Appendix

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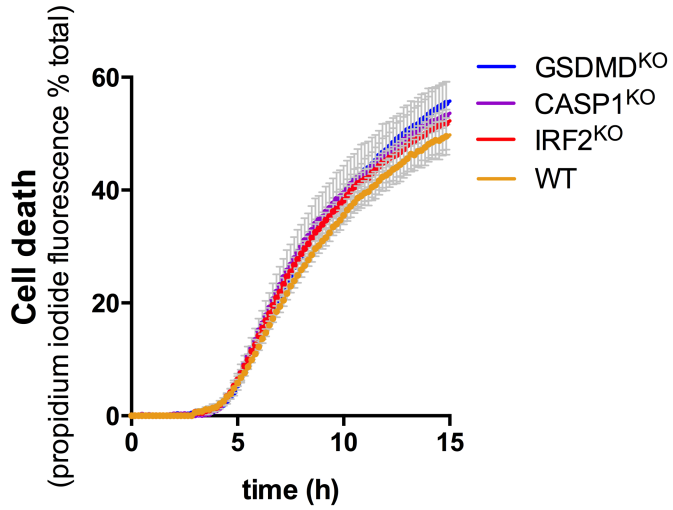
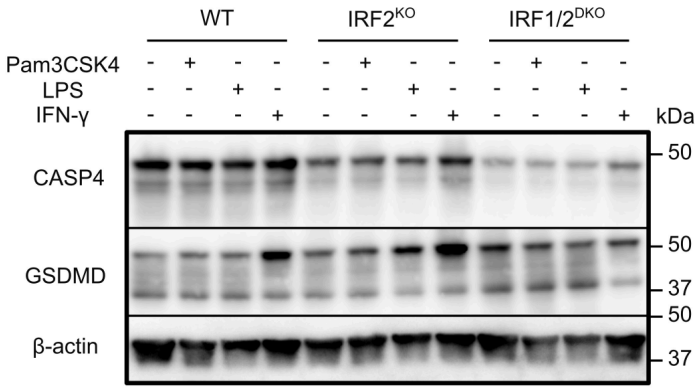


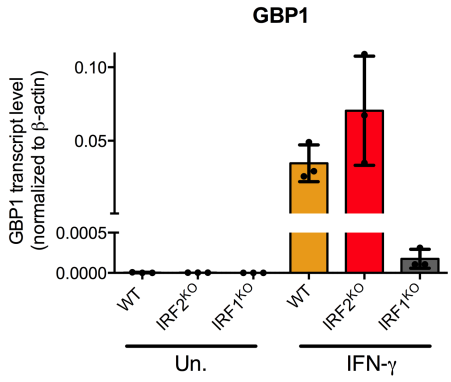
Appendix figure S1: Nigerin triggers inflammasome-independent necrosis in unprimed U937 cells

U937 cells of the indicated genotypes (in the absence of PMA and TLR agonist) were treated with nigericin. Cell death was monitored in real time by quantifying propidium iodide (PI) incorporation/fluorescence. Cell death was normalized using untreated and TX-100-treated sample. One experiment representative of two experiments is shown. Each dot represents the mean (+/-SD) of a biological triplicate.



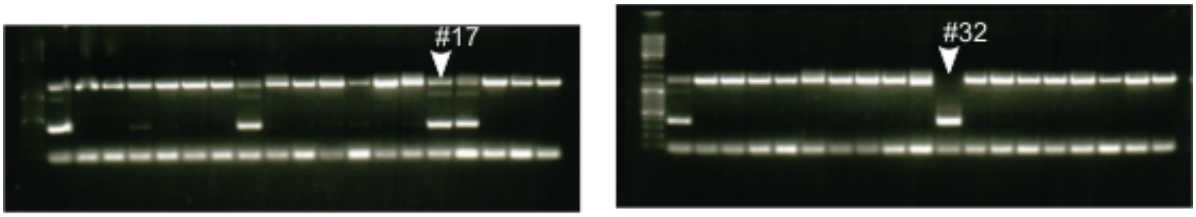
Appendix figure S2: TLR ligands in contrast to IFN-γ do not increase caspase-4 levels in *IRF2*^{KO} cells.

U937 cells of the indicated genotypes were treated with Pam3CSK4 (1 μg/ml), LPS (100 ng/ml) or IFN-γ (1000 u/ml) for 16 h. Caspase-4, gasdermin D and β-actin levels were determined by western blot analysis.



Appendix figure S3: *GBP1* transcript level is regulated by IRF1 in the presence of IFN-γ.

