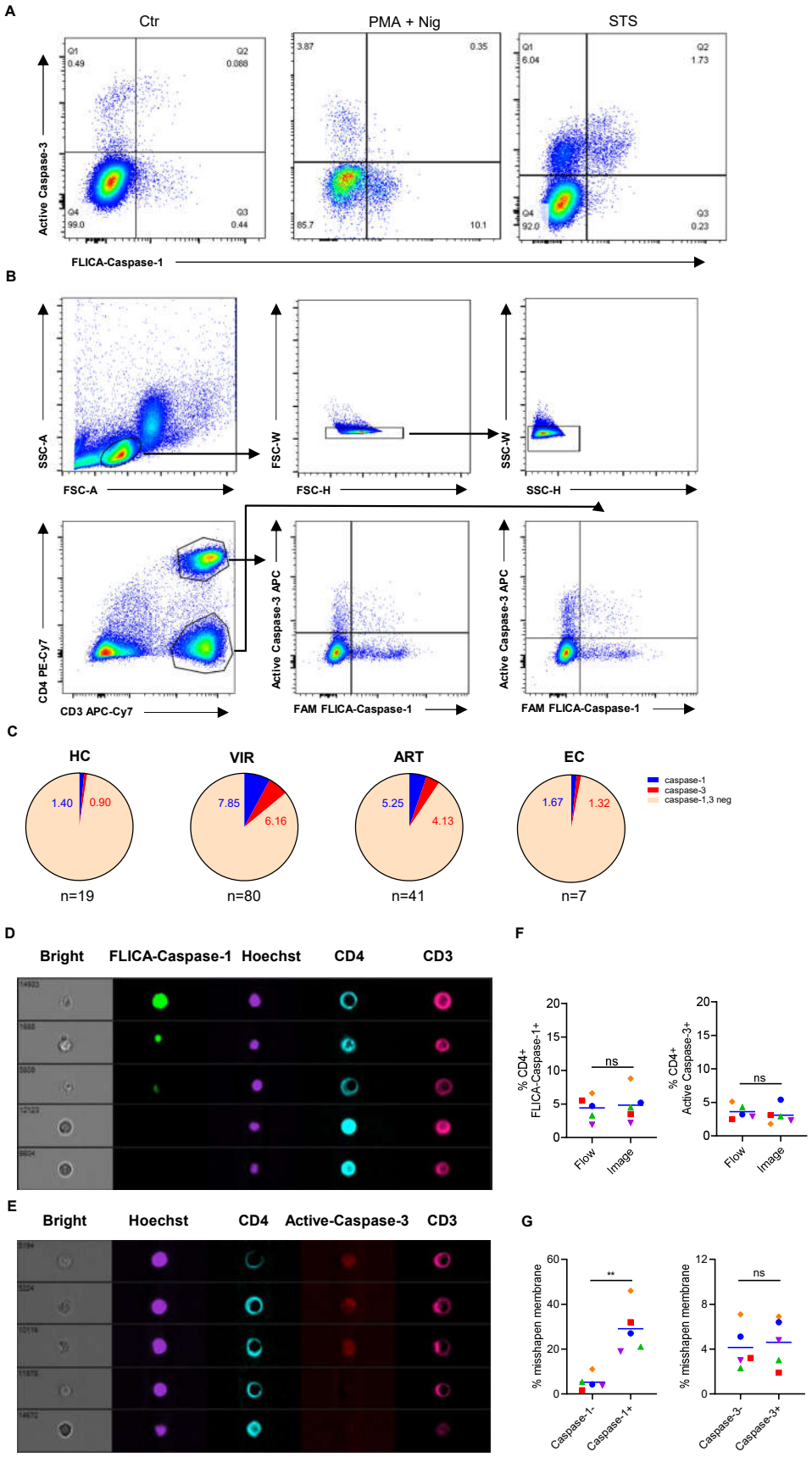
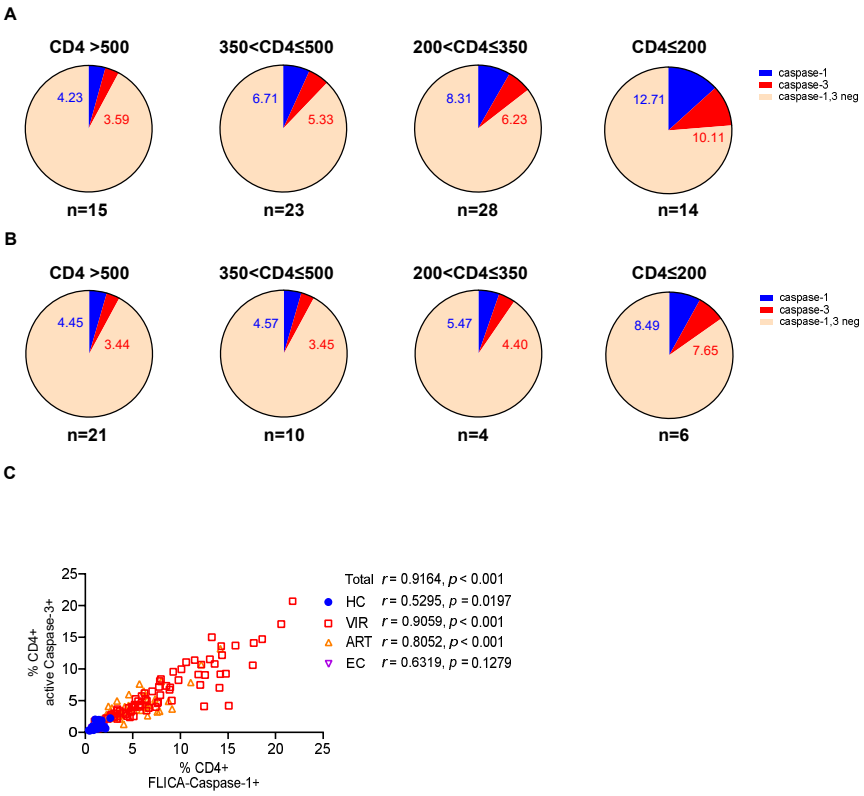
Supplemental Figure 1



Supplemental Figure 1. Detection of caspase-1 and caspase-3 activation in T cells.
(**A**) THP-1 cells were primed with PMA (100 ng/ml) for 24 h then stimulated with nigericin (Nig) (10 µM) or staurosporine (STS) (1 µM) for 1 h. Representative flow cytometry plot showing frequencies of FLICA-caspase-1+ and active caspase-3+ cells. (**B**) Gating strategies for analysis of caspases activation in T cells. (**C**) The contribution of FLICA-caspase-1+ and active caspase-3+ CD4+ T cells to the total CD4+ T cells in HCs (n = 19), VIRs (n = 80), ARTs (n = 27) and ECs (n = 7). (**D**) Representative image of ImageStream flow cytometric analysis of CD4+ T cells from a VIR patient and stained for FLICA-caspase-1 (green), Hoechst (purple), CD4 (blue), and CD3 (pink). (**E**) Representative image of ImageStream flow cytometric analysis of CD4+ T cells from a VIR patient and stained for Hoechst (purple), CD4 (blue), Active-Caspase-3 (red) and CD3 (pink). (**F**) Comparison of frequencies of caspases activating CD4+ T cells detected by flow cytometry and ImageStream flow cytometric analysis (n = 5). (**G**) Frequencies of misshapen membrane cells detected in caspase+ and caspase- CD4+ T cells in VIRs (n = 5). F-G: Wilcoxon signed-rank test; $^{**}p < 0.01$, ns, not significant.

