Immunity, Volume *55*

Supplemental information

Interferon-γ primes macrophages for pathogen ligand-induced killing via a caspase-8 and mitochondrial cell death pathway

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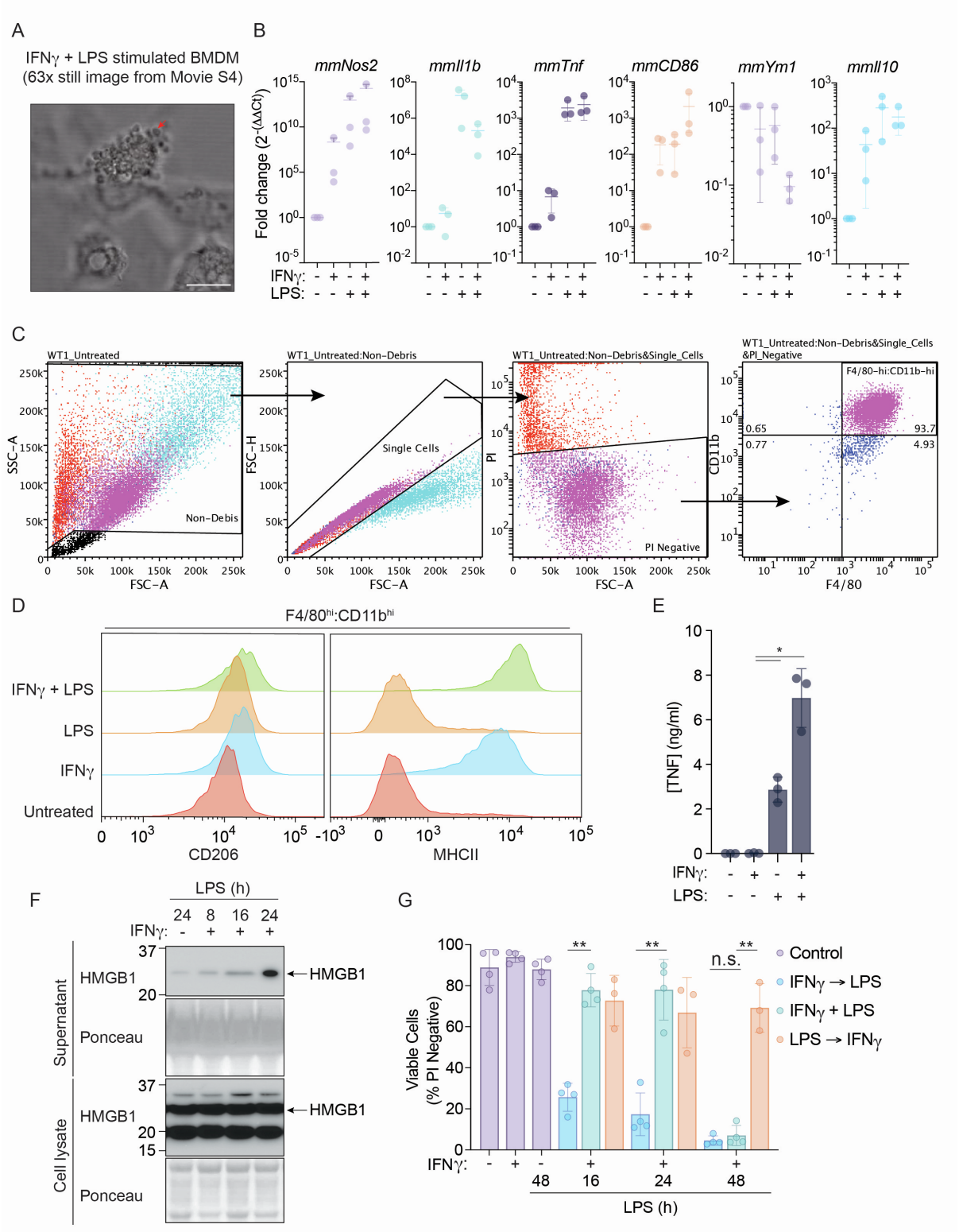

Figure S1. IFNγ primes macrophages for LPS-induced inflammatory-associated apoptotic cell death. Related to Figure 1.

(A) Representative still image from Movie S4 of WT BMDMs primed with IFNγ (50 ng/mL) overnight and then stimulated with LPS (50 ng/mL) for 24 h. Red arrow indicates apoptotic body formation. Scale bar (white) = 10 μm.

(B - E) WT BMDMs were treated with IFNγ (50 ng/mL) overnight, then with LPS (50 ng/mL) for 16 h (n = 3). (B) qPCR analysis was performed for expression of macrophage phenotypic markers and plotted as the fold-change expression relative to unstimulated BMDMs. *18s* was used as a housekeeping

gene expression control. (C and D) Macrophage receptor expression was assessed by flow cytometry. Gating strategy: Cell debris, doublets, and dead (PI positive cells) were removed. Representative data from one of three independent biological replicates displaying similar observations. (E) TNF secretion was measured by ELISA.
(F) Immunoblot analysis of cell supernatants and cell lysates of WT BMDMs primed with IFNγ (50 ng/mL) overnight and then stimulated with LPS (50 ng/mL) for 8 - 24 h. Ponceau staining is used as a loading control (n = 2).
(G) WT BMDMs were either primed with IFNγ (50 ng/mL) overnight and then stimulated with LPS (50 ng/mL) (IFNγ → LPS), co-stimulated at the same time with both IFNγ (50 ng/mL) and LPS (50 ng/mL) (IFNγ + LPS), or primed with LPS (50 ng/mL) overnight and then stimulated with IFNγ (50 ng/mL) (LPS → IFNγ) for 16, 24 or 48 h. Cell death was assessed by PI exclusion as measured by flow cytometry (n = 3 – 4).
Data represent the mean value ± SD, or a representative immunoblot or FACS plot, from indicated n independent experiments. $p > 0.05$ (n.s.), $p \leq 0.05$ (*), $p \leq 0.01$ (**).

