T. Ozawa, K. Matsuzono, T. Mashiko, R. Koide, S. Fujimoto, R. Tanaka, Complex aortic arch atherosclerosis in acute ischemic stroke patients with non-valvular atrial fibrillation, *J. Atherosclerosis Thromb.* 28 (2021) 776–785.
- [8] Y. Yin, X. Li, X. Sha, H. Xi, Y.F. Li, Y. Shao, J. Mai, A. Virtue, J. Lopez-Pastrana, S. Meng, D.G. Tilley, M.A. Monroy, E.T. Choi, C.J. Thomas, X. Jiang, H. Wang, X. F. Yang, Early hyperlipidemia promotes endothelial activation via a caspase-1-sirtuin 1 pathway, *Arterioscler. Thromb. Vasc. Biol.* 35 (2015) 804–816.
- [9] Y. Yin, J.L. Pastrana, X. Li, X. Huang, K. Mallilankaraman, E.T. Choi, M. Madesh, H. Wang, X.F. Yang, Inflammasomes: sensors of metabolic stresses for vascular inflammation, *Front. Biosci.* 18 (2013) 638–649.
- [10] Y. Yin, Y. Yan, X. Jiang, J. Mai, N.C. Chen, H. Wang, X.F. Yang, Inflammasomes are differentially expressed in cardiovascular and other tissues, *Int. J. Immunopathol. Pharmacol.* 22 (2009) 311–322.
- [11] Y.-F. Li, Xiao Huang, Xinyuan Li, Ren Gong, Ying Yin, Jun Nelson, Erhe Gao, Hongyu Zhang, Nicholas E. Hoffman, Steven R. Houser, Muniswamy Madesh, Douglas G. Tilley, Eric T. Choi, Xiaohua Jiang, Cong-Xin Huang, Hong Wang, Xiao-Feng Yang, Caspase-1 mediates hyperlipidemia-weakened progenitor cell vessel repair, *Front. Biosci.* 20 (2015), 1-1.
- [12] J. Lopez-Pastrana, L.M. Ferrer, Y.F. Li, X. Xiong, H. Xi, R. Cueto, J. Nelson, X. Sha, X. Li, A.L. Cannella, P.I. Imoukhuede, X. Qin, E.T. Choi, H. Wang, X.F. Yang, Inhibition of caspase-1 activation in endothelial cells improves angiogenesis: a novel therapeutic potential for ischemia, *J. Biol. Chem.* 290 (2015) 17485–17494.
- [13] Y. Sun, C. Johnson, J. Zhou, L. Wang, Y.F. Li, Y. Lu, G. Nanayakkara, H. Fu, Y. Shao, C. Sanchez, W.Y. Yang, X. Wang, E.T. Choi, R. Li, H. Wang, X.F. Yang, Uremic toxins are conditional danger- or homeostasis-associated molecular patterns, *Front. Biosci.* 23 (2018) 348–387.
- [14] M.A. Monroy, J. Fang, S. Li, L. Ferrer, M.P. Birkenbach, L.J. Lee, H. Wang, X. F. Yang, E.T. Choi, Chronic kidney disease alters vascular smooth muscle cell phenotype, *Front. Biosci.* 20 (2015) 784–795.
- [15] J. Yang, P. Fang, D. Yu, L. Zhang, D. Zhang, X. Jiang, W.Y. Yang, T. Bottiglieri, S. P. Kunapuli, J. Yu, E.T. Choi, Y. Ji, X. Yang, H. Wang, Chronic kidney disease induces inflammatory CD40+ monocyte differentiation via homocysteine elevation and DNA hypomethylation, *Circ. Res.* 119 (2016) 1226–1241.
- [16] M.M. Chan, X. Yang, H. Wang, F. Saaoud, Y. Sun, D. Fong, The microbial metabolite trimethylamine N-oxide links vascular dysfunctions and the autoimmune disease rheumatoid, *Arthritis. Nutrients.* 11 (2019).
- [17] L.M. Ferrer, A.M. Monroy, J. Lopez-Pastrana, G. Nanayakkara, R. Cueto, Y.F. Li, X. Li, H. Wang, X.F. Yang, E.T. Choi, Caspase-1 plays a critical role in accelerating chronic kidney disease-promoted neointimal hyperplasia in the carotid artery, *J Cardiovasc Transl Res* 9 (2016) 135–144.
- [18] R. Zhang, J. Saredy, Y. Shao, T. Yao, L. Liu, F. Saaoud, W.Y. Yang, Y. Sun, C. Johnson, Ct Drummer, H. Fu, Y. Lu, K. Xu, M. Liu, J. Wang, E. Cutler, D. Yu, X. Jiang, Y. Li, R. Li, L. Wang, E.T. Choi, H. Wang, X. Yang, End-stage renal disease is different from chronic kidney disease in upregulating ROS-modulated proinflammatory secretome in PBMCs - a novel multiple-hit model for disease progression, *Redox biology* 34 (2020) 101460.
