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IFNγ and TNFα Driven IRF1-Caspase 8 Dependent PANoptosis Promotes Keratinocyte Death in Psoriasis Independent of ZBP1

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Abstract

Psoriasis is a chronic inflammatory skin disorder characterized by dysregulated keratinocyte proliferation, differentiation, and immune activation. While aberrant immune signaling is well established, the contribution of inflammatory cell death pathways remains incompletely understood. Here, we identify PANoptosis, an integrated form of programmed cell death encompassing pyroptosis, apoptosis, and necroptosis, as a central feature of psoriasis. Integrative analysis of bulk and single-cell transcriptomic datasets revealed consistent upregulation of PANoptosis-associated genes in psoriatic lesions, predominantly in keratinocytes. Functional studies demonstrated that interferon-γ (IFNγ), particularly in combination with tumor necrosis factor-α (TNFα), robustly induces PANoptotic gene expression and inflammatory lytic cell death selectively in keratinocytes. This process is characterized by activation of caspase-8, caspase-3/7, GSDME, and necroptotic mediators, along with release of interleukin 1β. Mechanistically, cytokine-induced PANoptosis is dependent on the JAK–STAT1–IRF1 signaling axis but occurs independently of ZBP1 and nitric oxide. Inhibition of individual cell death pathways fails to rescue cell viability, whereas pan-caspase or JAK inhibition significantly attenuates PANoptosis, highlighting its coordinated nature. Furthermore, cytokine-induced PANoptosis is associated with downregulation of key epidermal barrier genes, linking inflammatory cell death to barrier dysfunction. Clinically, PANoptosis-associated gene expression decreases following anti-psoriatic therapy, while persistent caspase-8 expression correlates with poor treatment response. Furthermore, knockdown of either Caspase 8 or IRF1 attenuates PANoptosis. Collectively, these findings establish cytokine-driven PANoptosis as a critical mechanism underlying keratinocyte death and inflammation in psoriasis and identify the JAK–STAT1–IRF1-Caspase 8 axis as a potential therapeutic target for modulating inflammatory cell death.

Introduction

Psoriasis is a chronic, immune-mediated inflammatory skin disorder with complex etiology affecting approximately 125 million of the global population[1–3]. It is characterized by erythematous, scaly plaques that arise due to excessive proliferation and abnormal differentiation of epidermal keratinocytes, accompanied by a robust inflammatory response[2,4,5]. Although psoriasis was considered primarily a keratinocyte-driven disease, extensive research has established that it results from a complex interplay between the immune system and epidermal cells[2,3,5–7]. Activated immune cells, including dendritic cells, T cells, and macrophages, release pro-inflammatory cytokines such as tumor necrosis factor-α (TNFα), interferon-γ (IFNγ), interleukin-17 (IL-17), and interleukin-23 (IL-23), which sustain chronic inflammation and profoundly alter keratinocyte biology[2,3,5].

Keratinocytes are not only passive targets of immune signaling but also active participants in psoriasis pathogenesis[4,6–8]. Under inflammatory conditions, keratinocytes respond to cytokine stimulation by producing chemokines, antimicrobial peptides, and additional cytokines that amplify immune cell recruitment and activation[8–10]. Alongside hyperproliferation and impaired differentiation, increasing evidence indicates that dysregulated keratinocyte cell death contributes significantly to disease

pathology[4,11,12]. Several forms of programmed cell death, including apoptosis, necroptosis, and pyroptosis, have been implicated in psoriatic lesions[11–16]. These cell death pathways can promote the release of inflammatory mediators such as IL-1β, damage-associated molecular patterns (DAMPs), and other alarmins that further propagate skin inflammation[6,11]. Recent studies suggest that keratinocytes may undergo a coordinated form of inflammatory cell death known as PANoptosis, which integrates molecular components of pyroptosis, apoptosis, and necroptosis[17,18].

PANoptosis is an integrated form of inflammatory programmed cell death that coordinates molecular components of pyroptosis, apoptosis, and necroptosis[19,20]. Traditionally, these three pathways were considered distinct mechanisms of cell death, each regulated by specific molecular mediators such as inflammasomes and Gasdermin(s) in pyroptosis, caspases in apoptosis, and Receptor-interacting serine/threonine-protein kinase (RIPK) RIPK1–RIPK3–MLKL signaling in necroptosis[19–22]. However, accumulating evidence has revealed extensive crosstalk among these pathways, leading to the concept of PANoptosis as a unified inflammatory lytic cell death program. PANoptosis is typically triggered in response to pathogen infection, inflammatory cytokines, or cellular stress and is orchestrated through multi-protein signaling platforms termed PANoptosome[19,23,24]. These complexes integrate sensors, adaptors, and executioner molecules—including Z-DNA binding protein (ZBP1), caspase-8, RIPK3, and inflammasome components—to simultaneously engage multiple death pathway with the release of inflammatory cytokines and dama-associated molecular patterns (DAMPs) and other alarmins[19–21,25–27]. ZBP1 is a key sensor of Z-form of nucleic acids and is known to play functional role in infection-induced and sterile inflammatory conditions[28–30]. It engages with RIPK3, recruits Caspase 8 to form ZBP1-PANoptosome and thereby facilitates PANoptosis[28,29]. This integrated mechanism provides a robust host defense strategy by ensuring cell death even when pathogens or oncogenic processes block individual death pathways[22,23,25]. However, dysregulated PANoptosis can also contribute to inflammatory and autoimmune diseases by promoting excessive tissue damage and chronic inflammation[27]. Consequently, understanding the regulatory mechanisms governing PANoptosis has become increasingly important for elucidating the pathogenesis of diverse inflammatory conditions and for identifying new therapeutic targets to control aberrant inflammatory cell death. One of the first mechanistic study on IFNγ and TNFα-induced PANoptosis showed a JAK-STAT1-IRF1-Nitric oxide (NO) axis in murine macrophages[31]. However, no study till date have elucidated this pathway in PANoptosis of human cells. Induction of NO by IFNγ and/or TNFα mediated activation of STAT1-IRF1 transcription factors was well known for its cytotoxic activity and inhibition of NO generation prevented PANoptosis[31].

Despite increasing recognition of PANoptosis as a coordinated inflammatory cell death pathway in autoimmune diseases including psoriasis[27], the upstream regulatory mechanisms that initiate and control this process remain incompletely understood. Most studies have focused on the publicly available transcriptional datasets while functionally showed mostly the pyroptosis markers[17,18,32]. This knowledge gap in psoriasis warrants key attention and further study. During psoriasis, keratinocytes are constantly exposed to a cytokine-rich inflammatory environment dominated by mediators such as TNFα,

IFNγ, IL-17, and IL-23[1,2,5]. These cytokines strongly activate intracellular signaling pathways that influence both inflammatory responses and cell death programs. Identifying this mechanism is essential to understand how inflammatory signals translate into keratinocyte death and tissue damage in psoriasis. Moreover, uncovering the molecular mechanisms that control PANoptosis could reveal novel therapeutic targets capable of simultaneously modulating multiple inflammatory cell death pathways, thereby offering new strategies to mitigate chronic skin inflammation and restore epidermal homeostasis in psoriasis.

In this study, the expression of key PANoptosis-associated genes was found to be significantly higher in psoriasis compared with controls. It was further demonstrated that among the pro-inflammatory cytokines examined, IFNγ alone, and more prominently in combination with TNFα, transcriptionally reprogrammed keratinocytes to induce the expression of PANoptosis-related genes. Functionally, the combined stimulation with IFNγ and TNFα was shown to trigger PANoptosis in keratinocytes through a caspase-8–dependent but ZBP1-independent mechanism, in which interferon regulatory factor 1 (IRF1) played a critical regulatory role. Notably, inhibition of individual cell death pathways, including pyroptosis, apoptosis, or necroptosis, failed to reverse PANoptosis. In contrast, pharmacological inhibition of the Janus kinase–signal transducer and activator of transcription (JAK–STAT) pathway, but not the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, significantly attenuated cytokine-induced PANoptosis. Collectively, these findings demonstrate that IFNγ and TNFα play a central role in inducing keratinocyte death through PANoptosis, highlighting the potential contribution of this mechanism to the pathogenesis of psoriasis. Moreover, these results identify the JAK–STAT–IRF1-Caspase 8 signaling axis as a potential therapeutic target for modulating inflammatory cell death in psoriasis.

Materials and Methods

Chemicals and reagents

All the chemicals and reagents used in the study were either analytical grade, molecular biology grade or tissue culture grade depending on their usage. The details have been given in **supplementary Tables 2–4.**

Human sample collection

Generalized plaque type treatment naïve psoriasis patients (psoriasis vulgaris) from the

eastern India, were recruited (N = 18) in the study with the informed consent. All the recruited patients were verified for clinical diagnosis by two independent dermatologists and histopathological analyses. The demographic details of the patients including age, sex, age of onset, clinical diagnosis and HLA-Cw6 status were listed in **Supplementary Table 5**. The study was approved by the Institutional Ethics Committee for Human Research, Indian Statistical Institute. The tissue samples from the involved

psoriatic and uninvolved adjacent normal skin were obtained using a 4 mm punch biopsy and were collected in RNA later (Thermo Fisher, USA) and preserved at -80 C until further use.

Cell culture

All the human non-transformed cells HaCaT (keratinocytes), RPE1 (retinal pigment epithelial cells), HEK 293 (embryonic kidney epithelial cells) and MCF 10A (mammary epithelial) cells as well as THP1 monocytes were sourced from ATCC. HaCaT, RPE1 and HEK 293 cells were grown in DMEM Hi glucose supplemented with 10% Fetal bovine serum (FBS), L-glutamine (2 mM), 100 U/ml penicillin, 100 µg/ml streptomycin, 20 µg/ml gentamicin, and 10 mM HEPES. MCF10A cells were grown in similar media with 100 ng/mL cholera toxin (gift from Dr. Somsubhra Nath, Presidency University). THP1 cells were cultured in RPMI1640 supplemented with 10% FBS, L-glutamine (2 mM), 100 U/ml penicillin, 100 µg/ml streptomycin, 20 µg/ml gentamicin, and 10 mM HEPES. THP1 monocytes were differentiated into macrophages as described previously[33]. All the cells were grown at 37°C, 5% CO_2 inside an incubator. Cells were routinely checked for *Mycoplasma* contamination using PCR with specific primer pairs (**Supplementary Table 4**).