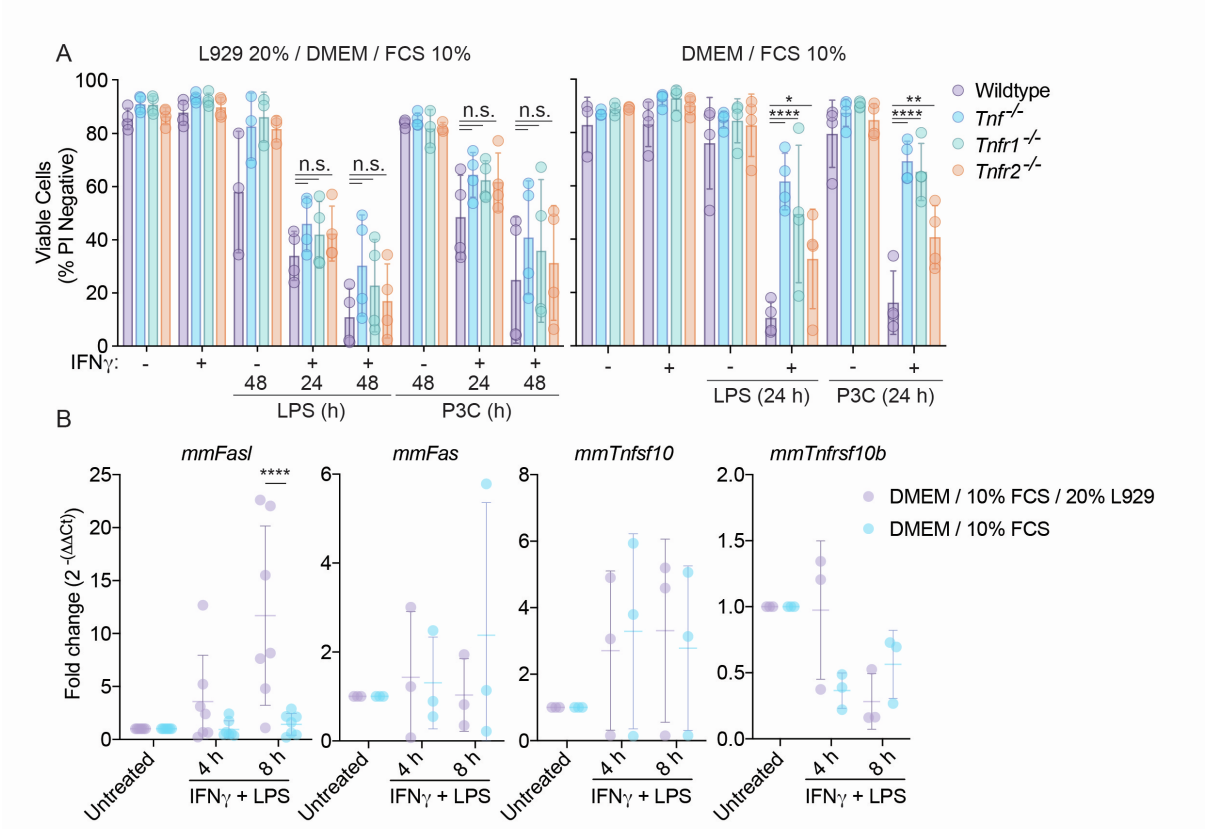

Figure S2. IFNγ/LPS-induced cell death correlates with L929 medium-dependent increases in *Fasl* expression and can occur independent of TNF signaling. Related to Figure 1.

(A) WT, *Tnf*$^{-/-}$, *Tnfrsf1a*$^{-/-}$ (TNFR1 deleted) *or Tnfrsf1b*$^{-/-}$ (TNFR2 deleted) BMDMs were treated with IFNγ (50 ng/mL) overnight, then with either LPS (50 ng/mL), or Pam3CSK4 (P3C, 500 ng/mL) for 24 or 48 h in either 20% L929 conditioned medium (vehicle medium: DMEM containing 10% FCS) (left graph) or DMEM containing 10% FCS (right graph). Cell death was assessed by PI exclusion as measured by flow cytometry (n = 4).

(B) WT BMDMs were treated with IFNγ (50 ng/mL) overnight, then with LPS (50 ng/mL) for 4 or 8 h, in either 20% L929 conditioned medium (vehicle medium: DMEM containing 10% FCS) or DMEM containing 10% FCS. qPCR analysis was performed for expression of *Fasl* (n = 7), *Fas* (n = 3), *Tnfsf10* (n = 3) or *Tnfsf10b* (n = 3) and plotted as the fold-change expression relative to unstimulated BMDMs. *Hprt* was used as a housekeeping gene expression control.

Data represent the mean value ± SD from indicated n independent experiments. $p > 0.05$ (n.s.), $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.0001$ (****).

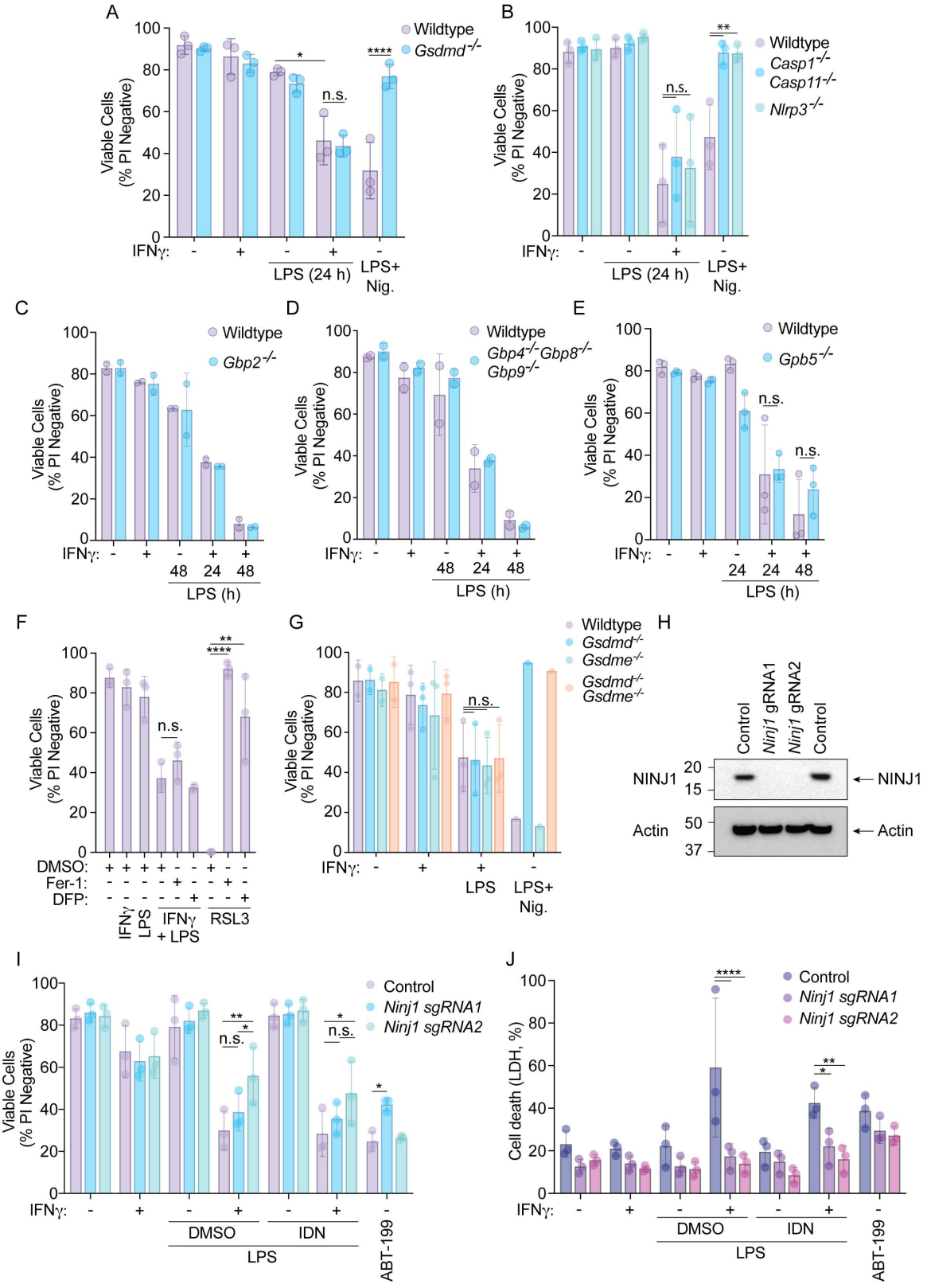

Figure S3. IFNγ/LPS-induced cell death proceeds in the absence of pyroptosis, ferroptosis and the cell lysis protein Ninj1. Related to Figure 2.