- [19] K. Xu, W.Y. Yang, G.K. Nanayakkara, Y. Shao, F. Yang, W. Hu, E.T. Choi, H. Wang, X. Yang, gaTa3, hDac6, and Bcl6 regulate FOXP3+ Treg Plasticity and Determine Treg conversion into either novel antigen-presenting cell-like Treg or Th1-Treg, *Front. Immunol.* 9 (2018) 45.
- [20] Q. Yang, G.K. Nanayakkara, C. Drummer, Y. Sun, C. Johnson, R. Cueto, H. Fu, Y. Shao, L. Wang, W.Y. Yang, P. Tang, L.W. Liu, S. Ge, X.D. Zhou, M. Khan, H. Wang, X. Yang, Low-intensity ultrasound-induced anti-inflammatory effects are mediated by several new mechanisms including gene induction, immunosuppressor cell promotion, and enhancement of exosome biogenesis and docking, *Front. Physiol.* 8 (2017) 818.
- [21] W.Y. Yang, Y. Shao, J. Lopez-Pastrana, J. Mai, H. Wang, X.F. Yang, Pathological conditions re-shape physiological Tregs into pathological Tregs, *Burns Trauma* 3 (2015).
- [22] D. Ni, T. Tang, Y. Lu, K. Xu, Y. Shao, F. Saaoud, J. Saredy, L. Liu, Ct Drummer, Y. Sun, W. Hu, J. Lopez-Pastrana, J.J. Luo, X. Jiang, E.T. Choi, H. Wang, X. Yang, Canonical secretomes, innate immune caspase-1-, 4/11-gasdermin D non-canonical secretomes and exosomes may contribute to maintain treg-ness for Treg immunosuppression, tissue repair and modulate anti-tumor immunity via ROS pathways, *Front. Immunol.* 12 (2021) 678201.
- [23] R. Zhang, K. Xu, Y. Shao, Y. Sun, J. Saredy, E. Cutler, T. Yao, M. Liu, L. Liu, C. Drummer, Y. Lu, F. Saaoud, D. Ni, J. Wang, Y. Li, R. Li, X. Jiang, H. Wang, X. Yang, Tissue Treg secretomes and transcription factors shared with stem cells contribute to a Treg niche to maintain treg-ness with 80% innate immune pathways, and functions of immunosuppression and tissue repair, *Front. Immunol.* 11 (2020) 632239.
- [24] J. Shen, Y. Yin, J. Mai, X. Xiong, M. Pansuria, J. Liu, E. Maley, N.U. Saqib, H. Wang, X.F. Yang, Caspase-1 recognizes extended cleavage sites in its natural substrates, *Atherosclerosis* 210 (2010) 422–429.
- [25] S. Gastaldello, X. Chen, S. Callegari, M.G. Masucci, Caspase-1 promotes Epstein-Barr virus replication by targeting the large tegument protein deneddylase to the nucleus of productively infected cells, *PLoS Pathog.* 9 (2013), e1003664.
- [26] A. Aries, J. Whitcomb, W. Shao, H. Komati, M. Saleh, M. Nemer, Caspase-1 cleavage of transcription factor GATA4 and regulation of cardiac cell fate, *Cell Death Dis.* 5 (2014) e1566.
- [27] F. He, J.A. Doucet, J.M. Stephens, Caspase-mediated degradation of PPARgamma proteins in adipocytes, *Obesity* 16 (2008) 1735–1741.
- [28] Y.F. Li, G. Nanayakkara, Y. Sun, X. Li, L. Wang, R. Cueto, Y. Shao, H. Fu, C. Johnson, J. Cheng, X. Chen, W. Hu, J. Yu, E.T. Choi, H. Wang, X.F. Yang, Analyses of caspase-1-regulated transcripts in various tissues lead to identification of novel IL-1beta-, IL-18- and sirtuin-1-independent pathways, *J. Hematol. Oncol.* 10 (2017) 40.
- [29] L. Wang, H. Fu, G. Nanayakkara, Y. Li, Y. Shao, C. Johnson, J. Cheng, W.Y. Yang, F. Yang, M. Lavalley, Y. Xu, X. Cheng, H. Xi, J. Yi, J. Yu, E.T. Choi, H. Wang, X. Yang, Novel extracellular and nuclear caspase-1 and inflammasomes propagate inflammation and regulate gene expression: a comprehensive database mining study, *J. Hematol. Oncol.* 9 (2016) 122.
- [30] M. Bruchard, C. Rebe, V. Derangere, D. Togbe, B. Ryffel, R. Boidot, E. Humblin, A. Hamman, F. Chalmin, H. Berger, A. Chevriaux, E. Limagne, L. Apetoh, F. Vegran, F. Ghiringhelli, The receptor NLRP3 is a transcriptional regulator of TH2 differentiation, *Nat. Immunol.* 16 (2015) 859–870.
- [31] U.M. Shikama, Y. T. Miyashita, M. Yamada, Comprehensive studies on subcellular localizations and cell death-inducing activities of eight GFP-tagged apoptosis-related caspases, *Exp. Cell Res.* 264 (2001) 315–325.
- [32] F. Liu, J. Fu, K. Bergstrom, X. Shan, J.M. McDaniel, S. McGee, X. Bai, W. Chen, L. Xia, Core 1-derived mucin-type O-glycosylation protects against spontaneous gastritis and gastric cancer, *J. Exp. Med.* (2020) 217.