In vitro stimulation of cells

HaCaT cells were initially treated with a range of concentration of IFNγ, TNFα, IFNβ and IFNλ3 (10 ng/mL to 200 ng/mL). Finally, the concentration of 50 ng/mL of IFNγ and TNFα was chosen for all the later experiments. THP1 cells treated with LPS (1 µg/mL for 4 h) followed with 5 mM ATP was chosen as a positive control for pyroptosis while HaCaT cells with TNFα (50 ng/mL) and Cycloheximide (50 µM) with and without Z-VAD-FMK (Pan-caspase inhibitor) were used as a positive control for apoptosis and necroptosis respectively.

All the mentioned cell death inhibitors (**Supplementary Table 2**) were used at a concentration of 50 µM and DMSO was as a vehicle control. The JAKs inhibitors, Upadacitinib and Baricitinib were both used at 1 µM concentration while the NF-κB inhibitors BAY11-7082 and JSH23 were used at a 3 µM and 30 µM respectively. For all the experiments, inhibitors were added first for 2 h and then IFNγ and TNFα were added for indicated time points.

si-RNA mediated knockdown

Targeted gene knockdown in HaCaT cells was done using three siRNAs against each target gene (**Supplementary Table 4**) with a si-RNA specific transfection reagent (**Supplementary Table 2**) according to the manufacturer's protocol (MedChemExpress, China). As a control, a non-targeted siRNA was used (**Supplementary Table 4**). The siRNA experiments were done three independent times and knockdown efficiency was calculated using immunoblotting for the target gene.

Cell viability assays

Cell viability was measured by multiple means using MTT, Zombie dye, lactate dehydrogenase-based assay and apoptosis-necrosis assay using Hoechst and PI. For MTT and LDH-based assays, HaCaT cells were seeded in 96-wells plate (2 x 10^4 cells/well) for overnight. Following day, either the inhibitors were added for 2 h and then subsequently the IFNγ, TNFα and other cytokines were added either alone or in combination for 24 h. Lactate dehydrogenase was measured from the culture supernatant using a commercially available kit (Supplementary Table 2), while MTT assay was performed from the cells as described previously[34]. Absorbance was measured at 490 nm for LDH assay and at 570 nm for MTT assay using a multimode plate reader (Biotek, Agilent, USA)

Cells were seeded on coverslips in 24-well plates (5 × 10^4 cells/well) and treated with cytokines for 24 h. Cells were stained with Zombie Red dye or Hoechst 33342 and PI for 30 min according to the manufacturer's instructions, washed with PBS, and fixed with 3% formaldehyde. After PBS washes, nuclei were stained with DAPI (4 µg/ml) for 30 min only for Zombie-Red assays. Coverslips were mounted with antifade reagent and imaged using a fluorescence microscope (Leica DMi8, Leica-microsystems, Wetzlar, Germany). Dead cells (Zombie Red–positive) were manually counted and expressed as a percentage of total cells. For apoptosis-necrosis assays, cells with strong blue (due to nuclear condensation) but weak red (indicative of apoptotic), strong blue and strong red (indicative of late apoptotic) and weak blue but strong red (indicative of necrotic) were counted and plotted.

Immunoblotting

HaCaT cells were collected as pellets and was lysed in RIPA cell lysis buffer on ice for 30 minutes with 1X protease-phosphatase inhibitor cocktail and centrifuged at 13000 rpm for ten minutes at 4^0C and the supernatant was collected[35]. The concentration of the protein samples were measured using BCA assay as per the manufacturer's protocol (**Supplementary Table 2**). Western blotting was performed as mentioned previously using 40 µg of protein samples[35]. Development of the images was done by using enhanced chemiluminescence reagent using a chemidoc (Bio-Rad MP, Bio-Rad, USA).

Cell fractionation method for cell lysate preparation was done to separate nuclear and cytoplasmic components using a rapid method using freshly prepared NP-40 lysis buffer[36]. The subsequent steps from sample collection remain similar to above.

Immunofluorescence

Immunofluorescence staining was done as described previously[33] using appropriate primary and secondary antibodies (**Supplementary Table 3**). For all the staining, fixation was done using methanol free 3% formaldehyde. The imaging was done in Upright fluorescence microscope (Leica microsystem,

Wetzlar, Germany). The images were taken with same intensities and exposure time. Fluorescence intensities (corrected total cell fluorescence, CTCF) were measured using ImageJ software (NIH, USA).

Enzyme-linked immunosorbent assay and Griess assay for nitrite generation

ELISA was performed to quantify IL1β, Caspase 1, Caspase 3 and Caspase 8 from the culture supernatant using commercially available kit (KRISHGEN Biosystems, India) in accordance with the protocols provided. Standard curves were constructed and the sample values were extrapolated from it.

Total nitrite level was measured from the culture supernatant of the cytokine treated keratinocytes using the Griess reagent as described previously[37]

Analysis of transcriptome datasets from Gene expression omnibus (GSE)

In the current study, the expression profile data of five non-inflammatory, eleven inflammatory and twenty-three psoriasis datasets were analyzed (**Supplementary Table 1**). Briefly, the datasets were downloaded from GEO (https://www.ncbi.nlm.nih.gov/geo/). Furthermore, six more datasets were studied where HaCaT or human primary keratinocytes were treated with different cytokines (**Supplementary Table 1**). All these data were publicly available; hence no ethical approval was required. The data were analyzed with Benjamini & Hochberg (False discovery rate/FDR) where $p < 0.05$ were considered significant. Pathway analysis was done using the String enrichment where top 20 pathways (Kyoto Encyclopedia of Genes and Genomes/KEGG pathways) were selected based on FDR and then sorted by fold enrichment. The genes for PANoptosis was selected based on literature and GeneCards database[38]. The graphs were made using SR plot[39].

RNA Seq analysis

The RNA sequencing was performed on an illumina Novaseq 6000 platform using paired-end technology. Total RNA was extracted using Pure Link RNA Mini Kit (Invitrogen, CA, USA). All samples have more than 600 ng/mL with a RIN value of over 9.5. The cDNA library was prepared KAPA RNA HyperPrep Kit with RiboErase (Illumina, CA, USA). the QC-approved libraries were subsequently sequenced. The FASTQ files were uploaded, reads are filtered based on their quality and are processed with Salmon software to obtain the quantification of reads per gene. These reads were normalized as TPM[40]. The differential expression analysis between conditions were analyzed using the DESeq2 analysis tool[40]. The functional analysis was done using String enrichment and KEGG pathways. The graphs were made using SR Plot[39].

Single cell RNA Seq analysis

Publicly available scRNA-seq dataset (GSE162183)[41] was processed from the https://yz-studio.shinyapps.io/shinyapph5ad/ webserver. Cell type specific gene expressions on reduced dimensions was visualized using Uniform Manifold Approximation and Projection (UMAP). Co-expression analysis of two genes was done using the same method. To perform the heatmap analysis, the expression patterns of the PANoptosis associated and skin barrier genes were grouped by categorical cell clustering information. The normalized expressions are averaged, log-transformed and then plotted.

RNA extraction and quantitative real-time PCR

Total RNA was isolated from paired psoriasis tissues or from cytokine-treated cells using the Trizol based method. The cDNA was prepared from the RNA samples using cDNA synthesis kit from Abclonal (MA, USA) and quantitative polymerase chain reaction (qPCR) was done using Abclonal SYBR qPCR master mix (MA, USA) with gene specific primers (**Supplementary Table 4**) using Bio-Rad CFX96 real time PCR (Bio-Rad, CA, USA). GAPDH was kept as an internal control for other gene expressions. Relative expression of gene of interest was measured by $2 - \Delta\Delta Ct$ method.

Statistical analysis

All the statistical analyses were done using GraphPad prism version 8.0. The data represented as mean ± SEM and derived from at least three independent experiments. P-value < 0.05 were considered as significant. Student's t test, and Mann–Whitney U test, based on the data distribution was performed for two groups. Experiments with more than two groups were compared by one-way ANOVA, followed by Tukey's multiple comparison test.

Results

PANoptosis signatures are activated in psoriasis patients and attributed primarily to keratinocytes

To determine whether PANoptosis activation is a general feature of inflammatory skin diseases, we analyzed transcriptomic datasets representing five non-inflammatory and twelve inflammatory dermatoses (**Supplementary Table 1**). We initially screened for PANoptosis gene from a list of 31 genes as described previously[38], within Psoriasis, Atopic Dermatitis and Lichen Planus (**Supplementary Fig. 1a**). Based on the abundance of expression, we shortened the list to 19 genes (**Supplementary Fig. 1b**). The pro-inflammatory cytokines were consistently elevated across inflammatory skin disorders, linking inflammatory signaling with activation of PANoptosis as well as in psoriasis cases (**Supplementary Figs. 1c,d**).

Pathway enrichment analysis of two large psoriasis cohorts (GSE13355 and GSE30999) revealed significant upregulation of genes involved in pyroptosis, apoptosis, and necroptosis compared with non-lesional skin, substantiating activation of PANoptosis-related pathways in psoriatic lesions (Figs. 1a,b).

Analysis of 14 independent psoriasis datasets further confirmed consistent upregulation of the PANoptosis panel (Fig. 1c). These findings were validated using our recently published dataset (GSE244679), which also showed significant elevation of PANoptosis genes, in psoriasis patients as compared with healthy controls (Fig. 1d). qRT-PCR analysis in an independent group of 18 paired samples from psoriasis patients (lesional and non-leisional biopsies) corroborated the significant upregulation of representative genes from pyroptotic, apoptotic, and necroptotic pathways and PANoptosis associated transcription factors (Fig. 1e). Furthermore, metadata analysis of patients with psoriasis patients with mild and severe lesions show high expression of PANoptosis association genes and pro-inflammatory cytokines indicating that PANoptosis may be an early and upstream event before chronicity of the disease (**Supplementary Figs. 1e,f**).

To identify the cellular source(s) of PANoptosis gene expression in psoriasis, we reanalyzed a single-cell RNA-seq dataset (GSE162183). UMAP analysis revealed distinct skin cell populations and clear transcriptional differences between healthy and psoriatic samples (**Supplementary Figs. 2a,b**). Among these, keratinocytes showed the strongest upregulation of the PANoptosis panel, particularly in the stratum basale indicating that cytokines from dermal cells might fuel the expression of PANoptotic genes (**Supplementary Fig. 2c**). Pathway-specific analysis further demonstrated a significant elevation of pyroptotic (CASP1, CASP4, GSDMD, GSDME), apoptotic (CASP7, CASP8, CASP10, BAX), and necroptotic (RIPK1, RIPK3, MLKL, ZBP1) genes in keratinocytes from patients with psoriasis (**Supplementary Fig. 3**). Co-expression analysis confirmed coordinated upregulation of PANoptosis genes in keratinocytes (**Supplementary Fig. 2d**).