(A – E) WT and (A) *Gsdmd*$^{-/-}$ (n = 3), (B) *Nlrp3*$^{-/-}$, *Casp1*$^{-/-}$*Casp11*$^{-/-}$ (n = 3), (C) *Gbp2*$^{-/-}$ (n = 2), (D) *Gbp4*$^{-/-}$*Gbp8*$^{-/-}$*Gbp9*$^{-/-}$ (n = 2), (E) *Gbp5*$^{-/-}$ (n = 3) BMDMs were treated with IFNγ (50 ng/mL) overnight then with LPS (50 ng/mL) for 24 or 48 h. (A and B) Treatment with LPS (200 ng/mL) for 2 h then with

Nigericin (Nig., 10 μM) for 15 min (pyroptosis) was used as a control. Cell death was assessed by PI exclusion as measured by flow cytometry.
(F) WT BMDMs were treated with IFNγ (50 ng/mL) overnight then with LPS (50 ng/mL), DMSO or inhibitors of ferroptosis: Ferrostatin-1 (Fer-1, 2 μM) or Deferiprone (DFP, 150 μM) for 24 h. Pre-treatment with DMSO, Ferrostatin-1 (Fer-1, 2 μM) or Deferiprone (DFP, 150 μM) for 15 minutes, then treatment with RSL3 (500 nM) for 24 h (ferroptosis) was used as a controls. Cell death was assessed by PI exclusion as measured by flow cytometry (n = 3).
(G) WT, *Gsdmd*$^{-/-}$, *Gsdme*$^{-/-}$, or *Gsdmd*$^{-/-}$*Gsdme*$^{-/-}$ BMDMs were treated with IFNγ (50 ng/mL) overnight then with LPS (50 ng/mL) for 24 or 48 h. Treatment with LPS (200 ng/mL) for 2 h then with Nigericin (Nig., 10 μM) for 15 min (pyroptosis) was used as a control. Cell death was assessed by PI exclusion as measured by flow cytometry (n = 1 – 3).
(H) Immunoblot confirming NINJ1 gene deletion in two *Ninj1*$^{-/-}$ cell lines *vs* Control (Cas9) iBMDMs generated on the IFNγ/LPS sensitive *Mlkl*$^{-/-}$ background (n = 2).
(I and J) Control or *Ninj1*$^{-/-}$ iBMDMs validated in (H) were treated with IFNγ (50 ng/mL) overnight then with LPS (50 ng/mL), DMSO, IDN-6556 (IDN, 5 μM) for 24 h, or as a control ABT-199 (25 μM for 16 h) (Kayagaki *et al*., 2021). (I) Cell death was assessed by PI exclusion as measured by flow cytometry and (J) late-stage membrane rupture was assessed by LDH release into cell supernatants (n = 3).
Data represent the mean value ± SD, or a representative immunoblot, from indicated n independent experiments. $p > 0.05$ (n.s.), $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.0001$ (****)..

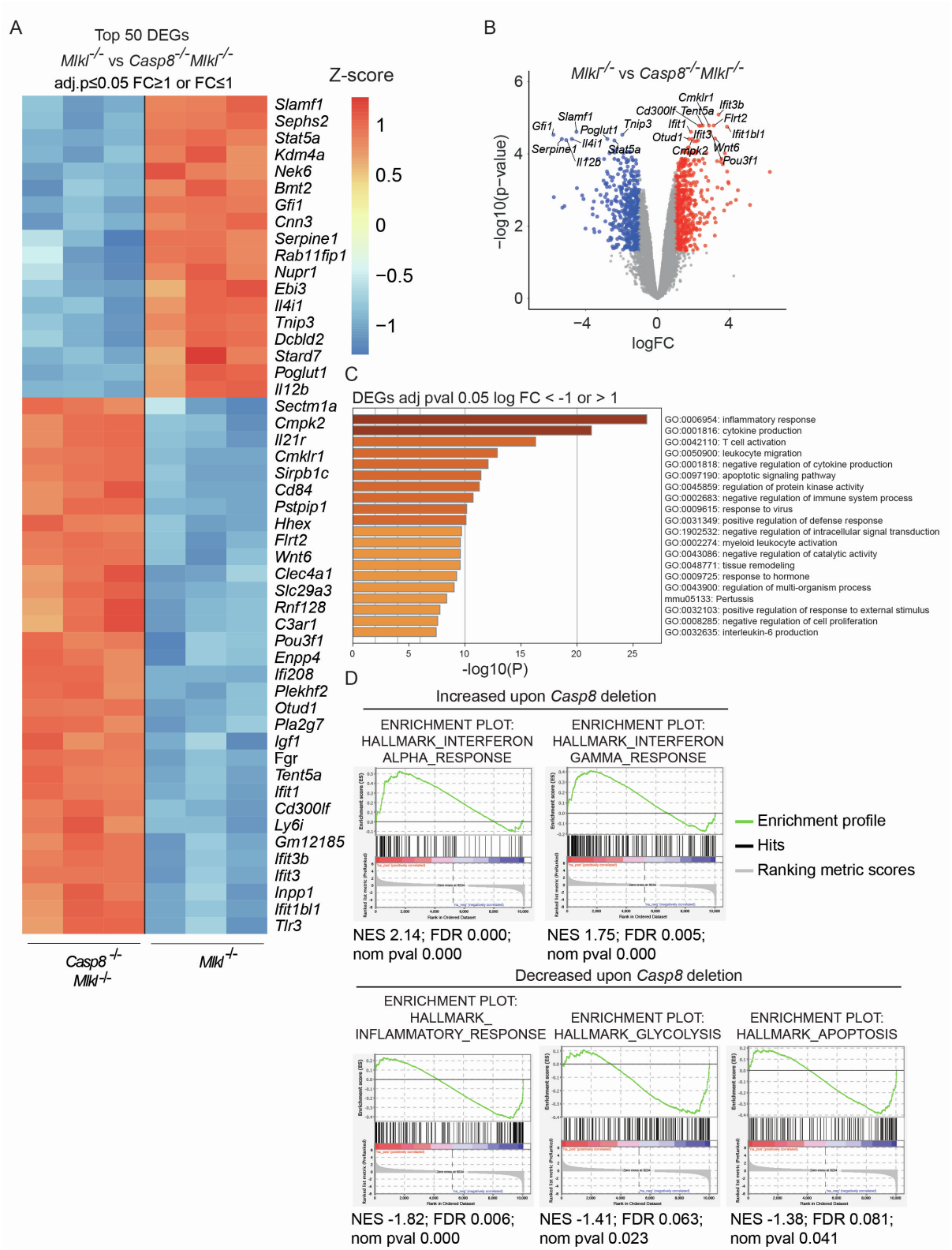

Figure S4. Caspase-8 mediates IFNγ/LPS-induced transcriptional programming in macrophages. Related to Figure 4.