- [33] P.L. Mao, Y. Jiang, B.Y. Wee, A.G. Porter, Activation of caspase-1 in the nucleus requires nuclear translocation of pro-caspase-1 mediated by its prodomain, *J. Biol. Chem.* 273 (1998) 23621–23624.
- [34] K.E. Johnson, L. Chikoti, B. Chandran, Herpes simplex virus 1 infection induces activation and subsequent inhibition of the IFI16 and NLRP3 inflammasomes, *J. Virol.* 87 (2013) 5005–5018.
- [35] S.M. Man, R. Karki, T.D. Kanneganti, DNA-sensing inflammasomes: regulation of bacterial host defense and the gut microbiota, *Pathog Dis* 74 (2016) ftw028.
- [36] C. Fankhauser, R.M. Friedlander, V. Gagliardini, Prevention of nuclear localization of activated caspases correlates with inhibition of apoptosis, *Apoptosis* 5 (2000) 117–132.
- [37] X. Wang, Y.F. Li, G. Nanayakkara, Y. Shao, B. Liang, L. Cole, W.Y. Yang, X. Li, R. Cueto, J. Yu, H. Wang, X.F. Yang, Lysophospholipid receptors, as novel conditional danger receptors and homeostatic receptors modulate inflammation-novel paradigm and therapeutic potential, *J Cardiovasc Transl Res* 9 (2016) 343–359.
- [38] Y. Shao, G. Nanayakkara, J. Cheng, R. Cueto, W.Y. Yang, J.Y. Park, H. Wang, X. Yang, Lysophospholipids and their receptors serve as conditional DAMPs and XAMP receptors in tissue oxidative and inflammatory injury, *Antioxidants Redox Signal.* 28 (2018) 973–986.
- [39] H. Xiao, M. Lu, T.Y. Lin, Z. Chen, G. Chen, W.C. Wang, T. Marin, T.P. Shentu, L. Wen, B. Gongol, W. Sun, X. Liang, J. Chen, H.D. Huang, J.H. Pedra, D. A. Johnson, J.Y. Shyy, Sterol regulatory element binding protein 2 activation of NLRP3 inflammasome in endothelium mediates hemodynamic-induced atherosclerosis susceptibility, *Circulation* 128 (2013) 632–642.
- [40] H. Xi, Y. Zhang, Y. Xu, W.Y. Yang, X. Jiang, X. Sha, X. Cheng, J. Wang, X. Qin, J. Yu, Y. Ji, X. Yang, H. Wang, Caspase-1 inflammasome activation mediates homocysteine-induced pyroptosis in endothelial cells, *Circ. Res.* 118 (2016) 1525–1539.
- [41] L.M. Ferrer, A.M. Monroy, J. Lopez-Pastrana, G. Nanayakkara, R. Cueto, Y.F. Li, X. Li, H. Wang, X.F. Yang, E.T. Choi, Caspase-1 plays a critical role in accelerating chronic kidney disease-promoted neointimal hyperplasia in the carotid artery, *J Cardiovasc Transl Res* 9 (2016) 135–144.
- [42] X. Li, P. Fang, Y. Li, Y.M. Kuo, A.J. Andrews, G. Nanayakkara, C. Johnson, H. Fu, H. Shan, F. Du, N.E. Hoffman, D. Yu, S. Eguchi, M. Madesh, W.J. Koch, J. Sun, X. Jiang, H. Wang, X. Yang, Mitochondrial reactive oxygen species mediate lysophosphatidylcholine-induced endothelial cell activation, *Arterioscler. Thromb. Vasc. Biol.* 36 (2016) 1090–1100.
- [43] X. Li, L. Wang, P. Fang, Y. Sun, X. Jiang, H. Wang, X.-F. Yang, Lysophospholipids induce innate immune transdifferentiation of endothelial cells, resulting in prolonged endothelial activation, *J. Biol. Chem.* (2018), 002752 jbc.RA118.
- [44] Y.F. Li, R.S. Li, S.B. Samuel, R. Cueto, X.Y. Li, H. Wang, X.F. Yang, Lysophospholipids and their G protein-coupled receptors in atherosclerosis, *Front. Biosci.* 21 (2016) 70–88.
- [45] X. Wang, Y.-F. Li, G. Nanayakkara, Y. Shao, B. Liang, L. Cole, W.Y. Yang, X. Li, R. Cueto, J. Yu, H. Wang, X.-F. Yang, Lysophospholipid receptors, as novel conditional danger receptors and homeostatic receptors modulate inflammation—novel paradigm and therapeutic potential, *Journal of Cardiovascular Translational Research* 9 (2016) 343–359.
- [46] Y. Shao, Z. Cheng, X. Li, V. Chernaya, H. Wang, X-f Yang, Immunosuppressive/anti-inflammatory cytokines directly and indirectly inhibit endothelial dysfunction—a novel mechanism for maintaining vascular function, *J. Hematol. Oncol.* 7 (2014) 80.