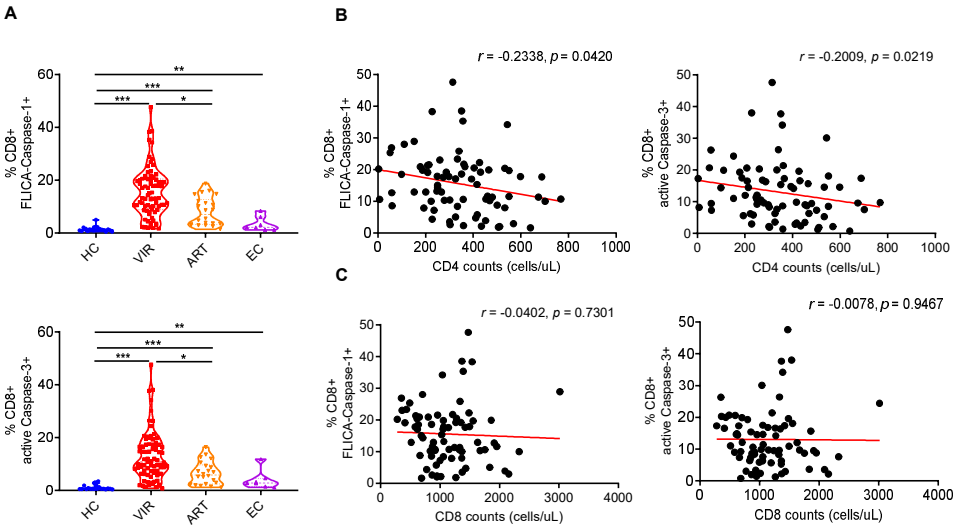
Supplemental Figure 2



Supplemental Figure 2. Activation of caspase-1 and caspase-3 in CD4+ T cells during chronic HIV-1 infection.

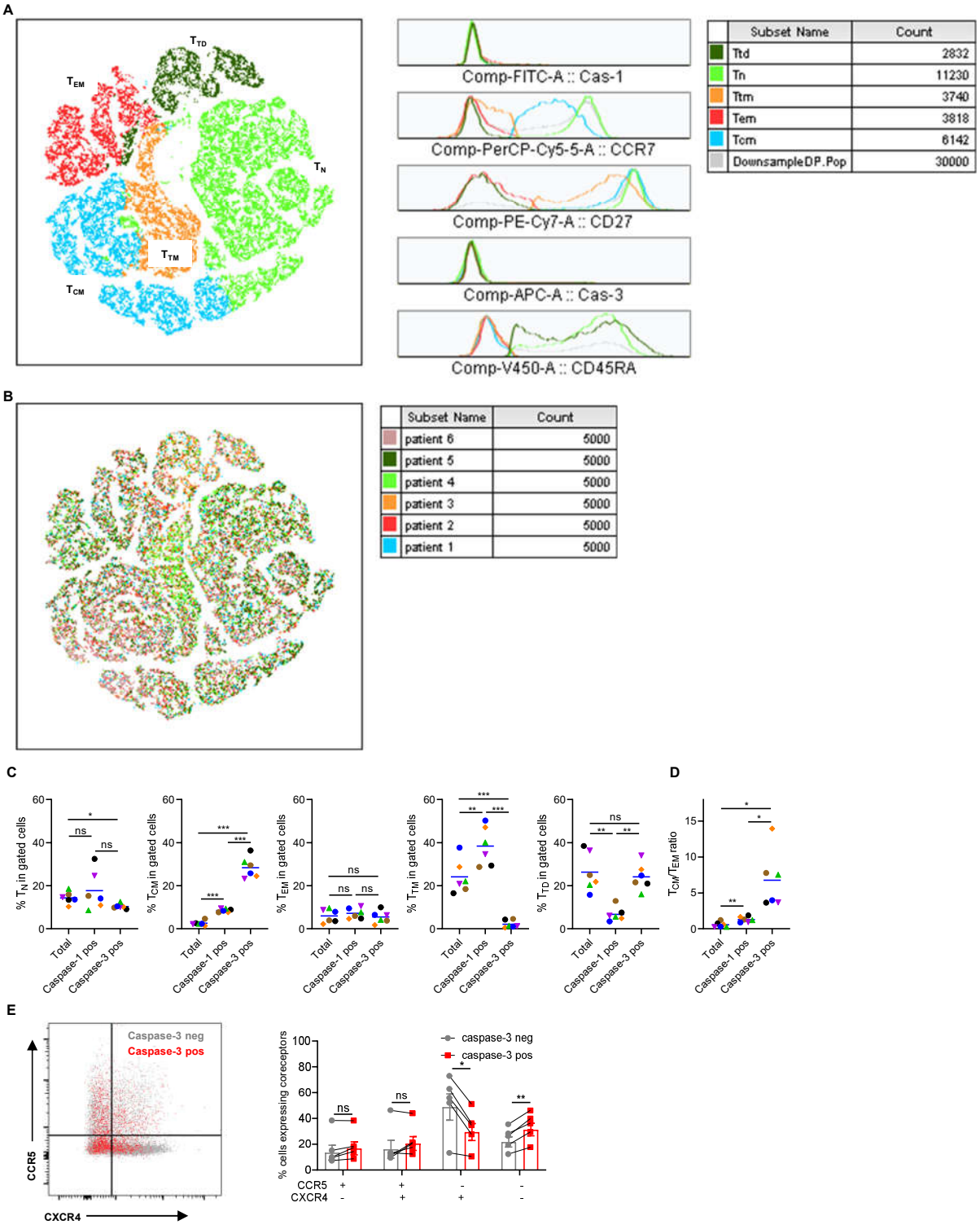
(**A**) VIRs were grouped according to the absolute CD4+ T cell count: CD4 ≤200 cells/µl (n = 14); 200< CD4 ≤350 cells/µl (n = 28); 350< CD4 ≤500 cells/µl (n = 23); CD4 >500 cells/µl (n = 15). Corresponding contribution of FLICA-caspase-1+ and active caspase-3+ CD4+ T cells to the total CD4+ T cells. (**B**) ARTs were grouped according to the absolute CD4+ T cell count: CD4 ≤200 cells/µl (n = 6); 200< CD4 ≤350 cells/µl (n = 4); 350< CD4 ≤500 cells/µl (n = 10); CD4 >500 cells/µl (n = 21). Corresponding contribution of FLICA-caspase-1+ and active caspase-3+ CD4+ T cells to the total CD4+ T cells. (**C**) Correlations of FLICA-caspase-1+CD4+ T cell frequencies with active caspase-3+CD4+ T cell frequencies in HCs (n = 19), VIRs (n = 80), ARTs (n = 41) and ECs (n = 7). *p* and Spearman's rho values are presented.