Mlkl$^{-/-}$ and *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs were treated with IFNγ (50 ng/mL) overnight then LPS (50 ng/mL) for 7 h followed by RNA isolation and 3'RNA-sequencing, as described in Figure 4 (n = 3).

(A) Heatmap for top 50 significant differentially expressed genes (DEGs) for IFNγ/LPS stimulated *Mlkl*$^{-/-}$ *vs Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs. Adjusted p ≤ 0.05 and cut-off values logFC ≥ 1 or logFC ≤ -1.

(B) Volcano plot highlighting DEGs that are up- or down-regulated in IFNγ/LPS stimulated *Casp8*$^{-/-}$ *Mlkl*$^{-/-}$ BMDMs in comparison to similarly treated *Mlkl*$^{-/-}$ BMDMs. The top 20 DEGs are labelled. Adjusted $p \leq 0.05$ and cut-off values logFC ≥ 1 or logFC ≤ -1.
(C) Gene Ontology analysis for significant DEGs for IFNγ/LPS stimulated *Mlkl*$^{-/-}$ *vs* *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs. Adjusted $p \leq 0.05$ and cut-off values logFC ≥ 1 or logFC ≤ -1.
(D) GSEA analysis of genes differentially regulated between IFNγ/LPS treated *Mlkl*$^{-/-}$ BMDMs *vs* *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs.

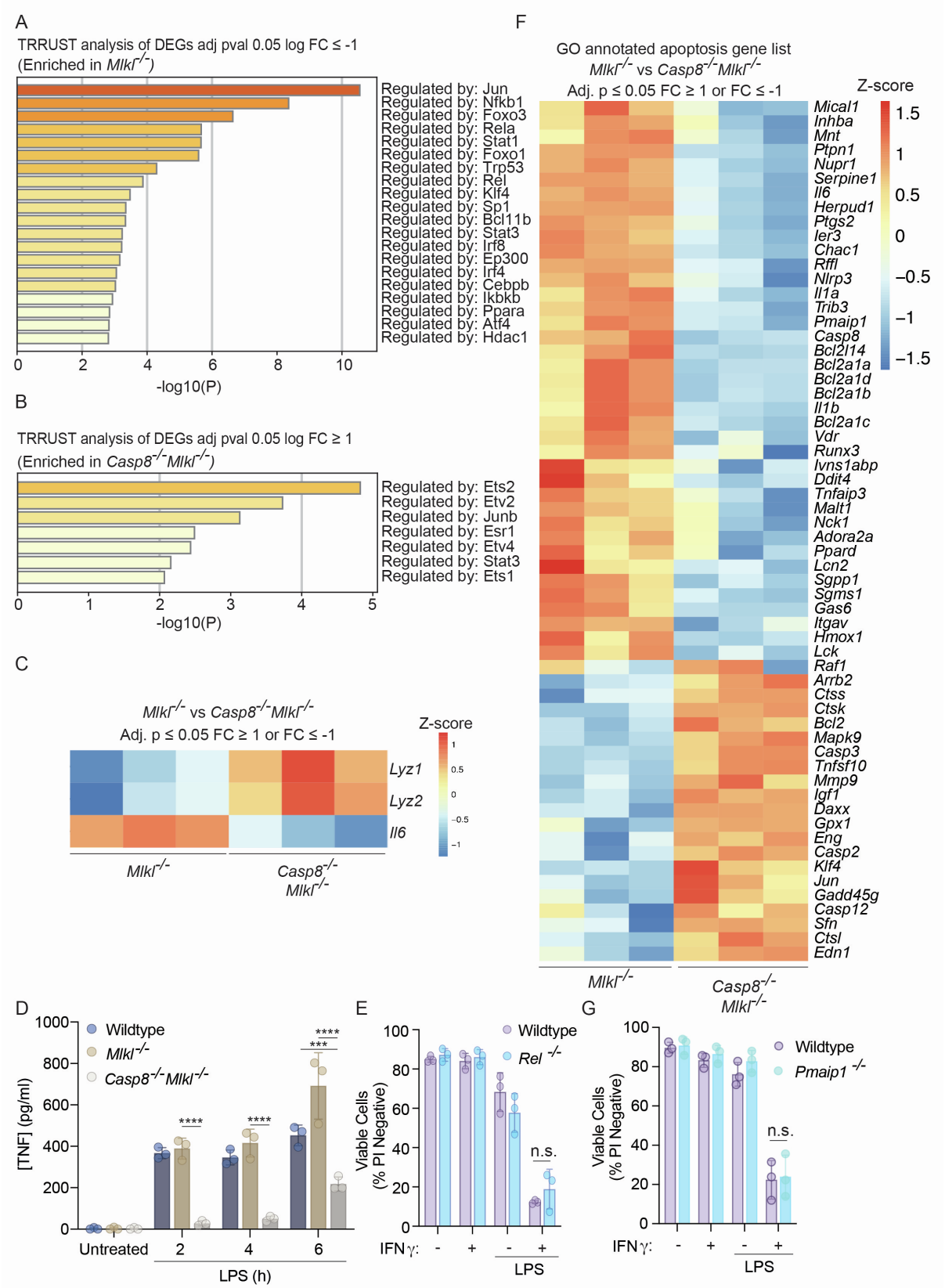

Figure S5. Upon IFNγ/LPS stimulation, caspase-8 regulates diverse transcriptional pathways to promote an inflammatory and metabolically active phenotype in macrophages. Related to Figure 4.

(A – C, F) *Mlkl*$^{-/-}$ and *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs were treated with IFNγ (50 ng/mL) overnight then LPS (50 ng/mL) for 7 h followed by RNA isolation and 3' mRNA-sequencing, as described in Figure 4 (n = 3).
(A) TRRUST analysis of significant DEGs downregulated in *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs as compared to *Mlkl*$^{-/-}$ BMDMs, displaying predicted transcription factors that regulate DEGs. Adjusted $p \leq 0.05$ and cut-off values logFC ≤ -1.
(B) TRRUST analysis of significant DEGs upregulated in *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs as compared to *Mlkl*$^{-/-}$ BMDMs, displaying predicted transcription factors that regulate DEGs. Adjusted $p \leq 0.05$ and cut-off values logFC ≥ 1.
(C) Heatmap for *Lyz1, Lyz2* and *Il6* expression in IFNγ/LPS-treated *Mlkl*$^{-/-}$ *vs Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs. Adjusted $p \leq 0.05$ and cut-off values logFC ≥ 1 or logFC ≤ -1.
(D) TNF secretion in wildtype (WT), *Mlkl*$^{-/-}$ or *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs stimulated with LPS (50 ng/mL) for 2, 4, or 6 h (n = 3).
(E) WT or *Rel*$^{-/-}$ BMDMs were treated with IFNγ (50 ng/mL) overnight then stimulated with LPS (50 ng/mL) for 24 h. Cell death was assessed by PI exclusion as measured by flow cytometry (n = 3).
(F) Heatmap for significant DEGs involved in GO-annotated apoptosis related pathways (see **Table S2 and S3**) that are enriched in IFNγ/LPS-treated *Mlkl*$^{-/-}$ BMDMs or *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs. Adjusted $p \leq 0.05$ and cut-off values logFC ≥ 1 or logFC ≤ -1.
(G) WT or *Pmaip1*$^{-/-}$ (lack the BH3-only protein NOXA) BMDMs were primed with IFNγ (50 ng/mL) overnight and then stimulated with LPS (50 ng/mL) for 24 h, as indicated. Cell death was assessed by PI exclusion as measured by flow cytometry (n = 3).
Data are presented as the mean ± SD of indicated n independent experiments. $p > 0.05$ (n.s.), $p \leq 0.001$ (***), $p \leq 0.0001$ (****).