- [47] G. Andonegui, H. Zhou, D. Bullard, M.M. Kelly, S.C. Mullaly, B. McDonald, E. M. Long, S.M. Robbins, P. Kubes, Mice that exclusively express TLR4 on endothelial cells can efficiently clear a lethal systemic Gram-negative bacterial infection, *J. Clin. Invest.* 119 (2009) 1921–1930.
- [48] Y. Yin, J.L. Pastrana, X. Li, X. Huang, K. Mallilankaraman, E.T. Choi, M. Madesh, H. Wang, X.F. Yang, Inflammasomes: sensors of metabolic stresses for vascular inflammation, *Front. Biosci.* 18 (2013) 638–649.
- [49] J.S. Pober, W.C. Sessa, Evolving functions of endothelial cells in inflammation, *Nat. Rev. Immunol.* 7 (2007) 803–815.
- [50] B. Lai, J. Wang, A. Fagenson, Y. Sun, J. Saredy, Y. Lu, G. Nanayakkara, W.Y. Yang, D. Yu, Y. Shao, Ct Drummer, C. Johnson, F. Saaoud, R. Zhang, Q. Yang, K. Xu, K. Mastascusa, R. Cueto, H. Fu, S. Wu, L. Sun, P. Zhu, X. Qin, J. Yu, D. Fan, Y. H. Shen, J. Sun, T. Rogers, E.T. Choi, H. Wang, X. Yang, Twenty novel disease group-specific and 12 new shared macrophage pathways in eight groups of 34 diseases including 24 inflammatory organ diseases and 10 types of tumors, *Front. Immunol.* 10 (2019) 2612.
- [51] Y. Shao, J. Saredy, W.Y. Yang, Y. Sun, Y. Lu, F. Saaoud, Ct Drummer, C. Johnson, K. Xu, X. Jiang, H. Wang, X. Yang, Vascular endothelial cells and innate immunity, *Arterioscler. Thromb. Vasc. Biol.* 40 (2020) e138–e152.
- [52] M. Liu, J. Saredy, R. Zhang, Y. Shao, Y. Sun, W.Y. Yang, J. Wang, L. Liu, Ct Drummer, C. Johnson, F. Saaoud, Y. Lu, K. Xu, L. Li, X. Wang, X. Jiang, H. Wang, X. Yang, Approaching inflammation paradoxes-proinflammatory cytokine blockages induce inflammatory regulators, *Front. Immunol.* 11 (2020) 554301.
- [53] Q. Yang, R. Zhang, P. Tang, Y. Sun, C. Johnson, J. Saredy, S. Wu, J. Wang, Y. Lu, F. Saaoud, Y. Shao, Ct Drummer, K. Xu, D. Yu, R. Li, S. Ge, X. Jiang, H. Wang, X. Yang, Ultrasound may suppress tumor growth, inhibit inflammation, and establish tolerogenesis by remodeling innate via pathways of ROS, immune checkpoints, cytokines, and trained immunity/tolerance, *J Immunol Res* 2021 (2021) 6664453.
- [54] Y. Shao, J. Saredy, K. Xu, Y. Sun, F. Saaoud, Ct Drummer, Y. Lu, J.J. Luo, J. Lopez-Pastrana, E.T. Choi, X. Jiang, H. Wang, X. Yang, Endothelial immunity trained by coronavirus infections, DAMP stimulations and regulated by anti-oxidant NRF2 may contribute to inflammations, myelopoiesis, COVID-19 cytokine storms and thromboembolism, *Front. Immunol.* 12 (2021) 653110.
- [55] Ct Drummer, F. Saaoud, Y. Shao, Y. Sun, K. Xu, Y. Lu, D. Ni, D. Atar, X. Jiang, H. Wang, X. Yang, Trained immunity and reactivity of macrophages and endothelial cells, *Arterioscler. Thromb. Vasc. Biol.* 41 (2021) 1032–1046.
- [56] X. Li, P. Fang, Y. Li, Y.-M. Kuo, A.J. Andrews, G. Nanayakkara, C. Johnson, H. Fu, H. Shan, F. Du, N.E. Hoffman, D. Yu, S. Eguchi, M. Madesh, W.J. Koch, J. Sun, X. Jiang, H. Wang, X. Yang, Mitochondrial reactive oxygen species mediate lysophosphatidylcholine-induced endothelial cell Activation Highlights, *Arterioscler. Thromb. Vasc. Biol.* 36 (2016) 1090–1100.
- [57] X. Li, P. Fang, W.Y. Yang, K. Chan, M. Lavallee, K. Xu, T. Gao, H. Wang, X. Yang, Mitochondrial ROS, uncoupled from ATP synthesis, determine endothelial activation for both physiological recruitment of patrolling cells and pathological recruitment of inflammatory cells, *Can. J. Physiol. Pharmacol.* 95 (2017) 247–252.
- [58] J. Cheng, G. Nanayakkara, Y. Shao, R. Cueto, L. Wang, W.Y. Yang, Y. Tian, H. Wang, X. Yang, Mitochondrial proton leak plays a critical role in pathogenesis of cardiovascular diseases, *Adv. Exp. Med. Biol.* 982 (2017) 359–370.
- [59] G.K. Nanayakkara, H. Wang, X. Yang, Proton leak regulates mitochondrial reactive oxygen species generation in endothelial cell activation and inflammation - a novel concept, *Arch. Biochem. Biophys.* 662 (2019) 68–74.