IFNγ and TNFα synergistically induced transcriptional activation of PANoptosis pathways in human keratinocytes

To identify cytokines that induce PANoptosis in keratinocytes, we reanalyzed transcriptomic datasets of cytokine-treated human keratinocytes. IFNγ alone upregulated several PANoptosis genes, and this effect was markedly enhanced when combined with TNFα (Fig. 2a). In contrast, other psoriasis-associated cytokines such as IL-17, IL-22, and the M5 cytokine cocktail had minimal effects on PANoptosis gene expression (Fig. 2a). We re-confirm this using RNA-seq analysis in HaCaT cells to show that IFNγ activated genes were associated with pyroptosis, apoptosis, and necroptosis, while TNFα co-treatment further amplified this response (Figs. 2b,c**)**. Combined cytokine treatment also induced the highest number of differentially expressed genes (3441) compared with IFNγ or TNFα treatment alone (**Supplementary Fig. 4**). These findings were supported by independent datasets showing enrichment of all the three programmed cell death pathways in HaCaT cells treated with IFNγ and TNFα for 24–48 h (**Supplementary Figs. 5a,c**), with consistent upregulation of the PANoptosis panel (Fig. 2d; **Supplementary Figs. 5b,d**).

The RNA-seq and qRT-PCR analysis demonstrated strong induction of inflammatory transcription factors STAT1, IRF1, RELA, NFKB1, and NFKB2 following combined cytokine stimulation (Fig. 2e; **Supplementary Fig. 5e**). This was accompanied by robust upregulation of pyroptotic, apoptotic, and necroptotic genes,

as well as inflammatory mediators including IL6, TNFα, IL1β, and TNFAIP3, with the strongest induction observed under IFNγ and TNFα co-treatment (Figs. 2f–h; **Supplementary Fig. 5e**).

IFNγ and TNFα synergistically induce inflammatory lytic cell death selectively in human keratinocytes

Following transcriptional activation of PANoptosis pathways, we examined whether IFNγ and TNFα induce cell death in keratinocytes. MTT-based cell viability assays demonstrated that the combinatorial treatment with IFNγ and TNFα significantly reduced cell viability of HaCaT cells in a dose- and time-dependent manner, with IFNγ alone inducing modest cytotoxicity whereas TNFα alone had minimal effect (Figs. 3a,b). In contrast, type I (IFNβ) and type III (IFNλ3) interferons, alone or with TNFα, did not reduce viability of keratinocytes, endorsing specificity of IFNγ-mediated signaling (**Supplementary Figs. 6a,b**).

Following combined cytokine treatment, LDH assays confirmed increased membrane damage (Fig. 3c), which was further supported by increased Zombie dye–positive cells and HMGB1 nuclear-to-cytoplasmic translocation (Fig. 3d; **Supplementary Fig. 6c**). Morphological analysis revealed increased apoptotic, late apoptotic, and necrotic cells, consistent with activation of multiple cell death pathways (Fig. 3e). Furthermore, co-treatment of IFNγ and TNFα significantly increased IL1β secretion, a feature of pyroptosis (Fig. 3f). Notably, this cytotoxic response was limited to keratinocytes as it was not observed in other epithelial cell lines (Fig. 3g).

IFNγ and TNFα induced functional activation of PANoptosis in keratinocytes and interfered with expression of skin barrier genes

The mediators of activation of pyroptosis, apoptosis, and necroptosis in keratinocytes following IFNγ and TNFα stimulation were examined. Western blot analysis showed no activation and cleavage of classical pyroptotic proteins CASP1 and GSDMD in HaCaT cells, although their cleavage was observed in LPS–nigericin–treated THP1 cells which served as a positive control (Fig. 4a). Instead, the gasdermin family protein GSDME and the membrane rupture mediator NINJ1 were robustly activated upon combined cytokine treatment (Fig. 4a). Apoptotic signaling was strongly induced, as evidenced by activation and cleavage of CASP8, CASP9, BID, CASP3, CASP7, and PARP (Fig. 4b). ELISA further confirmed increased levels of CASP1, CASP8, and CASP3 (Fig. 4c). Necroptotic signaling was also activated, with increased expression of CYLD, ZBP1, RIPK1, phosphorylated RIPK3, and phosphorylated MLKL (Fig. 4d). Fluorescence microscopy demonstrated co-expression of PANoptosis markers, including ZBP1–CASP8 and MLKL–CASP3, within individual cells following cytokine co-treatment (Fig. 4e).

As impaired skin barrier function is a hallmark of psoriasis, we examined the expression of barrier-associated genes. Single-cell transcriptomic analysis showed reduced expression of barrier genes in the stratum corneum of psoriasis compared with healthy controls (**Supplementary Fig. 7a**). Transcriptome reanalysis of cytokine-treated HaCaT cells revealed that IFNγ or TNFα alone increased barrier gene expression, whereas combined treatment markedly reduced genes such as TJP3, IVL, and FLG

(**Supplementary Fig. 7b**). Interestingly, CLDN1 expression remained elevated under combinatorial cytokine treatment, consistent with our RNA-seq data (**Supplementary Fig. 7c**). qPCR and western blot analysis validated decreased expression of TJP1, FLG, and IVL, while CLDN1 remained lower with cytokine treatment (**Supplementary Figs. 7d,e**).

Caspase-8 plays a central role in IFNγ- and TNFα-induced PANoptosis in keratinocytes and is associated with anti-psoriatic treatment response

Inhibitor studies confirmed that only the pan-caspase inhibitor Z-VAD-FMK significantly rescued viability of keratinocytes following treatment with IFNγ and TNFα, whereas inhibitors targeting individual pyroptotic (VX765), apoptotic (Z-DEVD-FMK, Z-IETD-FMK), or necroptotic pathways (Nec1, GSK872, NecS) provided little or partial protection (Fig. 5a), indicating coordinated activation of multiple cell death pathways consistent with PANoptosis. Additionally, inhibitor combinations failed to rescue cell death, with the exception with Necrosulfonamide, which produced a modest but significant 10% reduction (Fig. 5a) Furthermore, pan-caspase inhibition using Z-VAD-FMK but not others, markedly reduced activation of pyroptotic, apoptotic, and necroptotic markers following cytokine stimulation (Fig. 5b).

To determine the clinical relevance of these findings, we reanalyzed transcriptomic datasets from psoriasis patients before and after treatment and it showed that gene expression of PANoptosis markers decreased with anti-psoriatic treatment (**Supplementary Fig. 8a**). However, caspase-8 expression remained significantly elevated in non-responder patients as compared with responders (**Supplementary Figs. 8b,c**), suggesting a link between caspase-8–mediated PANoptosis and resistance to treatment. Given the pivotal role of caspase-8 as an upstream regulator of PANoptosis, targeted knockdown of caspase-8 using siRNA significantly reduced IFNγ- and TNFα-induced cytotoxicity and suppressed activation of pyroptotic, apoptotic, and necroptotic mediators (Figs. 5c,d; **Supplementary Fig. 5d**), confirming the central role of caspase-8 in coordinating PANoptosis in keratinocytes.

ZBP1 and nitric oxide (NO) is dispensable for keratinocyte PANoptosis

Caspase 8-dependent PANoptosis often involves ZBP1[19,21,23]. Furthermore, in a recent study, ZBP1 was shown to be critical for the keratinocytes PANoptosis under UVB-induced skin injury[28]. In this study, we also showed increased ZBP1 both at transcriptional and translational level (Fig. 2h and Figs. 4d and e). However, unlikely to Caspase 8, knockdown of ZBP1 was unable to restore the IFNγ- and TNFα-induced PANoptosis of keratinocytes (Figs. 6a **and b**). We further looked at Nitric oxide synthase 2 (NOS2) and NO, the other possible mediator of IFNγ and TNFα-induced PANoptosis as reported earlier[31] to determine whether it has any role in the keratinocyte PANoptosis. NOS2 expression was higher in Psoriasis patients (Fig. 6c) while *in vitro* its expression is induced with TNFα and combination of IFNγ and TNFα (Figs. 6c **and d**). However, blocking iNOS with inhibitors did not restore the cell death and the generation of NO is low in keratinocytes even with IFNγ and TNFα co-treatment (Fig. 6e **and f**).

JAK–STAT1 signaling is essential for IFNγ and TNFα–induced PANoptosis in keratinocytes, whereas NF-κB signaling is dispensable

To define upstream signaling driving PANoptosis in keratinocytes, we examined activation of inflammatory transcription factors following IFNγ and TNFα stimulation using western blotting and immunofluorescence. Early timepoint analysis (15–60 min) revealed rapid phosphorylation and nuclear translocation of STAT1 and IRF1 as well as activation of NF-κB (**Supplementary Figs. 9a-c**), while sustained activation of STAT1, IRF1, and NF-κB was observed up to 20 h post-stimulation (**Supplementary Figs. 9b–d**).

Pharmacological inhibition of JAK signaling with the FDA-approved inhibitors Upadacitinib and Baricitinib significantly rescued viability of keratinocytes following IFNγ and TNFα treatment and preserved cellular morphology (Fig. 7a). JAK inhibition suppressed STAT1 phosphorylation, reduced IRF1 expression, and blocked nuclear translocation of STAT1 and IRF1 but not NF-κB p65 (Figs. 7b–d). Consistently, JAK inhibition attenuated activation of pyroptotic (GSDME, NINJ1), apoptotic (CASP8, CASP3, CASP7, PARP), and necroptotic (ZBP1, pRIPK3, pMLKL) mediators and significantly reduced IL1β secretion, indicating suppression of PANoptosis (Figs. 7d–h).

In contrast, inhibition of NF-κB using BAY11-7082 or JSH23 blocked NF-κB translocation but did not rescue cell viability of keratinocytes (**Supplementary Figs. 10a–f**). Akin to this, IFNγ priming alone sensitized keratinocytes to cytotoxicity (**Supplementary Figs. 10g**), highlighting the dominant role of IFNγ signaling.

IRF1 is required for IFNγ and TNFα–induced PANoptosis in keratinocytes

Given the critical role of JAK–STAT1 signaling in PANoptosis, we examined whether its downstream target IRF1 is required for this process. siRNA-mediated knockdown of IRF1 significantly reduced IFNγ- and TNFα-induced cytotoxicity and preserved keratinocyte morphology compared with scrambled controls (Figs. 8a,b). Efficient IRF1 depletion was confirmed by Western blot (Figs. 8a,c) and resulted in marked attenuation of pyroptotic, apoptotic, and necroptotic markers, indicating that IRF1 is required for PANoptosis activation of keratinocytes (Fig. 8c).