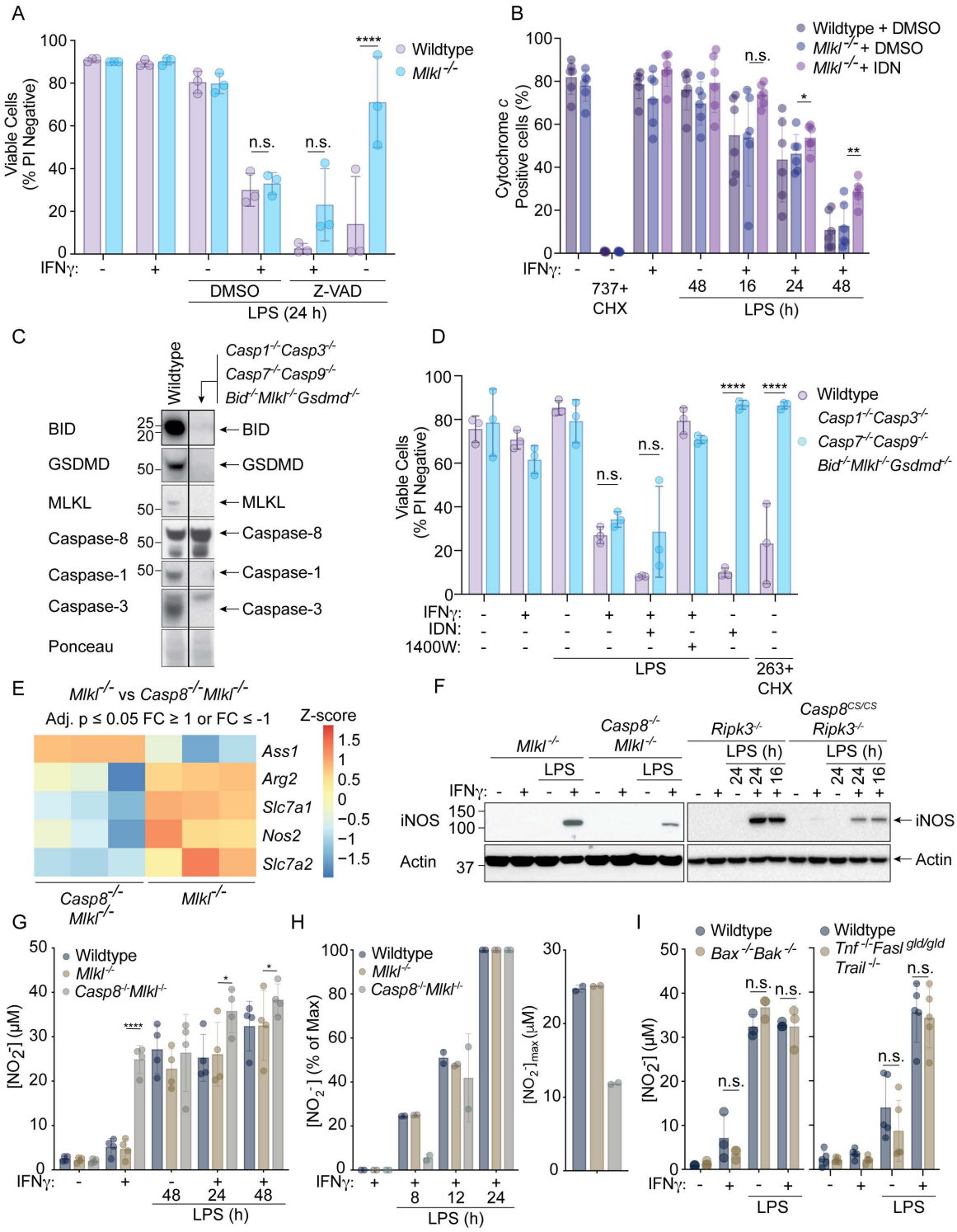

Figure S6. Caspase-8 activity promotes iNOS expression and IFNγ/LPS-induced cell death that is not blocked by pan-caspase inhibitor treatment. Related to Figure 5 and Figure 6.

(A) WT or *Mlkl*$^{-/-}$ BMDMs were treated with IFNγ (50 ng/mL) overnight then with LPS (50 ng/mL) for 24 h ± Z-VAD-fmk (Z-VAD, 20 μM). Treatment with LPS plus Z-VAD for 24 h (necroptosis) was used as a control (n = 3).

(B) WT or *Mlkl*$^{-/-}$ BMDMs were treated with IFNγ (50 ng/mL) overnight then with LPS (50 ng/mL) for 16, 24 or 48 h ± IDN-6556 (IDN, 5 μM). Treatment with ABT-737 (1 μM) plus cycloheximide (CHX, 10 μg/mL) for 6 h was used as a positive control for BAX/BAK activation. Cytochrome *c* retention was measured by intracellular cytochrome *c* staining and flow cytometric analysis (n = 6).

