

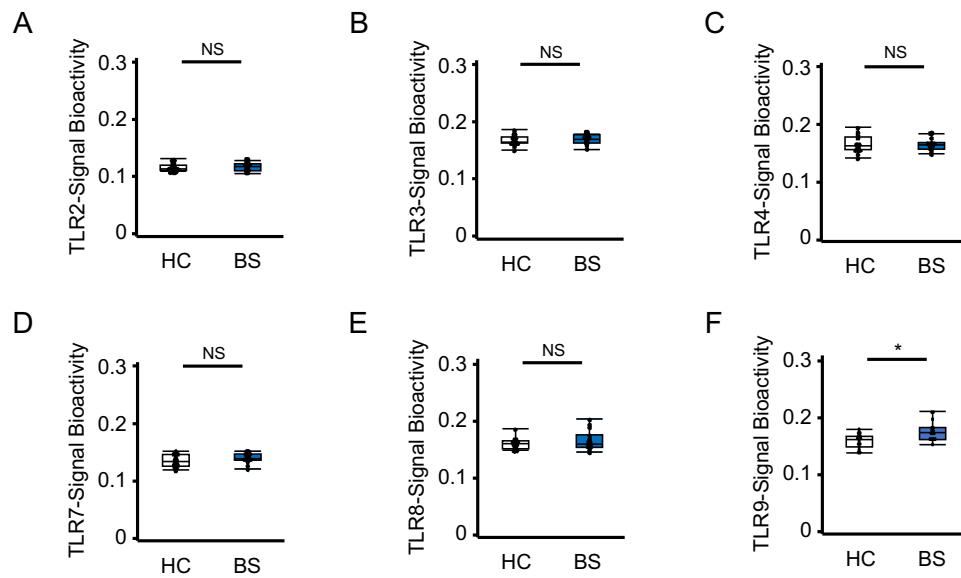
## **Appendix Figure and Tables**

### **Pyroptotic secretion of mitochondrial DNA via exosomes promotes inflammation in Behçet's syndrome**

Appendix Figure S1: **Lack of contamination by other TLR ligands.**

Appendix Table S1: **The summarized characterization of the enrolled patients**

Appendix Table S2: **Details of the reagents, antibodies, cell lines, and primers.**



**Appendix Figure S1: Lack of contamination by other TLR ligands.**

**A–F)** Response of TLR-reporter cells to HC and BS serum. HC and BS serum was added to the HEK-Blue-TLR2 (**A**), -TLR3 (**B**), -TLR4 (**C**), -TLR7 (**D**), -TLR8 (**E**), and -TLR9 (**F**) reporter cells, and SEAP levels in the CS were determined with QUANTI-Blue (OD<sub>620</sub>). Statistical analyses were performed using a Mann–Whitney U test (**A–F**) (mean  $\pm$  SD; \* $p < 0.05$ ; NS, not statistically significant). The data are representative of three independent experiments.

A

Characteristics and clinical manifestations	BS (n=59)	HC (n=15)
Age (years)	44 (37-55)	23 (22-24)
Sex (female)	25 (42.3)	2 (13.3)
Oral ulcers	21 (35.6)	
Uveitis	7 (11.9)	
Skin lesions	10 (16.9)	
Genital ulcers	8 (13.6)	
Arthralgias	7 (11.9)	
Intestinal lesions	7 (11.9)	
Vascular involvements	1 (1.7)	
Nervous system involvements	2 (3.4)	
WBC ( $\times 10^9/L$ )	7.510 (6.010-10.390)	
CRP (mg/dL)	0.1 (0.04-0.76)	
Corticosteroid	20 (36.3)	
Colchicine	27 (49.1)	
Infliximab	19 (34.5)	

B

Characteristics	RA (n=12)	SLE (n=22)	SjS (n=10)	AAV (n=14)	PM/DM (n=10)	SSc (n=9)
Age (years)	61.5 (51.5-70)	44.5 (38.5-60)	56 (48.5-67)	71 (68-77)	51 (41.3-60.5)	61 (59.4-64)
Sex (female)	11 (91.6)	15 (68.2)	9 (90)	5 (35.7)	8 (80)	6 (66.7)

**Appendix Table S1. The summarized characterization of the enrolled patients**

Enrolled patient's profiles of BS and HC (A), and other rheumatic diseases (B). Data are presented as n (%) or median (IQR). CRP, C-reactive protein; WBC, white blood cell; HC, healthy control; BS, Behçet's syndrome; RA, Rheumatoid Arthritis; SLE, Systemic Lupus erythematosus; SjS, Sjogren Syndrome; AAV, ANCA associated Vasculitis; PM/DM, Polymyositis/Dermatomyositis; SSc, Systemic sclerosis.