Discussion

Traditionally, psoriasis has been viewed as a hyperproliferative disorder of keratinocytes[1,42]; however, increasing evidence suggests that keratinocyte death is not merely a secondary consequence of inflammation but may actively contribute to disease initiation and amplification[2–4,11]. Thus, elucidating the molecular mechanisms governing inflammatory cell death in keratinocytes is essential for understanding psoriasis pathobiology and identifying new therapeutic targets.

In this study, PANoptosis is identified as a central feature of psoriasis, with keratinocytes acting as the primary executors of this integrated inflammatory cell death program. Through integrative analysis of

bulk and single-cell transcriptomic datasets, coupled with functional validation, a consistent upregulation of genes associated with pyroptosis, apoptosis, and necroptosis is demonstrated in psoriatic lesions, correlating with disease severity. These findings are consistent with previous reports implicating individual cell death pathways in psoriasis, including apoptosis dysregulation[11,12,15,43,44], necroptosis activation[11–13,15], and inflammasome-driven pyroptosis[14,16]. However, the present study extends these observations by demonstrating coordinated activation of these pathways, supporting the emerging concept of PANoptosis as a unified and regulated inflammatory cell death process.

At first glance, the involvement of cell death in a disease characterized by keratinocyte hyperproliferation may appear paradoxical. However, re-analysis of available single-cell transcriptomic datasets[41,45] reveals that PANoptotic gene expression is enriched in basal keratinocytes, which reside in close proximity to the dermis which consists of interspersed resident immune cells. This suggests that inflammatory signals originating from immune cells preferentially target basal keratinocytes, triggering PANoptosis and the release of pro-inflammatory mediators. This supports a model in which keratinocyte death and inflammation form a self-amplifying loop, rather than a linear cause–effect relationship[7,11]. Indeed, PANoptotic keratinocytes released inflammatory cytokine IL1β and damage-associated molecular patterns (DAMPs) such as HMGB1, thereby exacerbating inflammation. The observation that PANoptotic signatures are shared across multiple inflammatory dermatoses further suggests that this mechanism represents a common axis of chronic skin inflammation.

Cytokine-driven inflammation is central to psoriasis, with the IL-23/IL-17 axis and TNFα being well-established contributors[5,10,46]. However, their roles in regulating keratinocyte death remain incompletely understood. Previous studies have shown that TNFα can induce keratinocyte death via both apoptotic and pyroptotic mechanisms, including caspase-3/GSDME-dependent lytic death and NLRP3 inflammasome activation[14,16]. Variability in these findings may reflect differences in cytokine concentrations and exposure duration. In contrast, the present study demonstrates that IFNγ, particularly in combination with TNFα, is a potent inducer of PANoptosis in keratinocytes. Mechanistically, our study established IFNγ and TNFα as key upstream drivers of PANoptosis in keratinocytes. Although IFNγ alone was sufficient to induce partial PANoptotic transcriptional reprogramming, its combination with TNFα resulted in a synergistic amplification of PANoptosis-associated gene expression and cell death. This is consistent with emerging evidence highlighting a role for IFNγ responses in inflammatory skin diseases such as lichen planus and lupus erythematosus, where IFNγ and TNFα cooperatively induce RIPK3-dependent necroptosis[47].

The synergistic effect of IFNγ and TNFα on PANoptosis observed in this study are in line with prior reports in intestinal epithelial cells, cancer cells and macrophages, where these cytokines cooperatively induce PANoptosis through caspase-8 and IRF1-dependent mechanisms[31,48]. Caspase 8 is considered as a master regulator of the pyroptosis, apoptosis and necroptosis[49]. Furthermore, irrespective of the cellular context and stimulus, Caspase 8 remains conserved across different PANoptosome complex[50]. In the current study, Caspase 8 is induced transcriptionally through IFNγ-signaling and activated.

Strikingly, inhibition of Caspase 8 activation using small molecule inhibitor has no effect on the cell death, but siRNA-dependent knockdown of Caspase 8 inhibits PANoptosis. A similar observation was led by Woznicki et al.[51] and a non-enzymatic function of Caspase 8 in the cell death driven by JAK1/2-STAT1 pathway was hypothesized. However, in our study the role of Caspase 8 seems even more complex, as the PAN-caspase inhibitor Z-VAD-FMK was shown to inhibit Caspase 8 activation as well as keratinocyte PANoptosis. We think the bi-phasic activation of Caspase 8 might play an important role here where the initial activation can be prevented by the Z-VAD-IETD but the later activation by other caspases through the mitochondria-mediated intrinsic pathways can't be prevented by the Z-IETD-FMK. Since Z-VAD-FMK inhibits all the Caspases, the bi-phasic activation of Caspase 8 is prevented and thus the PANoptosis.

While ZBP1 has been identified as a key upstream sensor mediating PANoptosis in several contexts including keratinocytes upon UV treatment[28–31,52], the present findings indicate that ZBP1 is dispensable for IFNγ- and TNFα-induced PANoptosis in keratinocytes, despite its upregulation. This highlights the potential of cell and stimulus–specific differences in PANoptosis regulation and suggests the involvement of alternative upstream sensors in epithelial cells[51,53]. Similarly, although nitric oxide has been implicated in IFNγ- and TNFα-induced PANoptosis in murine systems[31], its role appears limited in human cells[48,53], which is consistent with the present findings showing minimal functional contribution despite increased NOS2 expression.

All the studies related to IFNγ, and TNFα-induced cell death highlighted the importance of JAK-STAT signaling in the process[31,51,53]. However, the involvement of NF-κB was never shown. In this study, we showed that TNF-mediated NF-κB signaling is dispensable for PANoptosis. TNFα essentially generate both pro and anti-survival signals depending on the ubiquitination of RIPK1 which is associated with TNFR1 at the cytoplasmic side[54]. While ubiquitination of RIPK1 forms the pro-survival protein complex, its de-ubiquitination triggers the formation of death signaling complexes which involves caspase 8[55]. IFNγ-potentiates the cytotoxicity of TNFα by induction of the deubiquitinase protein Cylindromatosis (CYLD) which deubiquitinates the RIPK1 and subsequently activates the TNFα-induced toxicity as also with induction of caspase 8 through IRF1-dependent pathway[55,56]. Our RNA Seq and qRT-PCR data revealed that in patients with psoriasis and in IFNγ-treated keratinocytes, the expression of CYLD and caspase 8 is significantly higher compared to healthy controls and untreated cells. Furthermore, we and others also observed that priming of the keratinocytes and tumor cells first with IFNγ followed with TNFα but not the opposite leading to more cell death[57]. The cell death by IFNγ alone that we observed is apoptosis, has also been observed in other inflammatory dermatoses[58–60] while combined IFNγ and TNFα-triggered IRF1-caspase 8 dependent PANoptosis of keratinocytes were not reported earlier. However, when this manuscript was in preparation, another parallel study showed activation of PARP, GSDMD, GSDME, CASP3 and MLKL in keratinocytes upon treatment with IFNγ and TNFα[61]. However, this study is far from any mechanistic details and also used extremely high levels of IFNγ and TNFα[61] thus technically differs from the current study.

The current study provides a link between PANoptosis and skin barrier dysfunction. The downregulation of barrier-associated genes such as *FLG*, *IVL*, and *TJP1* following IFNγ and TNFα treatment suggests that inflammatory cell death contributes directly to impaired barrier integrity. This is consistent with previous studies demonstrating that both IFNγ and TNFα disrupt keratinocyte differentiation and barrier formation[62–64]. Thus, PANoptosis may serve as a mechanistic bridge connecting inflammation, cell death, and barrier disruption in psoriasis.

From a clinical perspective, the reduction of PANoptosis-associated gene expression following anti-psoriatic therapy, coupled with persistent elevation of caspase-8 in non-responders, suggests that incomplete suppression of this pathway may contribute to treatment resistance. Furthermore, our data suggested that the expression of PANoptotic genes can be correlated with disease severity which may be used as a biomarker for the stratification of the patients. Indeed, GSDME, one of the key pyroptotic protein showed promise[65]. The identification of the JAK–STAT1–IRF1 axis as a critical upstream regulator of PANoptosis further integrates these findings into the broader framework of psoriasis pathogenesis. JAK–STAT signaling is already known to be activated in psoriatic lesions and is targeted therapeutically by JAK inhibitors[66–69]. The current study provides mechanistic insight into how this pathway may contribute to disease by driving inflammatory cell death in keratinocytes. We believe that careful monitoring of the patients following the JAK1 inhibitors would provide better efficacy and less relapses in psoriasis patients. Furthermore, identification and usage of specific inhibitors of STAT1, IRF1 and/or PANoptosis would also enhance the therapeutic options for the inflammatory dermatoses including psoriasis.

Declarations

Conflict of Interest: None declared

Availability of Data and Materials: All the data will be available upon request.The transcriptome datasets used in the study are available in the gene expression omnibus. The transcriptome data generated for this study is also available at GEO database (accession ID: GSE328995)

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conceptualization of human Psoriasis data, DM supervised and conceptualized the study, analyze the data, wrote the manuscript, obtained funding. All authors read and approved the final version.

Compliance with Ethics Requirement: All the experiments were performed with prior approval from Institutional Biosafety committee. The study related to Psoriasis clinical samples was approved by the Institutional Ethics Committee for Human Research, Indian Statistical Institute, Kolkata, India (ISI-IEC/2021/01/01), and IPGMER, Kolkata, India (IPGME&R/IEC/2021/001).