Supplemental Figure 3



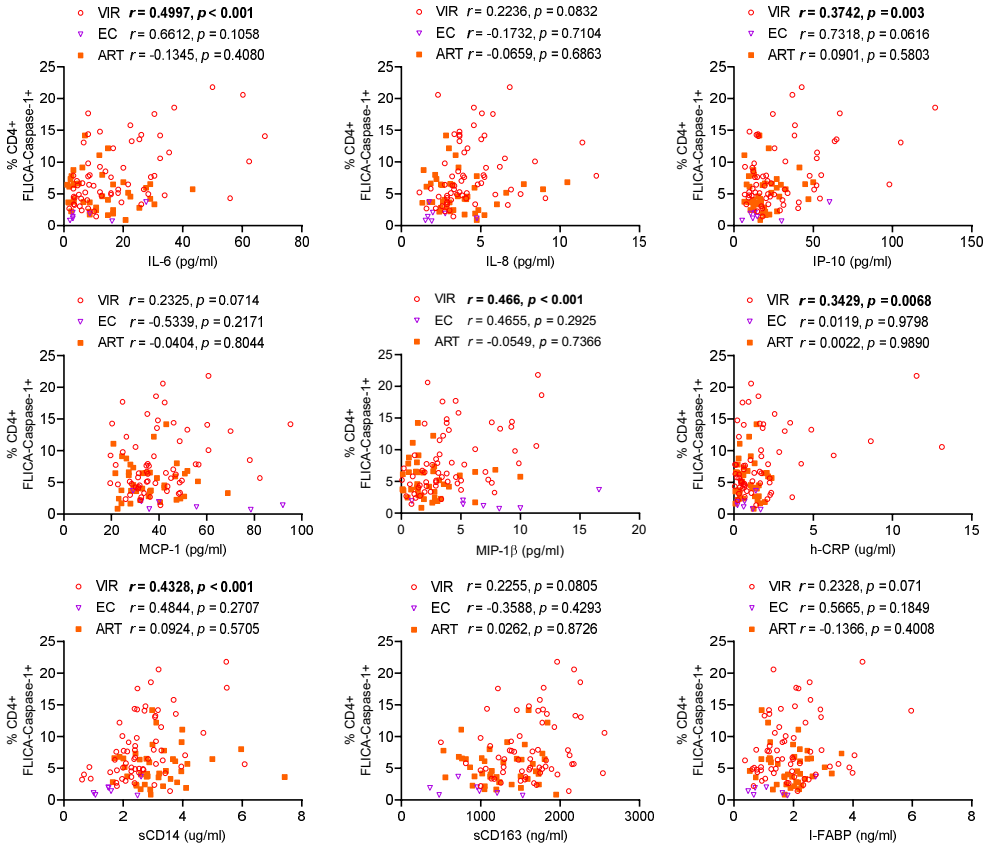
Supplemental Figure 3. Activation of caspase-1 and caspase-3 in CD8+ T cells during chronic HIV-1 infection.
(**A**) The frequencies of FLICA-caspase-1+CD8+ T cells and active caspase-3+CD8+ T cells from HCs (n = 19), VIRs (n = 80), ARTs (n = 27) and ECs (n = 7). (**B-C**) Association between the frequencies of FILICA-caspase-1+ CD8+ T cells and active caspase-3+ CD8+ T cells with the absolute CD4+ T cell count (**B**) and CD8+ T cell count (**C**) in VIRs. Each dot represents a single individual and associations were evaluated using Spearman correlation test. *p* and Spearman's rho values are presented. A: Mann–Whitney U-test; **p* < 0.05, ** *p* < 0.01, *** *p* < 0.001.

Supplemental Figure 4



Supplemental Figure 4. Phenotypes of apoptotic and pyroptotic CD8+ T cells.
(**A**) tSNE map of naïve/memory phenotype distribution (based on CD45RO, CCR7 and CD27) in HIV+ subjects (n = 6). Colors depict memory phenotypes. naïve (T_N: CD45RA+CCR7+CD27+), central memory (T_{CM}: CD45RA-CCR7+CD27+), transitional memory (T_{TM}: CD45RA-CCR7-CD27+), effector memory (T_{EM}: CD45RA-CCR7-CD27-) and terminally differentiated (T_{TD}: CD45RA+CCR7-CD27-) cells. (**B**) Distribution of cells based on memory populations in VIR patients (n = 6). Colors depict different individuals. (**C**) The proportion of T_N, T_{CM}, T_{TM}, T_{EM} and T_{TD} cells in total, FLICA-caspase-1+, and active caspase-3+ CD8+ T cells from VIRs (n = 6). (**D**) Value for T_{CM}/T_{EM} ratio was calculated. (**E**) The expression of HIV-1 coreceptor CCR5 and CXCR4 on active-caspase-3+ (red dots) and active-caspase-3- CD4+ T cells (gray dots) in VIRs (n = 5). C-E: Wilcoxon signed-rank test; *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant.