## Reagents and Kits

Name	Vender	Catalog number
Adenosine5-triphosphate (ATP) disodium salt hydrate	Sigma	A1852
Nigericin sodium salt Ready Made Solution (NIG)	Sigma	SML1779
Lipopolysaccharides from Escherichia coli O111:B4 (LPS)	Sigma	L3024
Phorbol 12-myristate 13-acetate (PMA)	Sigma	P1585-1MG
N-Formyl-Met-Leu-Phe	Sigma	F3506-5MG
DNase1 Amplification Grade	Sigma	AMPD1-1KT
IL-1R antagonist	Sigma	407616-25MGCN
ODN2006	InvivoGen	tlr-2006
2,3-cGAMP	InvivoGen	tlrl-cga23
Ac-YVAD-cmk	InvivoGen	178603-78-6
Monosodium urate crystals	InvivoGen	tlrl-msu-25
Recombinant human IL-12/p70	PeptoTech	200-12
Recombinant human IL-17	PeptoTech	200-17
Recombinant human TNF $\alpha$	PeptoTech	300-01A
Recombinant human IL1beta/IL1F2	R & D	201-LB-025
Recombinant human IL-23	R & D	1290-IL-010
Recombinant human IL-18/IL-1F4	R & D	9124-IL-010
IL-23R blocking peptide	BD BioScience	MBS9222188
Staurosporine	KOM	AG-CN-0022-C100
5-(N,N-dimethyl)-Amiride(DMA)	CAY	19100
Mito-TEMPO	ENZO	ALX-430-150-M005
Pertussis toxin(PTx)	List Biological Laboratories	LBL-0901-80
Ionomycin Ca2+ salt	Abcam	ab120116
Complete Freund's Adjuvant (CFA)	Chondrex	#7023
IRBP peptide (1-20) human	ANASPEC	AS-62297
Triton(R)X-100	Nacalai	35501-02
Q-Vai-Asp-Oph	R & D	OPH001-01M
Birinapant	CAY	19699
Amicon Ultra-15	Millipore	UFC901024, R9CA90257
<b>ELISA</b>		
Name	Vender	Catalog number
Human exosome CD63 ELISA kit	CosmoBio	HAK-HEL6363-1
Highly Sensitive ELISA kit for 8-OHdG	JalCA	KOG-HS10/E
<b>LDH Cytotoxicity assay</b>		
Name	Vender	Catalog number
CytoSelect LDH Cytotoxicity Assay kit	CosmoBio	CBA-241
<b>immune cells isolation</b>		
Name	Vender	Catalog number
Ficoll-Paque plus	Cytiva (GE)	17144003
Polymorphprep	CosmoBio	1114683
<b>DNA isolation</b>		
Name	Vender	Catalog number
MagNA Pure Compact	Roche Life Science	
DNA Extractor SP Kit	WAKO	296-60501
DNA Extractor WB kit	WAKO	291-50502
Quant-iT™ dsDNA Assay Kits, high sensitivity (HS) and broad range (BR)	InvitroGen	Q33120
<b>RNA isolation</b>		
Name	Vender	Catalog number
Rneasy Mini kit (250)	QIAGEN	74106
QIA shredder (250)	QIAGEN	79656
<b>Real-Time PCR</b>		
Name	Vender	Catalog number
TB Green Fast qPCR Mix	TaKaRa	RR430A
<b>Transfection Reagent</b>		
Name	Vender	Catalog number
LentiGuide-Puro	Addgene	
Avalanche-Omni Transfection Reagent	EZ biosystems	EZT-OMNI-1-1.5

Gene Editing Reagent		
Name	Vender	Catalog number
In-Fusion HD cloning kit	TaKaRa	Z9649N
DNA Ligation Kit (Mighty Mix)	TaKaRa	6023
PrimeSTAR GXL DNA polymerase	TaKaRa	R050A
CUGA7 gRNA Synthesis kit	Nippon Gene	314-08691
ISOSPIN PCR Product	Nippon Gene	315-08001
Establishment of p0 cell Reagent		
Name	Vender	Catalog number
L-Glutamine(200mM)	Thermo Fisher	A2916801
Sodium Pyruvate(100mM)	Thermo Fisher	11360070
Uridine>99%	Sigma	U3750-1G
Ethidium bromide solution (EtBr)	Sigma	E1385-5ML
Isolation of Mitochondria		
Name	Vender	Catalog number
Mitochondria/Cytosol Fractionation Kit	Bio Vision	#K256-25