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Figures

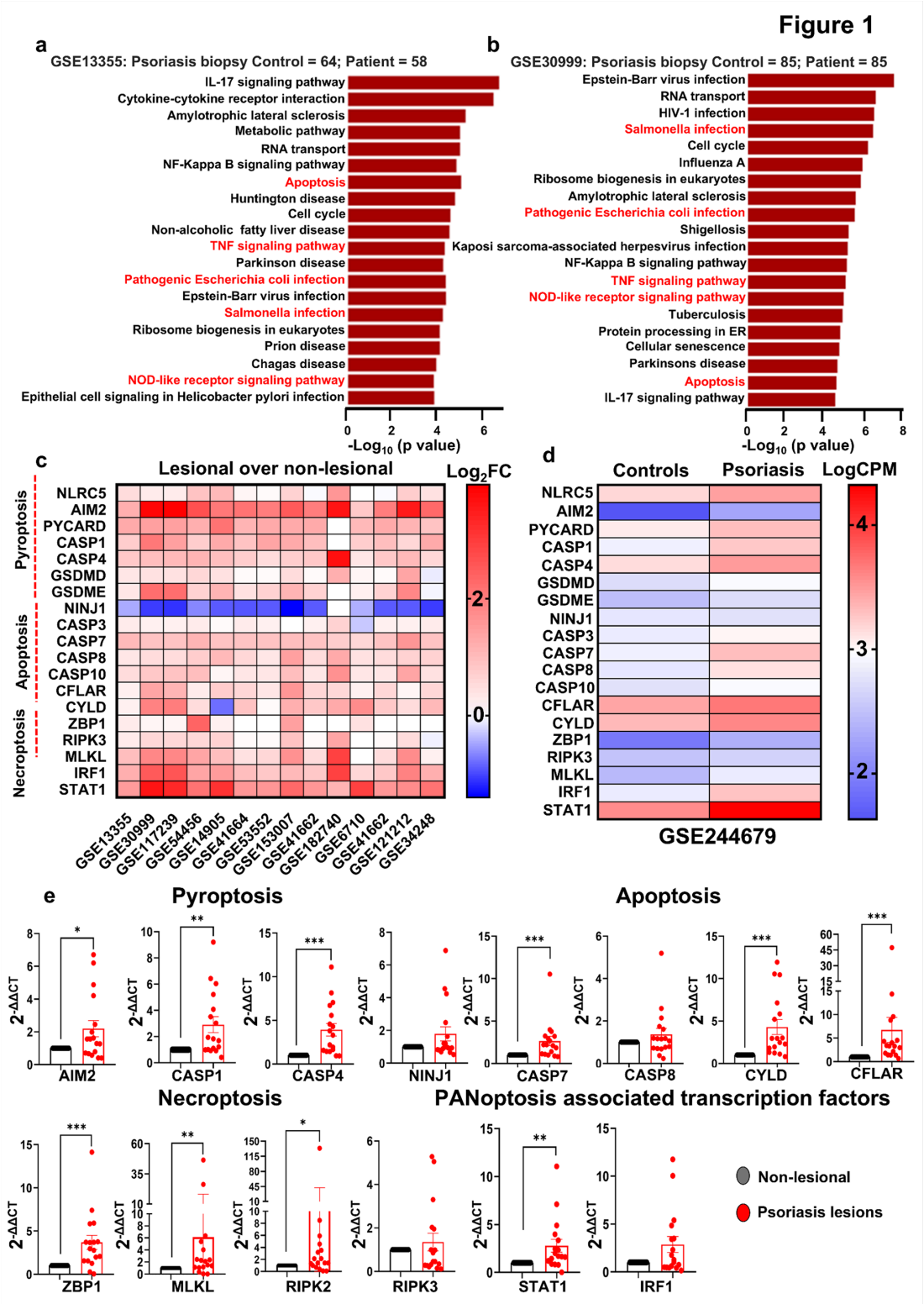


Figure 1

Enhanced PANoptosis Gene Signature in Psoriasis Patients. Pathway analysis of two large Psoriasis transcriptome datasets (GSE13355 and GSE30999) was performed, and the top 20 upregulated pathways were shown. Pathways marked in red contains PANoptosis associated DEGs (**a & b**). Heatmap showing the 19-gene PANoptosis panel across 14 published psoriasis transcriptomic datasets (**c**). Validation cohort for PANoptosis genes in our recently published RNA-Seq dataset (GSE244679) with

N=24 each of patients and control (**d**). Independent validation of the PANoptosis genes (4 genes each of pyroptosis, apoptosis and necroptosis and STAT1 as well as IRF1 in Psoriasis patients (paired lesional and non-lesional, N=18) using qRT-PCR (**e**). * Indicates significantly different than controls (for c and d). *, ** and *** indicates p<0.05, p<0.01 and p<0.001 respectively for (e). Wilcoxon Signed Rank test was performed using Graph-Pad Prism version 8.0.

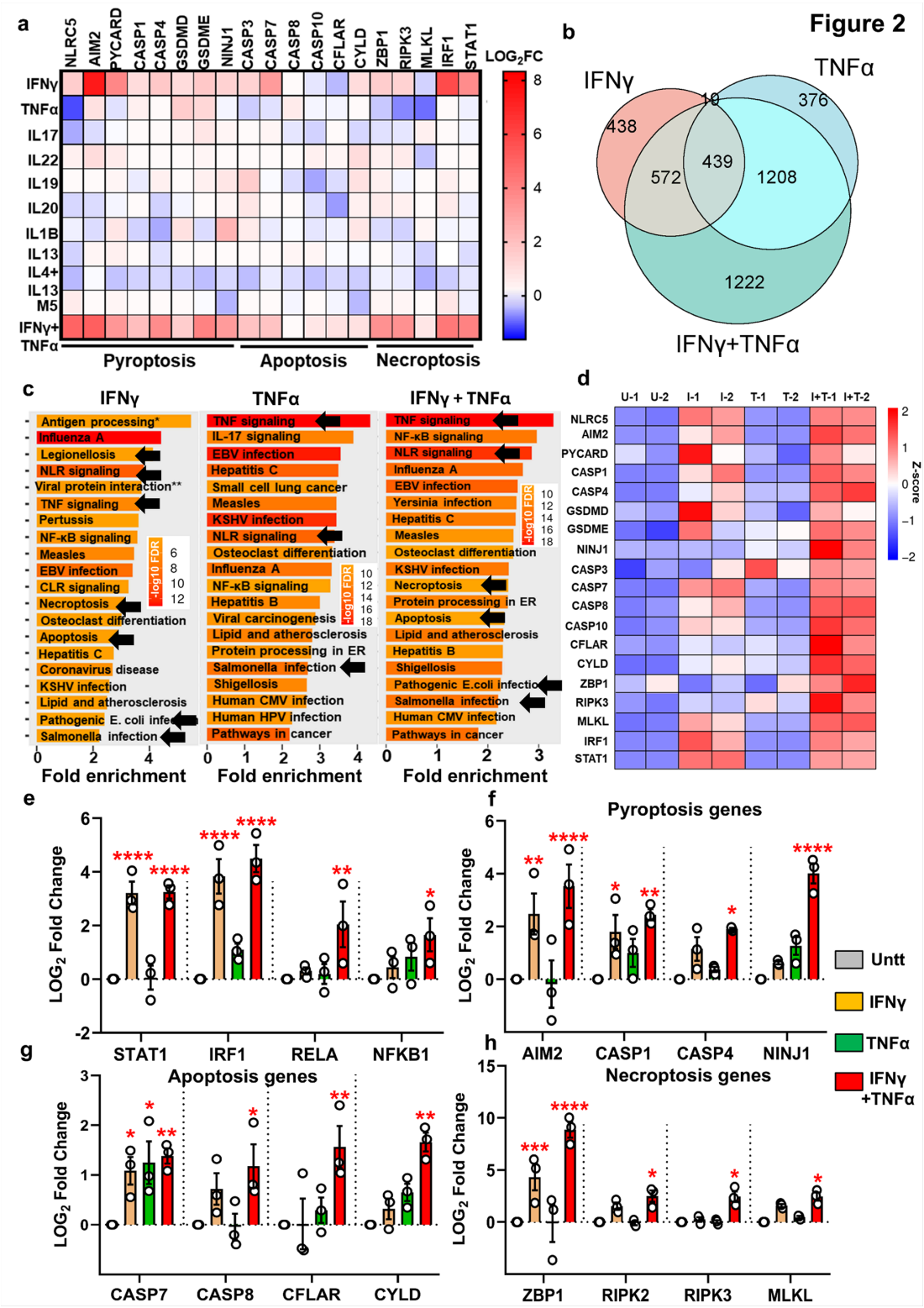


Figure 2

IFNγ and TNFα upregulated PANoptosis gene expression in human keratinocytes. Transcriptional analysis of PANoptosis genes in human keratinocytes was done by reanalyzing different available datasets of human keratinocytes treated with the cytokines as mentioned in the materials and methods (**a**). Overlapping inducible genes between IFNγ, TNFα and co-treatment of IFNγ and TNFα is shown using the Venn diagram (**b**). KEGG Pathway analysis of human keratinocytes (HaCaT) treated with either IFNγ or TNFα or combined IFNγ and TNFα (50 ng/mL each) for 24 h. Only top 20 upregulated pathways are shown (**c**). The arrow indicates the multiple cell death pathways that are activated. Heatmap showing the two biological replicates of unstimulated (U), IFNγ (I), TNFα (T) and IFNγ + TNFα (I+T)-treated samples for the 16 gene panel of PANoptosis (**d**). HaCaT cells were treated with IFNγ, TNFα individually or in combination for 24 h, RNA was isolated and then qRT-PCR was performed for Expression of the STAT1, IRF1, RELA and NFKB1 transcription factors (**e**), Pyroptotic genes (**f**), Apoptotic genes (**g**) and Necroptotic genes (**h**).

Each dot is an average of three technical replicates performed for each sample. All experiments were performed in three independent replicates (N=3). *, **, *** and **** indicates $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$ respectively. One-Way ANOVA followed with Tukey's multiple comparison test was performed using Graph-Pad Prism version 8.0.