- [60] Y. Sun, Y. Lu, J. Saredy, X. Wang, C. Drummer IV, Y. Shao, F. Saaoud, K. Xu, M. Liu, W.Y. Yang, X. Jiang, H. Wang, X. Yang, ROS systems are a new integrated network for sensing homeostasis and alarming stresses in organelle metabolic processes, *Redox biology* 37 (2020) 101696.
- [61] A. Virtue, C. Johnson, J. Lopez-Pastrana, Y. Shao, H. Fu, X. Li, Y.F. Li, Y. Yin, J. Mai, V. Rizzo, M. Tordoff, Z. Bagi, H. Shan, X. Jiang, H. Wang, X.F. Yang, MicroRNA-155 deficiency leads to decreased atherosclerosis, increased white adipose tissue obesity, and non-alcoholic fatty liver disease: a novel mouse model OF obesity paradox, *J. Biol. Chem.* 292 (2017) 1267–1287.
- [62] J. Mai, G. Nanayakkara, J. Lopez-Pastrana, X. Li, Y.F. Li, X. Wang, A. Song, A. Virtue, Y. Shao, H. Shan, F. Liu, M.V. Autieri, S.P. Kunapuli, Y. Iwakura, X. Jiang, H. Wang, X.F. Yang, Interleukin-17A promotes aortic endothelial cell activation via transcriptionally and post-translationally activating p38 mitogen-activated protein kinase (MAPK) pathway, *J. Biol. Chem.* 291 (2016) 4939–4954.
- [63] H. Fu, Y. Sun, Y. Shao, J. Saredy, R. Cueto, L. Liu, Ct Drummer, C. Johnson, K. Xu, Y. Lu, X. Li, S. Meng, E.R. Xue, J. Tan, N.C. Jhala, D. Yu, Y. Zhou, K.J. Bayless, J. Yu, T.J. Rogers, W. Hu, N.W. Snyder, J. Sun, X. Qin, X. Jiang, H. Wang, X. Yang, Interleukin 35 delays hindlimb ischemia-induced angiogenesis through regulating ROS-extracellular matrix but spares later regenerative angiogenesis, *Front. Immunol.* 11 (2020) 595813.
- [64] K.U. Malik, B.L. Jennings, F.A. Yaghini, S. Sahan-Firat, C.Y. Song, A.M. Estes, X. R. Fang, Contribution of cytochrome P450 1B1 to hypertension and associated pathophysiology: a novel target for antihypertensive agents, *Prostag. Other Lipid Mediat.* 98 (2012) 69–74.
- [65] D.P. Beiting, S. Hidano, J.E. Baggs, J.M. Geskes, Q. Fang, E.J. Wherry, C. A. Hunter, D.S. Roos, S. Cherry, The orphan nuclear receptor TLX is an enhancer of STAT1-mediated transcription and immunity to *Toxoplasma gondii*, *PLoS Biol.* 13 (2015).
- [66] X. Li, L. Wang, P. Fang, Y. Sun, X. Jiang, H. Wang, X.F. Yang, Lysophospholipids induce innate immune transdifferentiation of endothelial cells, resulting in prolonged endothelial activation, *J. Biol. Chem.* 293 (2018) 11033–11045.
- [67] A. Subramanian, P. Tamayo, V.K. Mootha, S. Mukherjee, B.L. Ebert, M.A. Gillette, A. Paulovich, S.L. Pomeroy, T.R. Golub, E.S. Lander, J.P. Mesirov, Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles, *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005) 15545–15550.
- [68] A. Liberzon, C. Birger, H. Thorvaldsdottir, M. Ghandi, J.P. Mesirov, P. Tamayo, The Molecular Signatures Database (MSigDB) hallmark gene set collection, *Cell Syst* 1 (2015) 417–425.
- [69] D. Bertheloot, E. Latz, HMGB1, IL-1 $\alpha$ , IL-33 and S100 proteins: dual-function alarmins, *Cell. Mol. Immunol.* 14 (2017) 43–64.
- [70] X. Sha, S. Meng, X. Li, H. Xi, M. Maddaloni, D.W. Pascual, H. Shan, X. Jiang, H. Wang, X.F. Yang, Interleukin-35 inhibits endothelial cell activation by suppressing MAPK-AP-1 pathway, *J. Biol. Chem.* 290 (2015) 19307–19318.
- [71] P. Broz, P. Pelegrin, F. Shao, The gasdermins, a protein family executing cell death and inflammation, *Nat. Rev. Immunol.* 20 (2020) 143–157.
- [72] X. Li, L. Wang, P. Fang, Y. Sun, X. Jiang, H. Wang, X.F. Yang, Lysophospholipids induce innate immune transdifferentiation of endothelial cells, resulting in prolonged endothelial activation, *J. Biol. Chem.* 293 (2018) 11033–11045.
- [73] D.H. Lin, A. Hoelz, The structure of the nuclear pore complex (an update), *Annu. Rev. Biochem.* 88 (2019) 725–783.