## Antibodies

Antibodies for western blotting			
Name	Vender	Catalog number	clone
ASC(B-3)	Santa Cruz	sc-514414	G0716
Anti-TLR9(26C593)	Santa Cruz	sc-52966	E0818
Rb mAb to ProCaspase1+p10+p12	Abcam	ab179515	GR3232708-2
Rb mAb to Gasdermin D	Abcam	ab210070	
Anti-cleaved N-terminal GSDMD Antibody	Abcam	ab215203	
Anti-TSG101 Ms mAb	Abcam	ab83	4A10
Anti-Calnexin (C5C9) Rabbit mAb	Cell Signaling	2679S	
Anti-Lamp1 Rabbit mAb	Abcam	ab24170	
Anti- $\beta$ actin Rabbit mAb (HRP Conjugate)	Cell Signaling	#5125S	
Anti-p38 MAPK Rabbit Ab	Cell Signaling	#9212S	
Anti-pp38 MAPK Rabbit Ab	Cell Signaling	#9211S	
Anti-p42/44 MAPK(Erk1/2) Rabbit Ab	Cell Signaling	137F5	
Anti-pp42/44 MAPK(Erk1/2) Rabbit Ab	Cell Signaling	T202/Y204	
VADC Rabbit Ab	Cell Signaling	#4866S	
Purified Mouse Anti-Tom20	BD BioScience	612278	
Purified Mouse Anti-Tim23	BD BioScience	611222	
Purified Mouse Anti-Flotillin-1	BD BioScience	610821	
mAb anti-FLAG M2-Peroxidase(HRP) antibody	Sigma	A8592-1MG	046K6075
Monoclonal Anti-acetylated Tublin antibody produced in mouse	Sigma	109M4831V	
pAb anti-NLRP3/NALP3	NOVUS	NBP2-12446	B-3
Anti-hIL-1b Affinity Purified Goat IgG	RSD	AF-201-NA	QM1018101
Ms mAb Anti-Hu CD9	Invivogen	AHS0902	
Anti-CD63 (LAMP3) mAb	MBL	MEX002-3	
Cleaved Caspase-8 Rabbit mAb	Cell Signaling Technology	#9496	
TFAM antibody	Cell Signaling Technology	#7495	
Anti-Sodium Potassium ATPase antibody	Abcam	ab76020	
Apolipoprotein A1 antibody	GeneTex	GTX112692	
Antibodies for flow cytometry and confocal microscopy			
Name	Vender	Catalog number	clone
Mito Tracker Green	InvitroGen	M7514	
Mito Tracker DeepRed	InvitroGen	M22426	
MitoSox Red Mitochondrial Superoxide Indicator	InvitroGen	M36008	2127454
Quanti-iT PicoGreen dsDNA reagent	InvitroGen	1094619	
Anti-CD11b PE (clone M1/70)	BD BioScience	553311	64266
Anti-Ly6G APC-Cy7 (clone 1A8)	Bio Legend	128031	B250488
Anti-Ly6C Brilliant Violet (clone HK1.4)	Sirigen	127623	B238261

Antibodies for Immune electron microscopy			
Name	Vender	Catalog number	clone
Anti-8-hydroxy-deoxyguanosine monoclonal antibody	JaLCA	MOG-020P	
Anti-dsDNA antibody	Abcam	ab27156	
Anti-mouse IgG(H+L) 10nm Gold	BBI Solutions	EM.GMHL10	
Nanogold®-Fab' Goat anti-Mouse IgG(H+L)	Nanoprobes	#2002	
Beads for AutoMACS			
Name	Vender	Catalog number	clone
Human CD14 microbeads	Miltenyi Biotec	130-050-201	
Beads for Cell culture			
Name	Vender	Catalog number	clone
Purified Anti-human CD28 (clone CD28.2)	BD BioScience	14-0289-82	E025695
Purified Anti-human CD3 (clone OKT3)	BD BioScience	16-0037-85	E019164

### Reporter cells for measurement of cytokine bioactivity and TLR signaling

Name	Vender	Catalog number
HEK-Blue IL-1 $\beta$	InvivoGen	hkb-il1b
HEK-Blue IL-12	InvivoGen	hkb-il12
HEK-Blue IL-17	InvivoGen	hkb-il17
HEK-Blue IL-18	InvivoGen	hkb-il18
HEK-Blue IL-23	InvivoGen	hkb-il23
HEK-Blue TNF $\alpha$	InvivoGen	hkb-tnfadmyd
HEK-Blue TLR2	InvivoGen	hkb-htlr2
HEK-Blue TLR3	InvivoGen	hkb-htlr3
HEK-Blue TLR4	InvivoGen	hkb-htlr4
HEK-Blue TLR7	InvivoGen	hkb-htlr7
HEK-Blue TLR8	InvivoGen	hkb-htlr8
HEK-Blue TLR9	InvivoGen	hkb-htlr9