(C) Immunoblot validation of Control WT (Cas-9) or *Casp1*$^{-/-}$*Casp3*$^{-/-}$*Casp7*$^{-/-}$*Casp9*$^{-/-}$*Bid*$^{-/-}$*Mlkl*$^{-/-}$ *Gsdmd*$^{-/-}$ hepta-gene targeted immortalized BMDMs (iBMDMs) confirming deletion of indicated genes. Unrelated lanes from each probe were removed and is indicated by a line (n = 1).
(D) Control WT (Cas-9) or *Casp1*$^{-/-}$*Casp3*$^{-/-}$*Casp7*$^{-/-}$*Casp9*$^{-/-}$*Bid*$^{-/-}$*Mlkl*$^{-/-}$*Gsdmd*$^{-/-}$ hepta-gene targeted iBMDMs were treated with IFNγ (50 ng/mL) overnight then with LPS (50 ng/mL) for 24 h ± 1400W (10 μM), IDN (10 μM), or vehicle (DMSO) as a control. Treatment with LPS plus IDN (10 μM) for 24 h (necroptosis) or ABT-263 (1 μM) plus cycloheximide (CHX, 10 μg/mL) for 6 h (apoptosis) were used as controls. Cell death was assessed by PI exclusion as measured by flow cytometry (n = 3).
(E) Heatmap for significant DEGs involved in arginine metabolism, as detailed (Young *et al*., 2018), in *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs *vs Mlkl*$^{-/-}$ BMDMs treated with IFNγ/LPS. Adjusted $p \leq 0.05$ and cut-off values logFC ≥ 1 or logFC ≤ -1.
(F) Immunoblot analysis of *Mlkl*$^{-/-}$ or *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs (left) or *Ripk3*$^{-/-}$ or *Casp8*$^{-/-}$*Ripk3*$^{-/-}$ BMDMs (right) treated with IFNγ (50 ng/mL) overnight then with LPS (50 ng/mL) for 24 h (left) or 16 or 26 h (right) (n = 3).
(G) Nitrite (NO_2^-) concentrations in cell supernatants from WT and *Mlkl*$^{-/-}$ *and Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs treated with IFNγ (50 ng/mL) overnight then with LPS (50 ng/mL) for 24 or 48 h as measured by the Griess assay (n = 4).
(H) WT, *Mlkl*$^{-/-}$ *and Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs were treated with IFNγ (50 ng/mL) overnight then with LPS (50 ng/mL) for 8, 12, 24 h. Nitrite (NO_2^-) production was measured by the Griess assay. Maximal, LPS-induced NO_2^- production $[NO_2^-]_{max}$ was determined by subtracting the concentration of NO_2^- post-IFNγ priming (i.e. LPS at time 0) (right). NO_2^- concentrations at 8 and 12 hours were similarly normalized to the IFNγ-priming amounts and displayed as a percentage of the $[NO_2^-]_{max}$ (left) (n = 2).
(I) Nitrite (NO_2^-) concentrations at 48h in cell supernatants from WT and *Bax*$^{-/-}$*Bak*$^{-/-}$ BMDMs analyzed in Figure 2C (left), or WT and *Tnf*$^{-/-}$*Fasl*$^{gld/gld}$*Trail*$^{-/-}$ BMDMs analyzed in Figure 1C (right), was measured by the Griess assay (n = 3).
Data represent the mean value ± SD, or a representative immunoblot, from indicated n independent experiments. $p > 0.05$ (n.s.), $p \leq 0.05$ (*), $p \leq 0.001$ (***), $p \leq 0.0001$ (****).

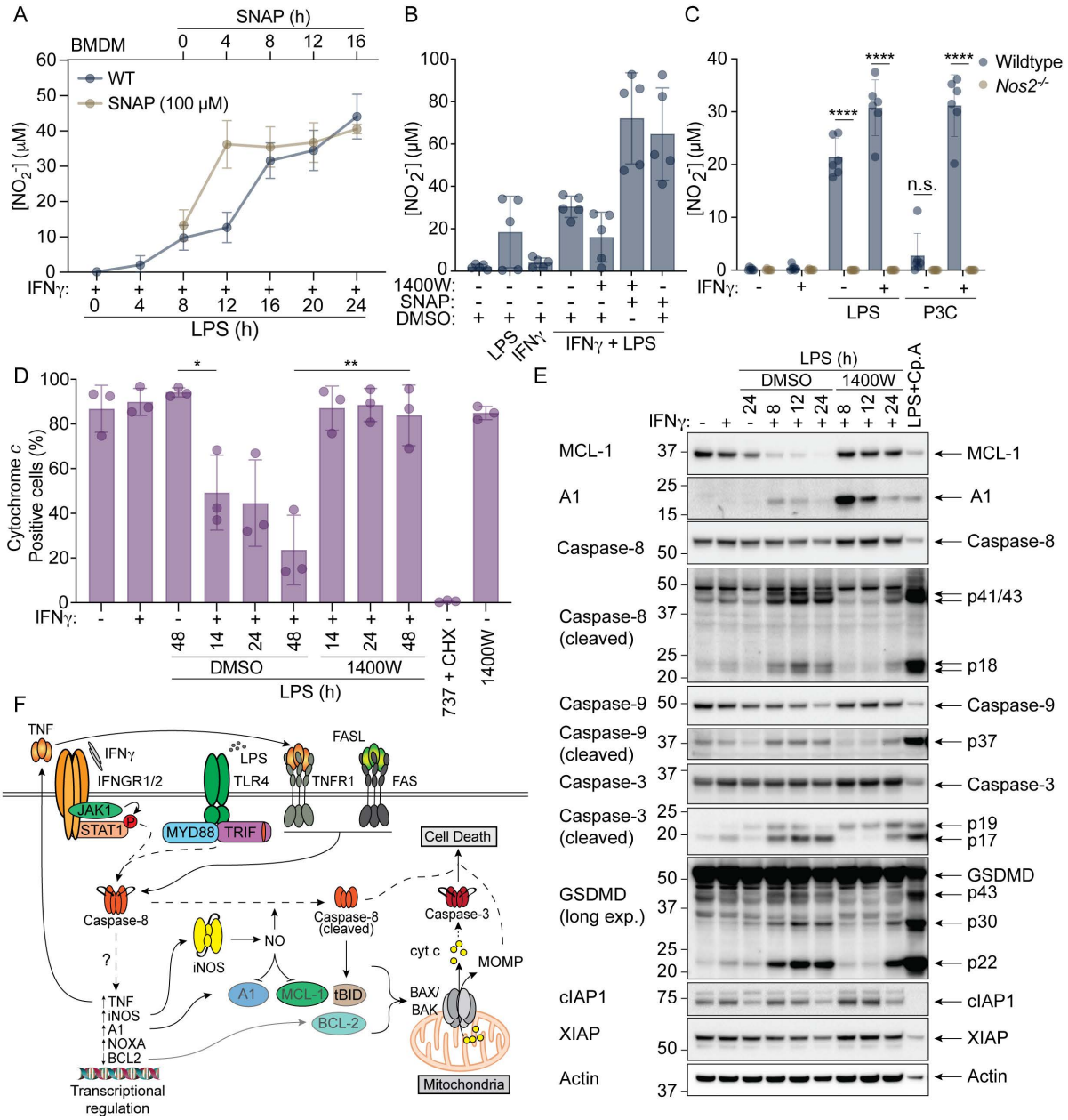

Figure S7. iNOS activity promotes caspase processing and destabilization of A1 and MCL-1 to orchestrate IFNγ/LPS-induced cell death. Related to Figure 6.

(A) WT BMDMs were treated with IFNγ (50 ng/mL) overnight then with LPS (50 ng/mL) (n = 4), or treated with SNAP (100 μM) only (n = 2). Time 0 is denoted as the addition of LPS (Bottom x-axis), or SNAP (Top x-axis). Nitrite (NO_2^-) production was measured by the Griess assay.

(B) Nitrite (NO_2^-) production of supernatants from cells analyzed in Figure 6D was measured by the Griess assay (n = 5).

(C) Nitrite (NO_2^-) production of supernatants from cells analyzed in Figure 6F was measured by the Griess assay (n = 6)

(D) WT BMDMs primed with IFNγ (50 ng/mL) overnight and then stimulated with LPS (50 ng/mL) ± 1400W (10 μM) or DMSO as a control. BMDMs treated with ABT-737 (1 μM) plus cycloheximide (CHX, 10 μg/mL) for 4 h are shown as a positive control for BAX/BAK-mediated cytochrome *c* release. Cytochrome *c* retention was measured by intracellular cytochrome *c* staining and flow cytometric analysis (n = 3).