- [74] F. Kapplusch, F. Schulze, S. Rabe-Matschewsky, S. Russ, M. Herbig, M. C. Heymann, K. Schoepf, R. Stein, U. Range, A. Rosen-Wolff, S. Winkler, C. M. Hedrich, J. Guck, S.R. Hofmann, CASP1 variants influence subcellular caspase-1 localization, pyroptosome formation, pro-inflammatory cell death and macrophage deformability, *Clin. Immunol.* 208 (2019) 108232.
- [75] J. Kalita, L.E. Kapinos, R.Y.H. Lim, On the asymmetric partitioning of nucleocytoplasmic transport - recent insights and open questions, *J. Cell Sci.* (2021) 134.
- [76] B. Nachmias, A.D. Schimmer, Targeting nuclear import and export in hematological malignancies, *Leukemia* 34 (2020) 2875–2886.
- [77] K. Rahmani, D.A. Dean, Leptomycin B alters the subcellular distribution of CRM1 (Exportin 1), *Biochem. Biophys. Res. Commun.* 488 (2017) 253–258.
- [78] A. Fittipaldi, M. Giacca, Transcellular protein transduction using the Tat protein of HIV-1, *Adv. Drug Deliv. Rev.* 57 (2005) 597–608.
- [79] D. Zhang, J. Wang, D. Xu, Cell-penetrating peptides as noninvasive transmembrane vectors for the development of novel multifunctional drug-delivery systems, *J. Contr. Release* 229 (2016) 130–139.
- [80] A.J. Bannister, T. Kouzarides, Regulation of chromatin by histone modifications, *Cell Res.* 21 (2011) 381–395.
- [81] A. Mayran, J. Drouin, Pioneer transcription factors shape the epigenetic landscape, *J. Biol. Chem.* 293 (2018) 13795–13804.
- [82] P.A. Colussi, N.L. Harvey, S. Kumar, Prodomain-dependent nuclear localization of the caspase-2 (Nedd2) precursor. A novel function for a caspase prodomain, *J. Biol. Chem.* 273 (1998) 24535–24542.
- [83] E.A. Prokhorova, G.S. Kopeina, I.N. Lavrik, B. Zhivotovsky, Apoptosis regulation by subcellular relocation of caspases, *Sci. Rep.* 8 (2018) 12199.
- [84] L. Besnault-Mascard, C. Leprince, M.T. Auffredou, B. Meunier, M.F. Bourgeade, J. Camonis, H.K. Lorenzo, A. Vazquez, Caspase-8 sumoylation is associated with nuclear localization, *Oncogene* 24 (2005) 3268–3273.
- [85] H. Shirakura, N. Hayashi, S. Ogino, K. Tsuruma, T. Uehara, Y. Nomura, Caspase recruitment domain of procaspase-2 could be a target for SUMO-1 modification through Ubc9, *Biochem. Biophys. Res. Commun.* 331 (2005) 1007–1015.
- [86] G.S. Kopeina, E.A. Prokhorova, I.N. Lavrik, B. Zhivotovsky, Alterations in the nucleocytoplasmic transport in apoptosis: caspases lead the way, *Cell Prolif* 51 (2018) e12467.
- [87] N.B. Bryan, A. Dorfleutner, S.J. Kramer, C. Yun, Y. Rojanasakul, C. Stehlik, Differential splicing of the apoptosis-associated speck like protein containing a caspase recruitment domain (ASC) regulates inflammasomes, *J. Inflamm.* 7 (2010) 23.
- [88] T. Fernandes-Alnemri, J. Wu, J.W. Yu, P. Datta, B. Miller, W. Jankowski, S. Rosenber, J. Zhang, E.S. Alnemri, The pyroptosome: a supramolecular assembly of ASC dimers mediating inflammatory cell death via caspase-1 activation, *Cell Death Differ.* 14 (2007) 1590–1604.
- [89] R. Stein, F. Kapplusch, M.C. Heymann, S. Russ, W. Staroske, C.M. Hedrich, A. Rosen-Wolff, S.R. Hofmann, Enzymatically inactive procaspase 1 stabilizes the ASC pyroptosome and supports pyroptosome spreading during cell division, *J. Biol. Chem.* 291 (2016) 18419–18429.
- [90] X. Li, P. Fang, W.Y. Yang, H. Wang, X. Yang, IL-35, as a newly proposed homeostasis-associated molecular pattern, plays three major functions including anti-inflammatory initiator, effector, and blocker in cardiovascular diseases, *Cytokine* 122 (2017), 154076–154076.
- [91] X. Li, J. Mai, A. Virtue, Y. Yin, R. Gong, X. Sha, S. Gutchigian, A. Frisch, I. Hodge, X. Jiang, H. Wang, X.F. Yang, IL-35 is a novel responsive anti-inflammatory cytokine—a new system of categorizing anti-inflammatory cytokines, *PLoS One* 7 (2012), e33628.
- [92] X. Li, Y. Shao, X. Sha, P. Fang, Y.M. Kuo, A.J. Andrews, Y. Li, W.Y. Yang, M. Maddaloni, D.W. Pascual, J.J. Luo, X. Jiang, H. Wang, X. Yang, IL-35 (Interleukin-35) suppresses endothelial cell activation by inhibiting mitochondrial reactive oxygen species-mediated site-specific acetylation of H3K14 (histone 3 lysine 14), *Arterioscler. Thromb. Vasc. Biol.* 38 (2018) 599–609.