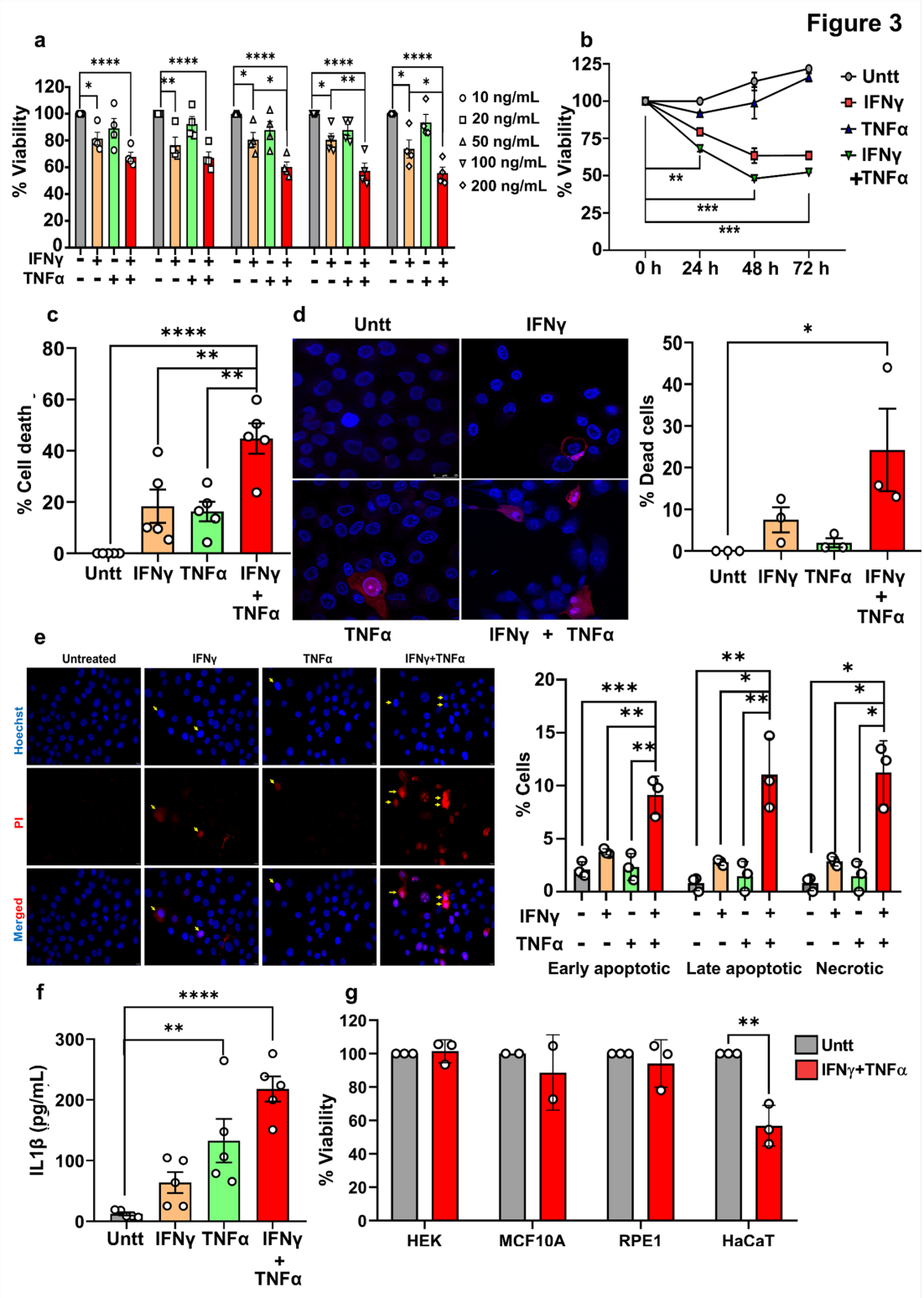


Figure 3

IFNγ and TNFα mediated lytic cell death specifically in keratinocytes. HaCaT cells were seeded in 96 well plates in triplicate (2X 10^4 cells/100 µL) for overnight. Cells were treated with indicated doses of IFNγ, TNFα alone or in combination for 24 h. Following that MTT assay was performed (**a**). Each dot is average of three technical replicates. Similar experiments were performed using 50 ng/mL each of IFNγ, TNFα alone or in combination for a time kinetics with indicated times (**b**). Similar to A, cells were treated

in 96 wells with individual or combined cytokine for 24 h and then lactate dehydrogenase assay was performed from the culture supernatant to check the lytic death of the cells (**c**). Each dot is average of three technical replicates. Zombie dye incorporation assay was performed using cells seeded on a coverslip within the 24 well plate followed with cytokine treatment as mentioned above. Cells were fixed and stained with Zombie dye and DAPI as mentioned in materials and methods and visualized and counted using confocal microscopy (**d**). The bar graph representing the dead cells were shown at the right-hand side. A microscopy based apoptotic-necrosis assay was performed on the keratinocytes that are seeded and treated similar to d. Only apoptotic cells (condensed chromosome), late apoptotic cells (condensed chromosome and PI positive) and necrotic cells (no condensed chromosome but PI positive) were counted under the microscope (**e**). For microscopy (d and e), from each coverslip, at least 100 cells were counted. IL1β was measured from culture supernatants of the keratinocytes that are either untreated or treated with single or double cytokines as mentioned in d (**f**). Effect of IFNγ and TNFα was tested on other immortal but non-cancerous epithelial cells HEK293 (kidney embryonic epithelial), MCF10A (breast epithelial cells), RPE1 (retinal pigment epithelial) alongside HaCaT (keratinocytes) (**g**). All the experiments were performed at least 3 independent times and *, **, *** and **** indicates $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$ respectively. One-Way ANOVA followed with Tukey's multiple comparison test (for **a, c, d, e, f**), Two Way ANOVA followed with Sidak's test (**b**) and Student's t test (**g**) was performed using Graph-Pad Prism version 8.0.

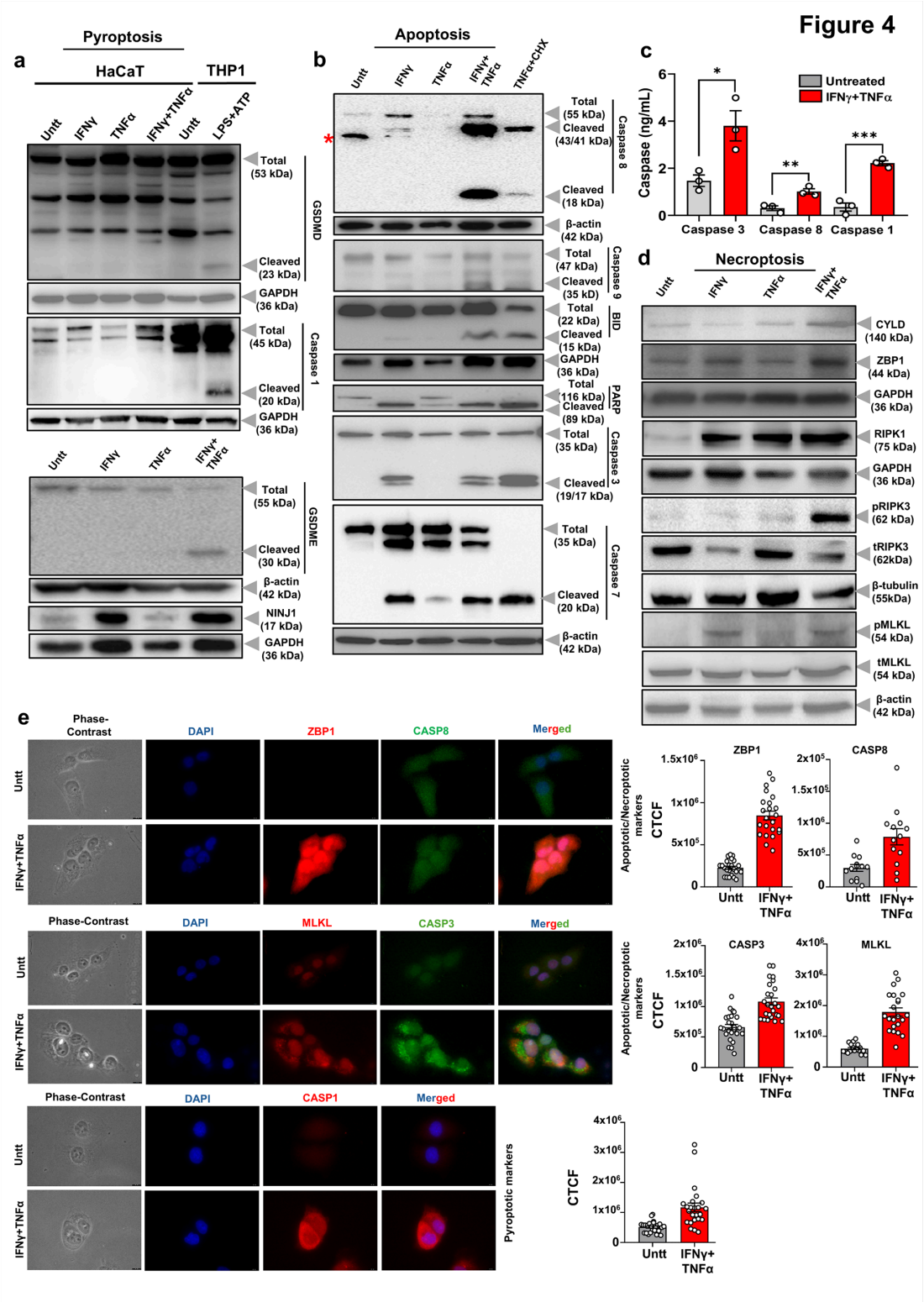


Figure 4

Activation of PANoptosis in keratinocytes upon stimulation with IFNγ and TNFα. Cells were seeded in 6 well plates and treated with either IFNγ or TNFα alone or in combination for 24 h with 50 ng/mL concentration of each cytokines. Functional activation of Pyroptosis genes (CASP1, GSDMD, GSDME, NINJ1) were checked using western blotting with specific primary antibodies (**a**), For Apoptosis CASP8, CASP9, BID, CASP3, CASP7, PARP were checked (**b**). The amount of CASP3, CASP8 and CASP1 were

checked using ELISA from the lysate against specific standards (**c**). Similar to A and B, activation of necroptosis genes (ZBP1, RIPK1, pRIPK3/RIPK3, pMLKL/MLKL) were checked from the lysates prepared from cytokine treated samples (**d**). Co-expression of ZBP1 and CASP8, MLKL and CASP3 as well as CASP1 was checked by fluorescence microscopy. Experiments were performed three times with similar results and representative images were shown here. Each dot indicates intensity from a single cell. *, **, and *** indicatesp<0.05, p<0.01, and p<0.001 respectively. Two tailed Student's t test (**c**) was performed using Graph-Pad Prism version 8.0.