(E) Immunoblot of WT BMDMs treated with IFNγ (50 ng/mL) overnight then with LPS (50 ng/mL) for 8, 12 or 24 h ± 1400W (10 μM) or vehicle. As controls for cell death pathway activation, BMDMs were also treated with LPS and Cp.A (1 μM) for 12 h (n = 3).

(F) Model depicting IFNγ priming and TLR activation induced macrophage cell death. IFNγ priming followed by LPS stimulation triggers caspase-8-regulated transcription of TNF, iNOS, A1 and NOXA. Caspase-8 also limits LPS-induced expression of *Bcl2*. Autocrine TNF and FASL production contribute to the caspase-8-mediated cell death response. iNOS expression licences caspase-8 processing via the activity of nitric oxide (NO), which in-turn promotes cleavage of BID. iNOS also limits expression of A1 and promotes MCL-1 degradation, which likely combines with reduced amounts of BCL-2 and activated BID to activate BAX and BAK-mediated mitochondrial outer membrane permeabilization (MOMP) and cytochrome *c* release into the cytosol. Cytochrome *c* release and apoptosome formation allows for caspase-3 and caspase-7 triggering and consequent rapid apoptosis. In the absence of BAX and BAK, caspase-8 activity drives MOMP-independent cell death.
Data represent the mean value ± SD, or a representative immunoblot, from indicated n independent experiments. $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.0001$ (****).

Table S1. Boutique list of genes associated with the major cell death pathways; Intrinsic and Extrinsic apoptosis, Pyroptosis and Necroptosis. Related to Figure 4C and 4D.

Extrinsic apoptosis		**Intrinsic apoptosis**		**Pyroptosis**		**Necroptosis**	
Gene	**Alias**	**Gene**	**Alias**	**Gene**	**Alias**	**Gene**	**Alias**
Tnf	TNF	*Casp9*	caspase-9	*Casp11*	caspase-11	*Ripk3*	RIPK3
Tnfrsf1a	TNFR1	*Apaf1*	APAF1	*Gsdme*	GSDME	*Mlkl*	MLKL
Tnfrsf1b	TNFR2	*Cycs*	cytochrome *c*	*Gsdmd*	GSDMD	*Zbp1*	ZBP1
Tnfrsf10a	DR4	*Bax*	BAX	*Casp1*	caspase-1		
Tnfrsf10b	DR5	*Bak*	BAK	*Nlrp3*	NLRP3		
Tnfsf10	TRAIL	*Bok*	BOK	*Aim2*	AIM2		
Fasl	FASL	*Bcl2*	BCL-2	*Mefv*	Pyrin		
Fas	FAS	*Bcl2l1*	BCL-XL	*Pycard*	ASC		
	TWEAK	*Bcl2a1a*	A1	*Nlrp1b*	NLRP1		
Tnfrsf12a	TWEAK Receptor	*Bcl2a1b*	A1	*Nek7*	NEK7		
Ripk1	RIPK1	*Bcl2a1c*	A1	*Birc1*	NAIP		
Tradd	TRADD	*Bcl2a1d*	A1	*Nlrc4*	NLRC4		
Traf2	TRAF2	*Bcl2l2*	BCL-W	*Il1b*	Il1β		
Birc2	cIAP1	*Bcl2l11*	BIM	*Il18*	Il18		
Birc3	cIAP2	*Bid*	BID				
Hoip	HOIP	*Bad*	BAD				
Hoil-1	HOIL	*Bbc3*	PUMA				
Sharpin	SHARPIN	*Pmaip1*	NOXA				
Tnfaip3	A20	*Bik*	BIK				
Mib1	MIB1	*Bmf*	BMF				
Mib2	MIB2	*Hrk*	HRK				
Birc4	XIAP						
Cyld	CYLD						
Spata2	SPATA2						
Map3k7	TAK1						
Mapk14	p38						
Mapkapk2	MK2						
Cflar	cFLIP						
Fadd	FADD						
Casp8	caspase-8						
Casp3	caspase-3						
Casp6	caspase-6						
Casp7	caspase-7						

Table S2. Apoptosis related Gene Ontology pathways enriched in IFNγ/LPSstimulated *Mlkl*$^{-/-}$ BMDMs or *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs[a]. Related to Figure 4 and Supplementary Figure S4.

GO Term	Description
GO:0008625	Extrinsic apoptotic signaling pathway via death domain receptors
GO:0008630	Intrinsic apoptotic signaling pathway in response to DNA damage
GO:0043154	Negative regulation of cysteine-type endopeptidase activity involved in apoptotic process
GO:0043281	Regulation of cysteine-type endopeptidase activity involved in apoptotic process
GO:0097190	Apoptotic signaling pathway
GO:0097191	Extrinsic apoptotic signaling pathway
GO:0097192	Extrinsic apoptotic signaling pathway in absence of ligand
GO:0097193	Intrinsic apoptotic signaling pathway
GO:1902041	Regulation of extrinsic apoptotic signaling pathway via death domain receptors
GO:1902042	Negative regulation of extrinsic apoptotic signaling pathway via death domain receptors
GO:2001233	Regulation of apoptotic signaling pathway
GO:2001234	Negative regulation of apoptotic signaling pathway
GO:2001236	Regulation of extrinsic apoptotic signaling pathway
GO:2001237	Negative regulation of extrinsic apoptotic signaling pathway
GO:0034392	Negative regulation of smooth muscle cell apoptotic process
GO:0008637	Apoptotic mitochondrial changes
GO:0090199	Regulation of release of cytochrome c from mitochondria
GO:0034390	Smooth muscle cell apoptotic process
GO:0034391	Regulation of smooth muscle cell apoptotic process
GO:0010660	Regulation of muscle cell apoptotic process
GO:0090201	Negative regulation of release of cytochrome *c* from mitochondria
GO:0043154	Negative regulation of cysteine-type endopeptidase activity involved in apoptotic process
GO:0010657	Muscle cell apoptotic process
GO:0010656	Negative regulation of muscle cell apoptotic process
mmu04210	Apoptosis

[a] Listed gene ontology pathways were significantly enriched (adjusted $p < 0.05$, logFC < - 1 or logFC > 1) in either *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ or *Mlkl*$^{-/-}$ BMDMs in our RNA-seq dataset.

Table S3. Apoptosis related Gene Ontology (GO) gene list[a] of significant differentially expressed genes in IFNγ/LPS stimulated *Mlkl*$^{-/-}$ BMDMs or *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ BMDMs. Related to Figure 4 and Supplemental Figure S5F.