### Primers

mtDNA primer for Real-Time PCR		
Human cytochrome C oxidase subunitIII ( <i>CoxIII</i> )	Fw	5'-ATGACCCACCAATCACATGC-3'
	Rv	5'-ATCACATGGCTAGGCCGGAG-3'
Human NADH dehydrogenase ( <i>NADH</i> )	Fw	5'-ATACCCATGGCCAACCTCCT-3'
	Rv	5'-GGGCCTTTCGTAGTTGTAT-3'
Primers and vector for gene cloning		
Human TLR9 cloning primer (infusion)	Fw	5'-GTGACAGATCCAAGGTGAAGT-3'
	Rv	5'-CTTCTCTACAAATGCATCACT-3'
3*FLAG Oligo promoter	Fw	5'-GACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGACTACAAGGATGACGATGACAAGTAGTGA-3'
	Rv	5'-TCACTACTTGTCTATCGTATCCTTGTAGTCGATGTCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTC-3'
Human caspase1+3*FLAG cloning primer (infusion)	Fw (lenti-Casp)	5'-AATTCTGCAGCGGCCGCGCCACCATGGCCGACAAG-3'
	Rv(Casp-FLAG)	5'-CCGTCATGGTCTTTGTAGTCATGTCCTGGGAAGAGGTAGA-3'
	Rv (FLAG-Lenti)	5'-GGAGAGGGGCGGATCCCTACCAAGAAGGCTCAAAG-3'
Human NLRP3+3*FLAG cloning primer (infusion)	Fw (Lenti-NLRP3)	5'-AATTCTGCAGCGGCCGCGCCACCATGAAGATGGCAAGCACCCG-3'
	Rv (NLRP3-FLAG)	5'-CCGTCATGGTCTTTGTAGTCCTCAAGAAGGCTCAAAGACGA-3'
	Rv (FLAG-Lenti)	5'-GGAGAGGGGCGGATCCCTACCAAGAAGGCTCAAAG-3'
Human mutant NLRP3(Val200Met) cloning primer	Fw (Lenti-NLRP3)	5'-AATTCTGCAGCGGCCGCGCCACCATGAAGATGGCAAGCACCCG-3'
	Rv1 (mutation sequence)	5'-AATGGGACTCATGGGGCTCT-3'
	Fw2 (mutation sequence)	5'-AGAGCCCCATGAGTCCATT-3'
	Rv (FLAG-Lenti)	5'-GGAGAGGGGCGGATCCCTACCAAGAAGGCTCAAAG-3'
Caspase1 p10+3*FLAG cloning primer (infusion)	Fw	5'-AATTCTGCAGCGGCCGCGCCACCATGGCTATTAAGA-3'
	Rv	5'-CCGTCATGGTCTTTGTAGTCATGTCCTGGGAAGAGGTAGA-3'
Caspase1 p20+3*FLAG cloning primer (infusion)	Fw	5'-AATTCTGCAGCGGCCGCGCCACCATGAACCCAGCT-3'
	Rv	5'-CCGTCATGGTCTTTGTAGTCATCTTTAAACACACCCACAC-3'
Gasmd-NT-3*FLAG	Eurofins Genomics	
TSG101-mCherry	VectorBuilder	pLV[Exp]-Puro-EF1A>mCherry/hTSG101[NM_006292.4]

Target sequence for Generation of ko-cell-lines		
Generation of KO-NLRP3 THP1 cell	sgRNA sequence	5'- CGAAGCAGCACTCATGCGAG-3' 5'- GTCTGATTCCGAAGTCACCG-3' 5'- ATTCTCCCCACATAGAGTC-3'.
Generation of KO-Caspase1 THP1 cell	sgRNA sequence	5'- GACAGTATTCCTAGAAGAAC-3' 5'- TAATGAGAGCAAGACGTGTG-3', 5'- GCTCCCTAGAAGAAGCTCAA-3'.
Generation of KO-GasderminD THP1 cell	sgRNA sequence	5'- CGGCTCTCACCTGTGCGGG-3' 5'- CTTGCTTTAGACGTGCAGCG-3' 5'- ACCTCCAGCCACCCGCGAC-3'.

**Appendix Table S2: Details of the reagents, antibodies, cell lines, and primers.**