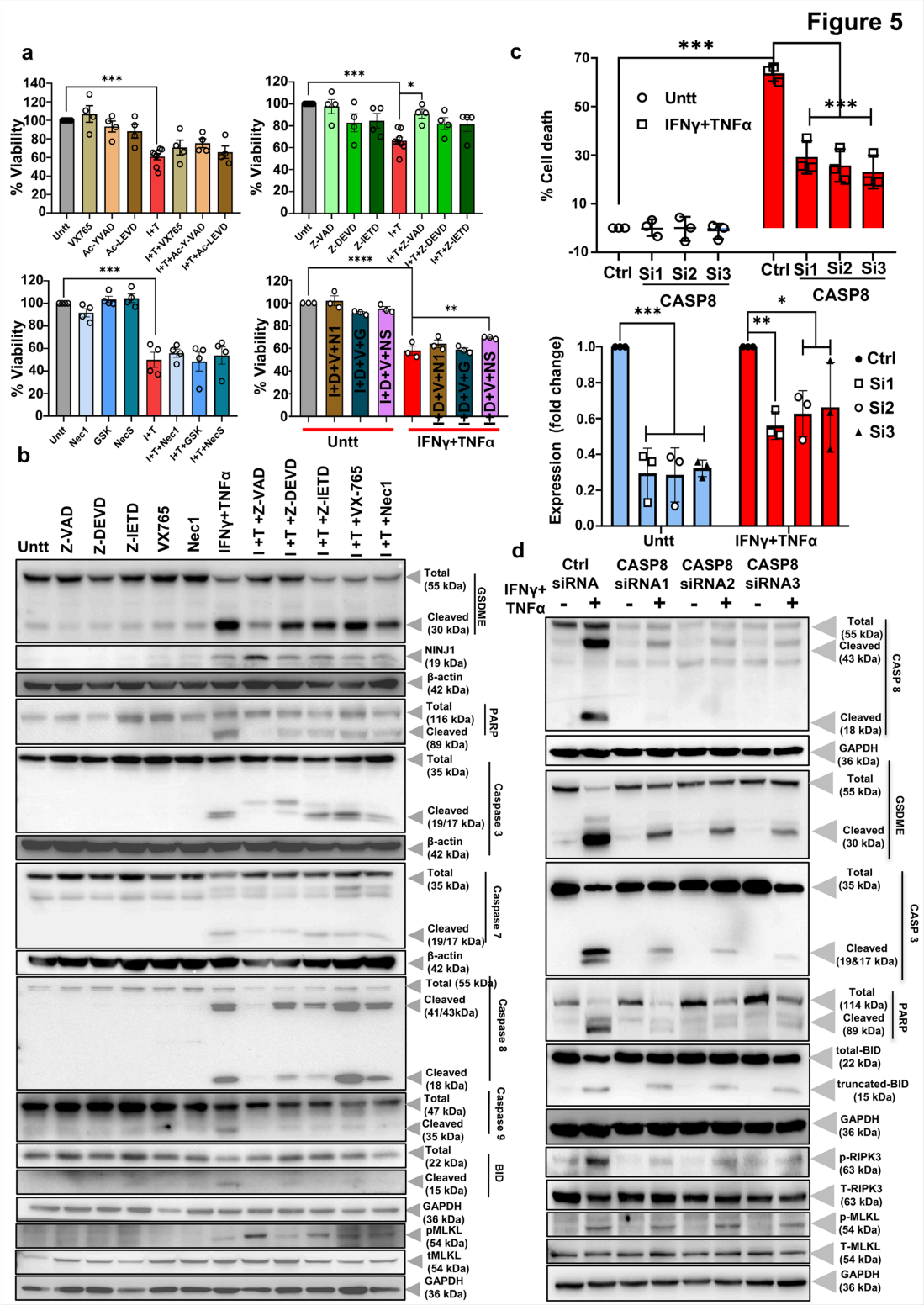


Figure 5

IFNγ and TNFα induced keratinocyte PANoptosis can be partially inhibited by Pan-caspase inhibitors where Caspase 8 plays a central role. HaCaT cells were seeded in 96 well plates, treated with Pyroptosis, Apoptosis and Necroptosis inhibitors alone or in combination as indicated, 2 h prior to stimulation with IFNγ and TNFα with 50 ng/mL concentration of each cytokines. Z-IETD-FMK (I), Z-DEVD-FMK (Z), VX765 (V), Necrostatin 1(Nec1), GSK-872 (G), and Necrostatin S (NS). Cell viability was measured using MTT assay after 24 h (**a**). Each dot is average of three technical replicate per condition per experiment. Experiments were conducted at least three independent times. HaCaT cells that are treated with IFNγ and TNFα in the presence and absence of Pyroptosis, Apoptosis and Necroptosis inhibitors, were subject to lysis for western blotting for markers of PANoptosis using target specific primary and secondary antibodies as mentioned in materials and methods (**b**). HaCaT cells were seeded in 6 well plates, transfected with three different siRNAs targeting Caspase 8 or with scrambled siRNA for 24 h as described in materials and method. Following that, cells were treated with IFNγ and TNFα for 24 h. The culture supernatant was used to measure cytotoxicity using LDH assay (**c**). Each dot is an average of two technical replicates per experiment. Experiments were conducted three independent times. The bar diagram below shows the knockdown efficiency. Similar to c, the lysates were prepared and western blotting was performed for the indicated PANoptosis markers using specific primary and secondary antibodies as described in materials and methods (**d**). *, **, ***, and **** indicates $p<0.05$, $p<0.01$, $p<0.001$, and $p<0.0001$ respectively. One Way ANOVA followed with Tukey's multiple comparison test (**a** and **c**) was performed using Graph-Pad Prism version 8.0.

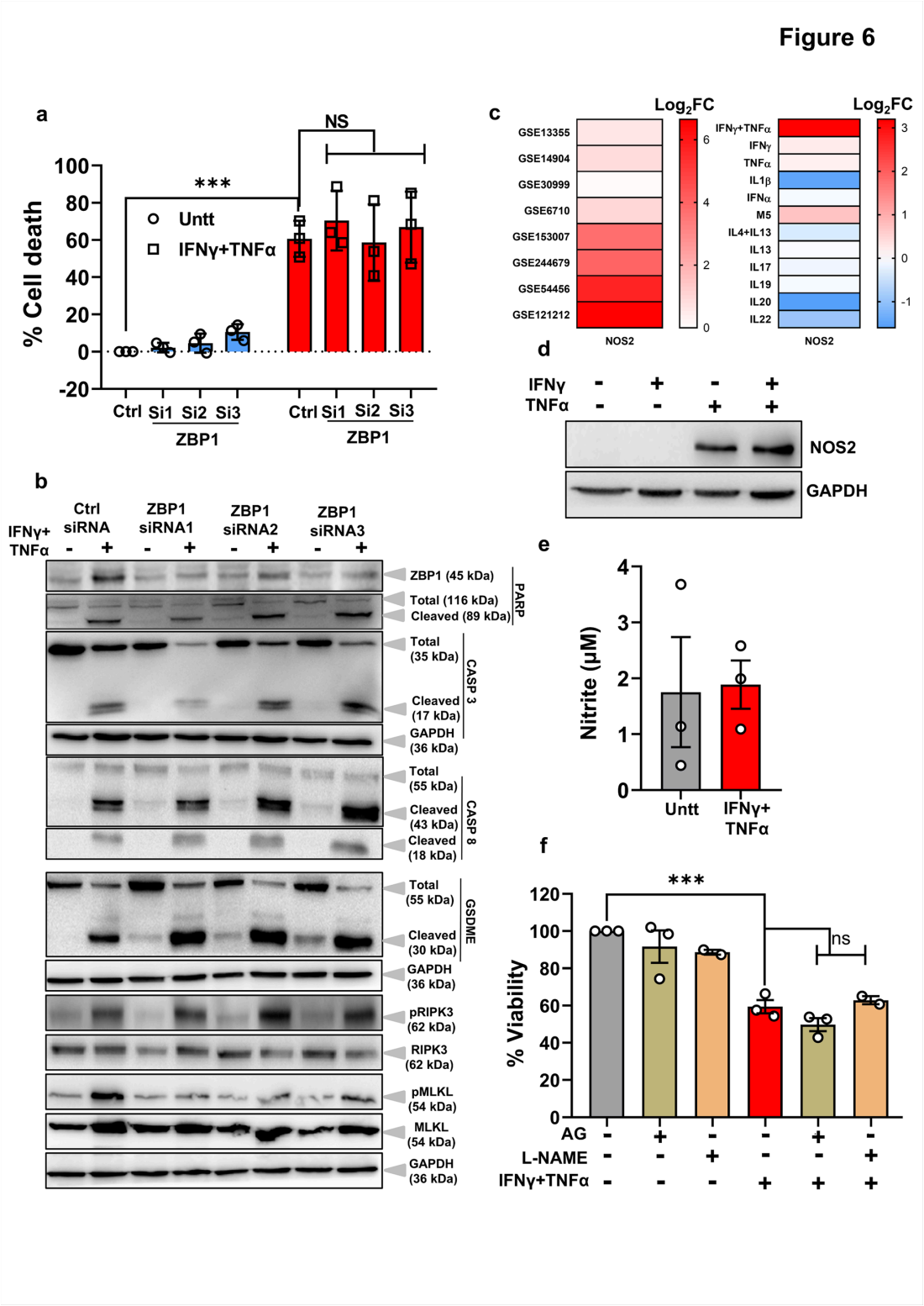


Figure 6

ZBP1 and nitric oxide (NO) are dispensable for IFNγ and TNFα induced keratinocyte PANoptosis. HaCaT cells were seeded in 6 well plates, transfected with three different siRNAs targeting ZBP1 or with scrambled siRNA for 24 h as described in materials and methods. Following that, cells were treated with IFNγ and TNFα for 24 h. The culture supernatant was used to measure cytotoxicity using LDH assay (**a**). Each dot is an average of two technical replicates per experiment. Experiments were conducted three

independent times. The bar diagram below shows the knockdown efficiency. Similar to a, the lysates were prepared and western blotting was performed for the indicated PANoptosis markers using specific primary and secondary antibodies as described in materials and methods (**b**). Transcriptome re-analysis was done for NOS2 in Psoriasis patient datasets (left) and in human keratinocytes treated with indicated cytokines (**c**). HaCaT cells were treated with IFNγ, TNFα alone or in combination for 24 h, lysates were prepared and western blotting was performed for inducible nitric oxide synthase (iNOS/NOS2) (**d**). Nitrite levels were measured from the culture supernatant of the untreated as well as IFNγ and TNFα treated keratinocytes (**e**). Cell viability was performed using MTT assay with and without the indicated NOS inhibitors as described in materials and methods (**f**). Experiments were performed three independent times with three technical replicates each time. Each dot represents mean of technical replicates from a single experiment. *** indicates $p<0.001$. One Way ANOVA followed with Tukey's multiple comparison test (**a** and **d**) was performed using Graph-Pad Prism version 8.0.