- [93] X. Sha, S. Meng, X. Li, H. Xi, M. Maddaloni, D.W. Pascual, H. Shan, X. Jiang, H. Wang, X.F. Yang, Interleukin-35 inhibits endothelial cell activation by suppressing MAPK-AP-1 pathway, *J. Biol. Chem.* 290 (2015) 19307–19318.
- [94] X. Li, P. Fang, Y. Sun, Y. Shao, W.Y. Yang, X. Jiang, H. Wang, X. Yang, Anti-inflammatory cytokines IL-35 and IL-10 block atherogenic lysophosphatidylcholine-induced, mitochondrial ROS-mediated innate immune

- activation, but spare innate immune memory signature in endothelial cells, *Redox biology* 28 (2020) 101373.
- [95] C. Zhao, D.D. Gillette, X. Li, Z. Zhang, H. Wen, Nuclear factor E2-related factor-2 (Nrf2) is required for NLRP3 and AIM2 inflammasome activation, *J. Biol. Chem.* 289 (2014) 17020–17029.
- [96] R. Zhang, J. Saredy, Y. Shao, T. Yao, L. Liu, F. Saaoud, W.Y. Yang, Y. Sun, C. Johnson, Ct Drummer, H. Fu, Y. Lu, K. Xu, M. Liu, J. Wang, E. Cutler, D. Yu, X. Jiang, Y. Li, R. Li, L. Wang, E.T. Choi, H. Wang, X. Yang, End-stage renal disease is different from chronic kidney disease in upregulating ROS-modulated proinflammatory secretome in PBMCs - a novel multiple-hit model for disease progression, *Redox Biol* (2020) 101460.
- [97] M. Keller, A. Ruegg, S. Werner, H.D. Beer, Active caspase-1 is a regulator of unconventional protein secretion, *Cell* 132 (2008) 818–831.
- [98] M.B. Lorey, K. Rossi, K.K. Eklund, T.A. Nyman, S. Matikainen, Global characterization of protein secretion from human macrophages following non-canonical caspase-4/5 inflammasome activation, *Mol. Cell. Proteomics* 16 (2017) S187–S199.
- [99] C. Wilyard, New human gene tally reignites debate, *Nature* 558 (2018) 354–355.
- [100] D.E. Place, T.D. Kanneganti, The innate immune system and cell death in autoinflammatory and autoimmune disease, *Curr. Opin. Immunol.* 67 (2020) 95–105.
- [101] J. Wang, B. Lai, G. Nanayakkara, Q. Yang, Y. Sun, Y. Lu, Y. Shao, D. Yu, W. Y. Yang, R. Cueto, H. Fu, H. Zeng, W. Shen, S. Wu, C. Zhang, Y. Liu, E.T. Choi, H. Wang, X. Yang, Experimental data-mining analyses reveal new roles of low-intensity ultrasound in differentiating cell death regulatome in cancer and non-cancer cells via potential modulation of chromatin long-range interactions, *Front Oncol* 9 (2019) 600.
- [102] M.G. Kazanietz, M. Durando, M. Cooke, CXCL13 and its receptor CXCR5 in cancer: inflammation, immune response, and beyond, *Front. Endocrinol.* 10 (2019) 471.
- [103] C.M. de Winde, A.L. Matthews, S. van Deventer, A. van der Schaaf, N. D. Tomlinson, E. Jansen, J.A. Eble, B. Nieswandt, H.M. McGettrick, C.G. Figdor, M.G. Tomlinson, S.E. Acton, A.B. van Spriel, C-type lectin-like receptor 2 (CLEC-2)-dependent dendritic cell migration is controlled by tetraspanin CD37, *J. Cell Sci.* 131 (2018).
- [104] G. Cavalli, C.A. Dinarello, Anakinra therapy for non-cancer inflammatory diseases, *Front. Pharmacol.* 9 (2018) 1157.
- [105] N. Yatim, J. Boussier, R. Chocron, J. Hadjadj, A. Philippe, N. Gendron, L. Barnabei, B. Charbit, T.A. Szwebel, N. Carlier, F. Pene, C. Azoulay, L. Khider, T. Mirault, J.L. Diehl, C.L. Guerin, F. Rieux-Laucat, D. Duffy, S. Kerneis, D. M. Smadja, B. Terrier, Platelet activation in critically ill COVID-19 patients, *Ann. Intensive Care* 11 (2021) 113.
- [106] D.C. Yanez, S. Ross, T. Crompton, The IFITM protein family in adaptive immunity, *Immunology* 159 (2020) 365–372.
- [107] J. Rivera-Correa, M.S. Mackroth, T. Jacobs, J. Schulze Zur Wiesch, T. Rolling, A. Rodriguez, Atypical memory B-cells are associated with Plasmodium falciparum anemia through anti-phosphatidylserine antibodies, *Elife* 8 (2019).