GBP1 transcript levels were quantified by qRT-PCR in the indicated cell lines in the presence or absence (Un.) of IFN-γ (1000 u/ml, 16 h) and normalized to β-actin levels. One dot represents the average of three technical qRT-PCR values of one experiment, the bar represents the mean (+/- SD) of three independent experiments.



Appendix figure S4: Profile of iPSC clones invalidated for *IRF2*.

IRF2 sequence flanking the two sgRNA was amplified by PCR in different iPSC clones. Clones showing a PCR fragments differing from WT iPSC were selected and sequenced. Clones 17 (presenting a 900 bp on one allele and a 900pb inversion on the second allele) and 32 (homozygous 900pb deletion) were selected for futher investigations

Cell line	Targeted gene	Validation	Ref
U937 Cas9 clone	*None*	WB[1]	(lagrange et al. 2018)
U937-CASP1	*CASP1*	WB[1]	(lagrange et al. 2018)
U937-CASP4	*CASP4*	WB[1]	(lagrange et al. 2018)
U937-GSDMD	*GSDMD*	WB[1]	(lagrange et al. 2018)
U937-IRF1^{KO}	*IRF1*	WB[1]	This work
U937-IRF2^{KO}	*IRF2*	WB[1]	This work
U937-IRF3^{KO}	*IRF3*	88%[2]	This work
U937-IRF4 ^{KO}	*IRF4*	93%[2]	This work
U937-IRF5 ^{KO}	*IRF5*	AG[3]	This work
U937-IRF6 ^{KO}	*IRF6*	91%[2]	This work
U937-IRF7 ^{KO}	*IRF7*	>50%[4]	This work
U937-IRF8 ^{KO}	*IRF8*	85%[2]	This work
U937-IRF9 ^{KO}	*IRF9*	95%[2]	This work
U937-IRF1/2^{DKO}	*IRF1*	WB[1]	This work
	IRF2	WB[1]	
U937-IRF2/3^{DKO}	*IRF2*	WB[1]	This work
	IRF3	89%[2]	
U937-IRF2/4^{DKO}	*IRF2*	WB[1]	This work
	IRF4	98%[2]	
U937-IRF2/5^{DKO}	*IRF2*	WB[1]	This work
	IRF5	AG[3]	
U937-IRF2/6^{DKO}	*IRF2*	WB[1]	This work
	IRF6	93%[2]	
U937-IRF2/7^{DKO}	*IRF2*	WB[1]	This work
	IRF7	>50%[4]	
U937-IRF2/8^{DKO}	*IRF2*	WB[1]	This work
	IRF8	89%[2]	
U937-IRF2/9^{DKO}	*IRF2*	WB[1]	This work
	IRF9	93%[2]	
U937-INTS6^{KO}	*INTS6*	84%[2]	This work
U937-PDHA1^{KO}	*PDHA1*	88%[2]	This work
U937-DET1^{KO}	*DET1*	43%[2]	This work
U937-WDR73 ^{KO}	*WDR73*	80%[2]	This work
U937-IKBKE ^{KO}	*IKBKE*	94%[2]	This work
U937-COP1 ^{KO}	*COP1*	93%[2]	This work
U937-TFAP4 ^{KO}	*TFAP4*	98%[5]	This work
U937-ARPC1A ^{KO}	*ARPC1A*	80%[2]	This work
U937-FIG4 ^{KO}	*FIG4*	83%[2]	This work
U937-IFI27L1 ^{KO}	*IFI27L1*	77%[2]	This work
U937-MTF1 ^{KO}	*MTF1*	88%[2]	This work
U937-RUNX1 ^{KO}	*RUNX1*	85%[2]	This work
iPSC-WT	*NA*		NIMH
iPSC-CASP1^{KO}#C14	*CASP1*	WB[1], AG[3], 100%[2]	This work
iPSC- IRF2^{KO}#C17	*IRF2*	WB[1], AG[3], 100%[2]	This work
iPSC- IRF2^{KO}#C32	*IRF2*	WB[1], AG[3], 100%[2]	This work

1: WB: Validation by Western blotting analysis
2: Overall efficiency determined for 1 sgRNA by sequencing of the flanking region and analysis by tide.
3: AG: gene deletion visible by DNA electrophoresis on agarose gel
4: Overall efficiency determined for 1 sgRNA by sequencing of the flanking region and visual analysis (due to high GC content and poor sequencing quality, TIDE automatic analysis could not be used)
5: Including 72% of non-disrupting deletions (-3 or -9 nucleotides)
NA: Not applicable

Appendix Table 1: Cell lines generated by CRISPR/Cas9 with the validation method. The two cell lines in red were not validated due to low CRISPR efficiency <50% or due to a high frequency of non ORF-disrupting indels.