Gene	Alias	Gene	Alias
Adora2a	Adenosine receptor A2a	*Il6*	Interleukin-6
Arrb2	β-arrestin-2	*Inhba*	Inhibin beta A chain
Bcl2	BCL-2	*Itgav*	Integrin alpha-V
Bcl2a1a	Bcl-2-related protein A1	*Ivns1abp*	Influenza virus NS1A-binding protein
Bcl2a1b	B-cell leukemia/lymphoma 2 related protein A1b	*Jun*	Transcription factor AP-1
Bcl2a1c	B-cell leukemia/lymphoma 2 related protein A1c	*Klf4*	Krueppel-like factor 4
Bcl2a1d	A1-d protein	*Lck*	Tyrosine-protein kinase Lck
Bcl2l14	Apoptosis facilitator Bcl-2-like protein 14	*Lcn2*	Neutrophil gelatinase-associated lipocalin
Casp12	caspase-12	*Malt1*	Mucosa-associated lymphoid tissue lymphoma translocation protein 1
Casp2	caspase-2	*Mapk9*	Mitogen-activated protein kinase 9
Casp3	caspase-3	*Mical1*	[F-actin]-monooxygenase MICAL1
Casp8	caspase-8	*Mmp9*	Matrix metalloproteinase-9
Chac1	Glutathione-specific gamma-glutamylcyclotransferase 1	*Mnt*	Max-binding protein MNT
Ctsk	Cathepsin K	*Nck1*	Cytoplasmic protein NCK1
Ctsl	Pro-cathepsin L	*Nlrp3*	NACHT, LRR and PYD domains-containing protein 3
Ctss	Cathepsin S	*Nupr1*	Nuclear protein 1
Daxx	Death domain associated protein 6	*Pmaip1*	Phorbol-12-myristate-13-acetate-induced protein 1 (NOXA)
Ddit4	DNA-damage inducible transcript 4	*Ppard*	Peroxisome proliferator-activated receptor delta
Ddx3x	ATP-dependent RNA helicase DDX3X	*Ptgs2*	Prostaglandin G/H synthase 2
Dipk2a	Divergent protein kinase domain 2A	*Ptpn1*	Tyrosine-protein phosphatase non-receptor type 1
Edn1	Endothelin-1	*Raf1*	RAF proto-oncogene serine/threonine-protein kinase
Eng	Endoglin	*Rffl*	E3 ubiquitin-protein ligase rififylin
Gadd45g	Growth arrest and DNA damage-inducible protein GADD45 gamma	*Runx3*	Runt-related transcription factor 3
Gas6	Growth arrest-specific protein 6	*Serpine1*	Plasminogen activator inhibitor 1
Gpx1	Glutathione peroxidase 1	*Sfn*	14-3-3 protein sigma
Herpud1	Homocysteine-responsive endoplasmic reticulum-resident ubiquitin-like domain member 1 protein	*Sgms1*	Phosphatidylcholine:ceramide cholinephosphotransferase 1
Hmox1	Heme oxygenase 1	*Sgpp1*	Sphingosine-1-phosphate phosphatase 1
Hspa1b	Heat shock 70 kDa protein 1B	*Tnfaip3*	Tumor necrosis factor alpha-induced protein 3 (A20)
Ier3	Radiation-inducible immediate-early gene IEX-1	*Tnfsf10*	Tumor necrosis factor ligand superfamily member 10 (TRAIL)
Igf1	Insulin-like growth factor I	*Trib3*	Tribbles homolog 3
Il1a	Interleukin-1-alpha	*Vdr*	Vitamin D3 receptor
Il1b	Interleukin-1-beta		

[a] This composite genes list was derived from all GO pathways identified in Table S2.

Table S4. Table of oligonucleotide primers used to assess gene expression by qPCR. Related to Figure 4F, S1B and S2B.

Oligonucleotide	Source	Identifier
qPCR primer for mm*18S (*For: 5' GTAACCCGTTGAACCCCATT)	Integrated DNA Technologies	N/A
qPCR primer for mm*18S (*Rev: 5' CCATCCAATCGGTAGTAGCG)	Integrated DNA Technologies	N/A
qPCR primer for mm*Bcl2 (*For: 5' CCTGTGGATGACTGAGTACCTG)	Integrated DNA Technologies	*OriGene Technologies Inc.* Cat#: MP201255
qPCR primer for mm*Bcl2 (*Rev: 5' AGCCAGGAGAAATCAAACAGAGG)	Integrated DNA Technologies	*OriGene Technologies Inc.* Cat#: MP201255
qPCR primer for mm*CD86 (*For: 5' TCAGTGATCGCCAACTTCAG)	Integrated DNA Technologies	N/A
qPCR primer for mm*CD86 (*Rev: 5' TTAGGTTTCGGGTGACCTTG)	Integrated DNA Technologies	N/A
qPCR primer for mm*Fasl (*For: 5' GAAGGAACTGGCAGAACTCCGT)	Integrated DNA Technologies	*OriGene Technologies Inc.* Cat#: MP204632
qPCR primer for mm*Fasl (*Rev: 5' GCCACACTCCTCGGCTCTTTTT)	Integrated DNA Technologies	*OriGene Technologies Inc.* Cat#: MP204632
qPCR primer for mm*Fas (*For: 5' CTGCGATTCTCCTGGCTGTGAA)	Integrated DNA Technologies	*OriGene Technologies Inc.* Cat#: MP204625
qPCR primer for mm*Fas (*Rev: 5' CAACAACCATAGGCGATTTCTGG)	Integrated DNA Technologies	*OriGene Technologies Inc.* Cat#: MP204625
qPCR primer for mm*Hprt (*For: 5' TGAAGTACTCATTATAGTCAAGGGCA)	Integrated DNA Technologies	N/A
qPCR primer for mm*Hprt (*Rev: 5' CTGGTGAAAAGGACCTCTCG)	Integrated DNA Technologies	N/A
qPCR primer for mm*Il10 (*For: 5' GGTTGCCCAGCCTTATCGGA)	Integrated DNA Technologies	N/A
qPCR primer for mm*Il10 (*Rev: 5' ACCTGCTCCACTGCCTTGCT)	Integrated DNA Technologies	N/A
qPCR primer for mm*Il1b (*For: 5' AGTTGACGGACCCCAAAAG)	Integrated DNA Technologies	N/A

qPCR primer for mm*Il1b (*Rev: 5' AGCTGGATGCTCTCATCAGG)	Integrated DNA Technologies	N/A
qPCR primer for mm*Nos2 (*For: 5' GCCACCAACAATGGCAACA)	Integrated DNA Technologies	N/A
qPCR primer for mm*Nos2 (*Rev: 5' CGTACCGGATGAGCTGTGAATT)	Integrated DNA Technologies	N/A
qPCR primer for mm *Tnfrsf10b (*For: 5' TGTGTCGATGCAAACCAGGCAC)	Integrated DNA Technologies	*OriGene Technologies Inc.* Cat#: MP217750
qPCR primer for mm*Tnfrsf10b (*Rev: 5' GCCGTTTTGGAGACACACTTCC)	Integrated DNA Technologies	*OriGene Technologies Inc.* Cat#: MP217750
qPCR primer for mm*Tnfsf10 (*For: 5' GGAAGACCTCAGAAAGTGGCAG)	Integrated DNA Technologies	*OriGene Technologies Inc.* Cat#: MP217736
qPCR primer for mm*Tnfsf10 (*Rev: 5' TTTCCGAGAGGACTCCCAGGAT)	Integrated DNA Technologies	*OriGene Technologies Inc.* Cat#: MP217736
qPCR primer for mm*Ym1 (*For: 5' GGGCATACCTTTATCCTGAG)	Integrated DNA Technologies	N/A
qPCR primer for mm*Ym1 (*Rev: 5' CCACTGAAGTCATCCATGTC)	Integrated DNA Technologies	N/A