- [108] A. Kubota, A. Suto, K. Suga, A. Iwata, S. Tanaka, K. Suzuki, Y. Kobayashi, H. Nakajima, Inhibition of Interleukin-21 prolongs the survival through the promotion of wound healing after myocardial infarction, *J. Mol. Cell. Cardiol.* 159 (2021) 48–61.
- [109] W. Niu, Y. Xu, X. Zha, J. Zeng, S. Qiao, S. Yang, H. Zhang, L. Tan, L. Sun, G. Pang, T. Liu, H. Zhao, N. Zheng, Y. Zhang, H. Bai, IL-21/IL-21R signaling aggravated respiratory inflammation induced by intracellular bacteria through regulation of CD4(+) T cell subset responses, *J. Immunol.* 206 (2021) 1586–1596.
- [110] F. Kleefeldt, U. Rueckschloss, S. Ergun, CEACAM1 promotes vascular aging processes, *Aging (Albany NY)* 12 (2020) 3121–3123.
- [111] A. Sadlon, P. Takousis, P. Alexopoulos, E. Evangelou, I. Prokopenko, R. Perneczky, miRNAs identify shared pathways in Alzheimer's and Parkinson's diseases, *Trends Mol. Med.* 25 (2019) 662–672.
- [112] A. Hirayama, S. Joshita, K. Kitahara, K. Mukawa, T. Suga, T. Umemura, E. Tanaka, M. Ota, Lymphocyte antigen 75 polymorphisms are associated with disease susceptibility and phenotype in Japanese patients with inflammatory bowel disease, *Dis. Markers* 2016 (2016) 6485343.
- [113] S. Cascio, O.J. Finn, Intra- and extra-cellular events related to altered glycosylation of MUC1 promote chronic inflammation, tumor progression, invasion, and metastasis, *Biomolecules* 6 (2016).
- [114] A.N. Carrera, M.K.O. Grant, B.N. Zordoky, CYP1B1 as a therapeutic target in cardio-oncology, *Clin. Sci.* 134 (2020) 2897–2927.
- [115] C.V. Vaz, S. Correia, H.J. Cardoso, M.I. Figueira, R. Marques, C.J. Maia, S. Socorro, The emerging role of Regucalcin as a tumor suppressor: facts and views, *Curr. Mol. Med.* 16 (2016) 607–619.
- [116] Z. Liu, S. Gu, T. Lu, K. Wu, L. Li, C. Dong, Y. Zhou, IFI6 depletion inhibits esophageal squamous cell carcinoma progression through reactive oxygen species accumulation via mitochondrial dysfunction and endoplasmic reticulum stress, *J. Exp. Clin. Oncol.* 39 (2020) 144.
- [117] S.K. George, Y. Jiao, C.E. Bishop, B. Lu, Mitochondrial peptidase IMP2L mutation causes early onset of age-associated disorders and impairs adult stem cell self-renewal, *Aging Cell* 10 (2011) 584–594.
- [118] M. El Assar, J. Angulo, L. Rodriguez-Manas, Oxidative stress and vascular inflammation in aging, *Free Radical Biol. Med.* 65 (2013) 380–401.
- [119] M. Wang, M. Topalovski, J.E. Toombs, C.M. Wright, Z.R. Moore, D.A. Boothman, H. Yanagisawa, H. Wang, A. Witkiewicz, D.H. Castrillon, R.A. Brekken, Fibulin-5 blocks microenvironmental ROS in pancreatic cancer, *Canc. Res.* 75 (2015) 5058–5069.
- [120] C. Zhong, X. Yang, Y. Feng, J. Yu, Trained immunity: an underlying driver of inflammatory atherosclerosis, *Front. Immunol.* 11 (2020) 284.
- [121] V.V. Singh, N. Kerur, V. Bottero, S. Dutta, S. Chakraborty, M.A. Ansari, N. Paudel, L. Chikoti, B. Chandran, Kaposi's sarcoma-associated herpesvirus latency in endothelial and B cells activates gamma interferon-inducible protein 16-mediated inflammasomes, *J. Virol.* 87 (2013) 4417–4431.
- [122] P.G. Coulon, N. Dhanushkodi, S. Prakash, R. Srivastava, S. Roy, N.I. Alomari, A. M. Nguyen, W.R. Warsi, C. Ye, E.A. Carlos-Cruz, U.T. Mai, A.C. Cruel, K. M. Ekmekciyan, E. Pearlman, BenMohamed L. NLRP3, NLRP12, and IFI16 inflammasomes induction and caspase-1 activation triggered by virulent HSV-1 strains are associated with severe corneal inflammatory herpetic disease, *Front. Immunol.* 10 (2019) 1631.
- [123] H. Zeng, G.K. Nanayakkara, Y. Shao, H. Fu, Y. Sun, R. Cueto, W.Y. Yang, Q. Yang, H. Sheng, N. Wu, L. Wang, W. Yang, H. Chen, L. Shao, J. Sun, X. Qin, J.Y. Park, K. Drosatos, E.T. Choi, Q. Zhu, H. Wang, X. Yang, DNA checkpoint and repair factors are nuclear sensors for intracellular organelle stresses-inflammations and cancers can have high genomic risks, *Front. Physiol.* 9 (2018) 516.