Gene target	sgRNA #	Sequence (5'-3')
GSDMD	1	TGAGTGTGGACCCTAACACC
	2	AGGTTGACACACTTATAACG
CASP1[1]	1	ACAGACAAGGGTGCTGAACA
	2	ATGGAAACAAAAGTCGGCAG
CASP1[2]	1	TATTGAGAGCAAGACGTGTG
	2	GACAGTATTCCTAGAAGAAC
CASP4	1	AGTTATCCAAAACACCAGTG
	2	TGCAGCTCATCCGAATATGG
IRF1	1	GAACTCCCTGCCAGATATCG
	2	TCTAGGCCGATACAAAGCAG
IRF2[1,2]	1	CAGCATTCGGTAGACCCTGA
	2	GGATGCATGCGGCTAGACAT
IRF3	1	AGAAGGGTTGCGTTTAGCAG
	2	GAGGTGACAGCCTTCTACCG
IRF4	1	CAGACCCGTACAAAGTGTAC
	2	CCCATGACGTTTGGACCCCG
IRF5	1	AGAAGCGCTGCTTGTCACTG
	2	AGATCAAGTTTCAGTACCGG
IRF6	1	AAGGGCTGTATAGGTGCCTG
	2	CAAGTTTCAGTACCGTGGGA
IRF7	1	CACCTTGAAGATGCGCGCGT
	2	GATGCACTCACCTTGCACCG
IRF8	1	ATGGCTCGGAAATGTCCAGT
	2	CTTCTGTGGACGATTACATG
IRF9	1	AACTGAGGCCCCCTTTCAAG
	2	AATTTAAGGAGGTTCCTGAG
INTS6	1	AAAGTCCGGAGCCGATCACA
	2	ACTACAGGATGAGATGTACG
PDHA1	1	AGCACTGATTACTACAAGAG
	2	GATGCAGACTGTACGCCGAA
DET1	1	AATCAGTGACCCCCAACCCA
	2	ACGCACGTTCAAGTGTGACA
RUNX1	1	TAGATGATCAGACCAAGCCC
	2	CTGATCGTAGGACCACGGTG
MTF1	1	GCACATTCGAACTCATACAG
	2	AATGCACTTCCACAACACAA
PACS1	1	AGATCTGGGCCACAGCACGC
	2	GATGAACCTGTACGCCACCT

ZNF699	1	TTTGTGTTCTGTGAGCGATG
	2	CAAAACTCACTGTACATGGG
WDR73	1	AAAGACGCCCATCTGAGCCA
	2	GATTTCAAAGTGCGCCATGG
IKBKE	1	AGCATCCCGACATGTATGAG
	2	CGTGCACAAGCAGACCAGTG
TRIM28	1	CCAGCGGGTGAAGTACACCA
	2	CTTCCCAGGCAGTACCACTG
COP1	1	GATTCTTATGGAATTCCTCA
	2	TACCAATCTAGACAACTCAG
TFAP4	1	ACAGCTCAAGCGCTTCATCC
	2	AGGCTCCCCGGACATCTGGG
ARPC1A	1	CATGTGACTTCAAATGCAGG
	2	GAGCACAACGGACACATCAC
FIG4	1	CACAGGTGGAATGAACTAGG
	2	GAAGGATTAATTACACAAGG
IFI27L1	1	GAGAGTGGATGGGACTCAGG
	2	GGCTGTGGGGACTGTGCTCG

Appendix table 2: Sequences of all sgRNA used to generate knock-out cell lines.

[1]sgRNA used to generate U937 mutated cell lines

[2]sgRNA used to generate iPSC deleted clones

gene	Sequence (5'-3')	Ref
CASP1	CTGGGGACTCTCAGCAGATCA	(Lagrange et al., 2018)
	ATAGCTGGGTTGTCCTGCAC	
CASP4	GGCAGGACAAATGCTTCTTC	(Lagrange et al., 2018)
	GACAAAGCTTGAGGGCATCT	
NLRP3	CACCTGTTGTGCAATCTGAAG	(Lagrange et al., 2018)
	GCAAGATCCTGACAACATGC	
GBP1	CCAGTGCTCGTGAACTAAGGA	(Lagrange et al., 2018)
	TGTCAGTGGATCTCTGATGC	
ASC	GGGACCAAGGGTGTAGTAAGG	this work
	ACTCTGGTCTCCCGACTCC	
β-actin	ATTGGCAATGAGCGGTTC	(Lagrange et al., 2018)
	CGTGGATGCCACAGGACT	

Appendix table 3: qRT-PCR primers used in this study