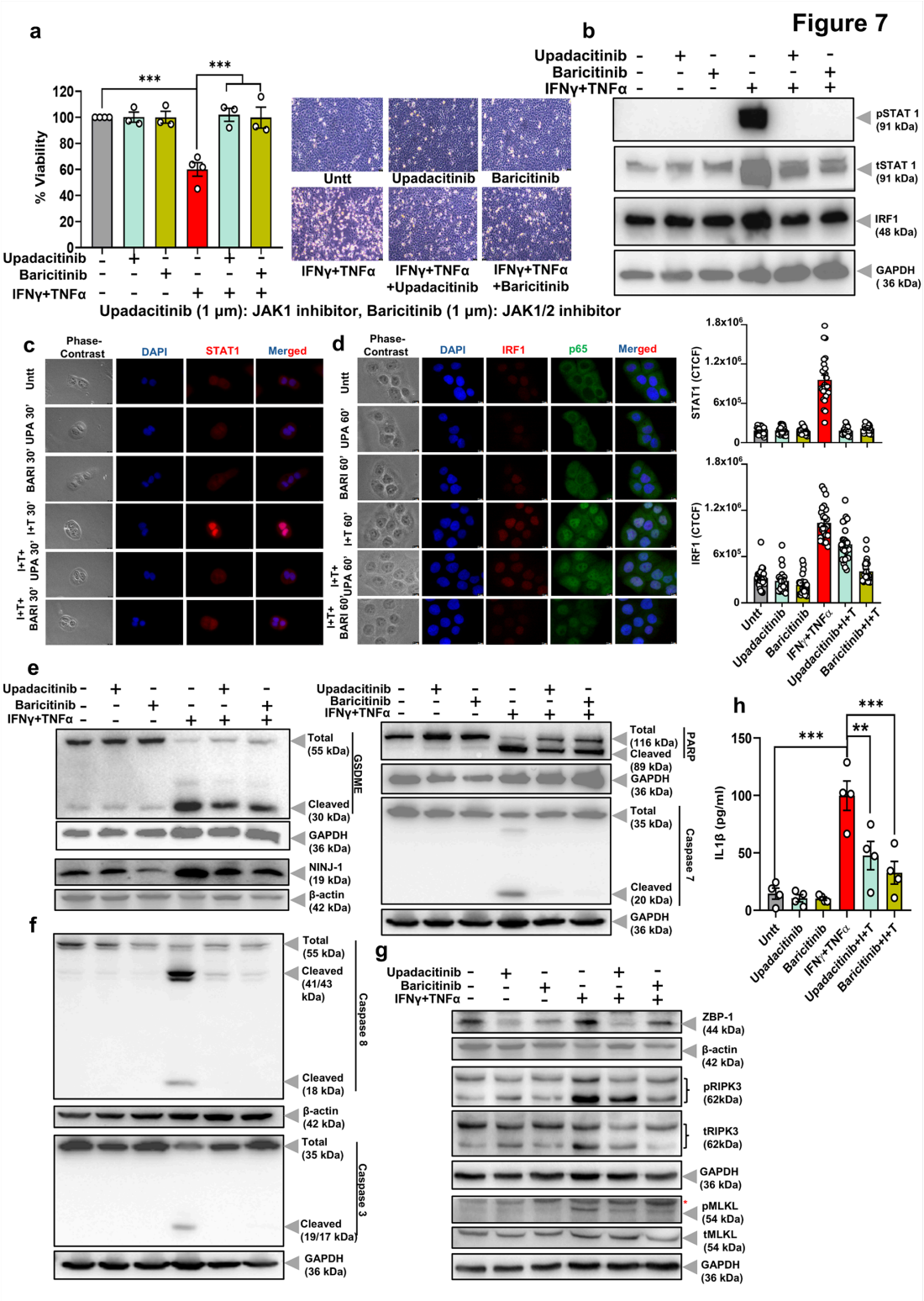


Figure 7

IFNγ-induced JAK-STAT signaling plays a central role in keratinocyte PANoptosis. HaCaT cells were seeded in 96 well plates, incubated with JAK inhibitors Upadacitinib and Bariticinib and 2 h later treated with IFNγ and TNFα for 24 h. Cell viability was measured using MTT assay (**a**). At the right-hand side of the graph, the cellular morphology of the cells were shown after IFNγ and TNFα treatment in presence and absence of the Upadacitinib and Baricitinib. Each dot represents average of three technical

replicates of each time. HaCaT cells were treated similarly to a, cell lysates were prepared and western blotting was performed for phospho STAT1, total STAT1 and IRF1 (**b**). Immunofluorescence was performed in the cells after IFNγ and TNFα treatment (30 minutes for STAT1 and 60 minutes for IRF1 and NF-κB-p65) in presence and absence of the Upadacitinib and Baricitinib as described in materials and methods (**c and d**). The corresponding fluorescence intensity profiles were shown at the right-hand side. Similar to b, cell lysates were prepared after IFNγ and TNFα treatment in presence and absence of the Upadacitinib and Baricitinib and then western blotting was performed for markers of Pyroptosis (GSDME and NINJ-1 in **e**), Apoptosis (Caspase 8, Caspase 3, PARP and Caspase 7 in **f**), and Necroptosis (ZBP1, pRIPK3/RIPK3 and pMLKL/MLKL in **g**). Each experiments were performed at least twice with similar results. IL1β was measured from the culture supernatant of the cells after IFNγ and TNFα treatment in presence and absence of the Upadacitinib and Baricitinib (**h**). Each dot is average of the three technical replicates performed in each experiment. *, **, and *** indicates $p<0.05$, $p<0.01$, and $p<0.001$ respectively. One Way ANOVA followed with Tukey's multiple comparison test (**a** and **h**) was performed using Graph-Pad Prism version 8.0.

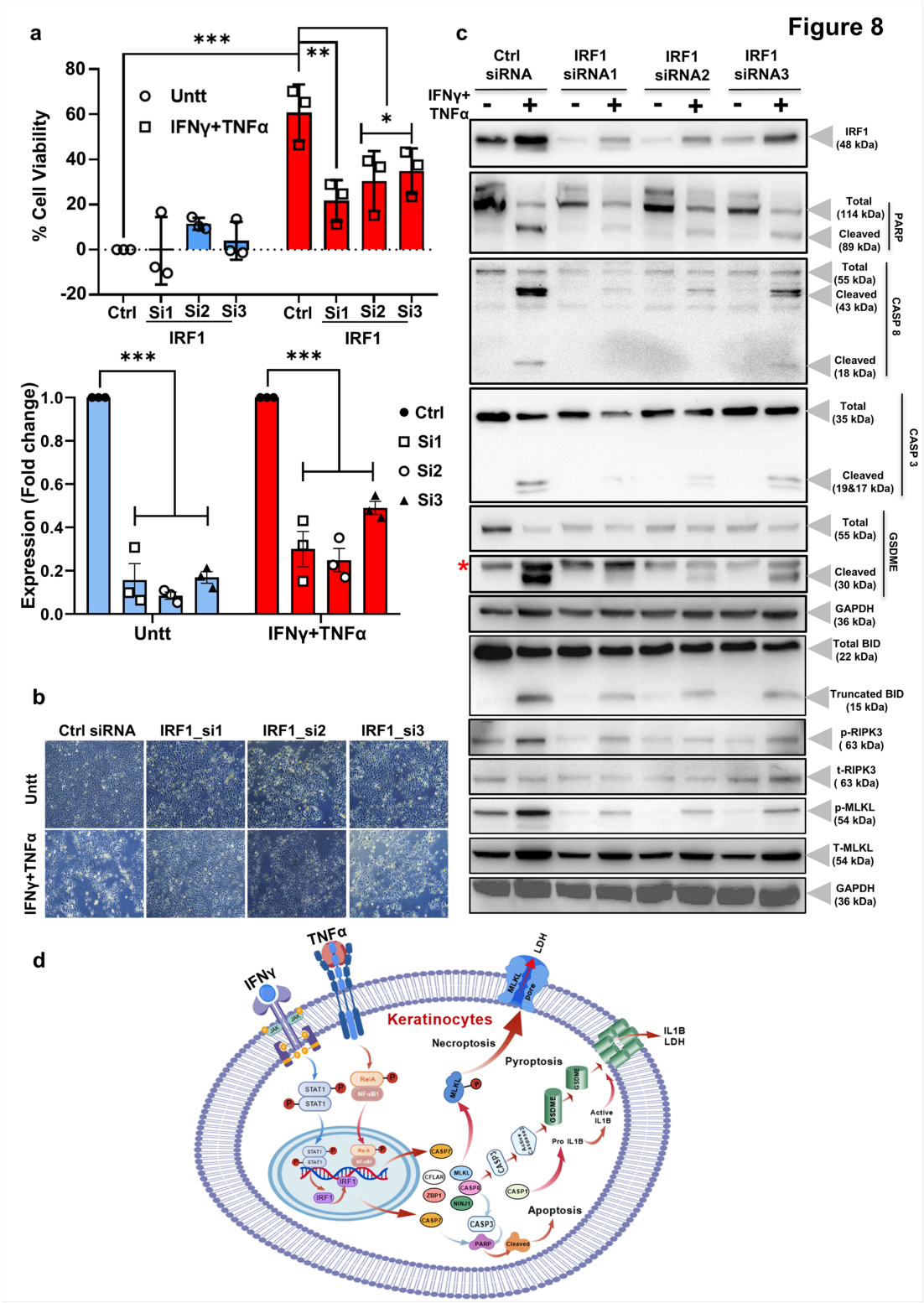


Figure 8

IFNγ-induced IRF1 plays a critical role in the PANoptosis of keratinocytes. HaCaT cells were seeded in 6 well plates, transfected with three different siRNAs targeting IRF1 or with scrambled siRNA for 24 h as described in materials and method. Following that, cells were treated with IFNγ and TNFα for 24 h. The culture supernatant was used to measure cytotoxicity using LDH assay, top panel (**a**). The knockdown efficiency was performed using western blotting from each transfection, bottom panel (**b**). Each dot is an

average of two technical replicates per experiment. Experiments were conducted three independent times. Morphological analysis was done for the IRF1 knockdown and scrambled siRNA transfected cells that are being treated with IFNγ and TNFα as described above (**b**), and cell lysates were used to measure the knockdown efficiency and indicated PANoptosis markers (**c**). Proposed mechanism of PANoptosis in keratinocytes by IFNγ and TNFα (**d**)*, **, and *** indicates $p<0.05$, $p<0.01$, and $p<0.001$ respectively. * Indicates nonspecific band. Two Way ANOVA followed with Tukey's multiple comparison test was performed using Graph-Pad Prism version 8.0.

Supplementary Files

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- SupplementaryTables.docx
- SupplementaryFigure6.tif
- Supplementaryfigure7.tif
- SupplementaryFigure1.tif
- Datasetanalysis.xlsx
- SupplementaryFigure3.tif
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- SupplementaryFigure8.tif
- SupplementaryFigure4.tif
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