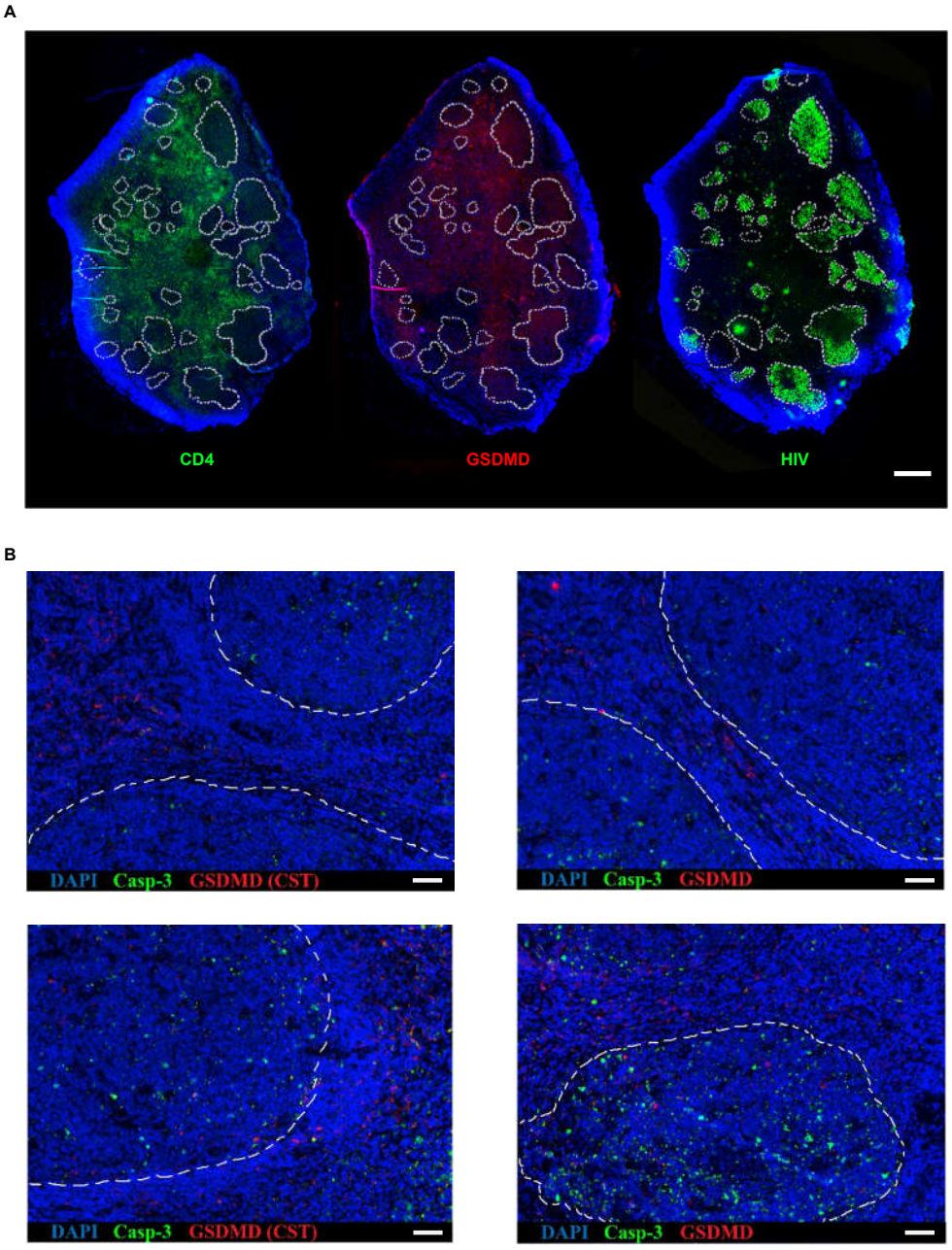
Supplemental Figure 5



Supplemental Figure 5. Correlations of FLICA-caspase-1 frequency in CD4+ T cells and plasma inflammatory markers in VIR and ART patients.

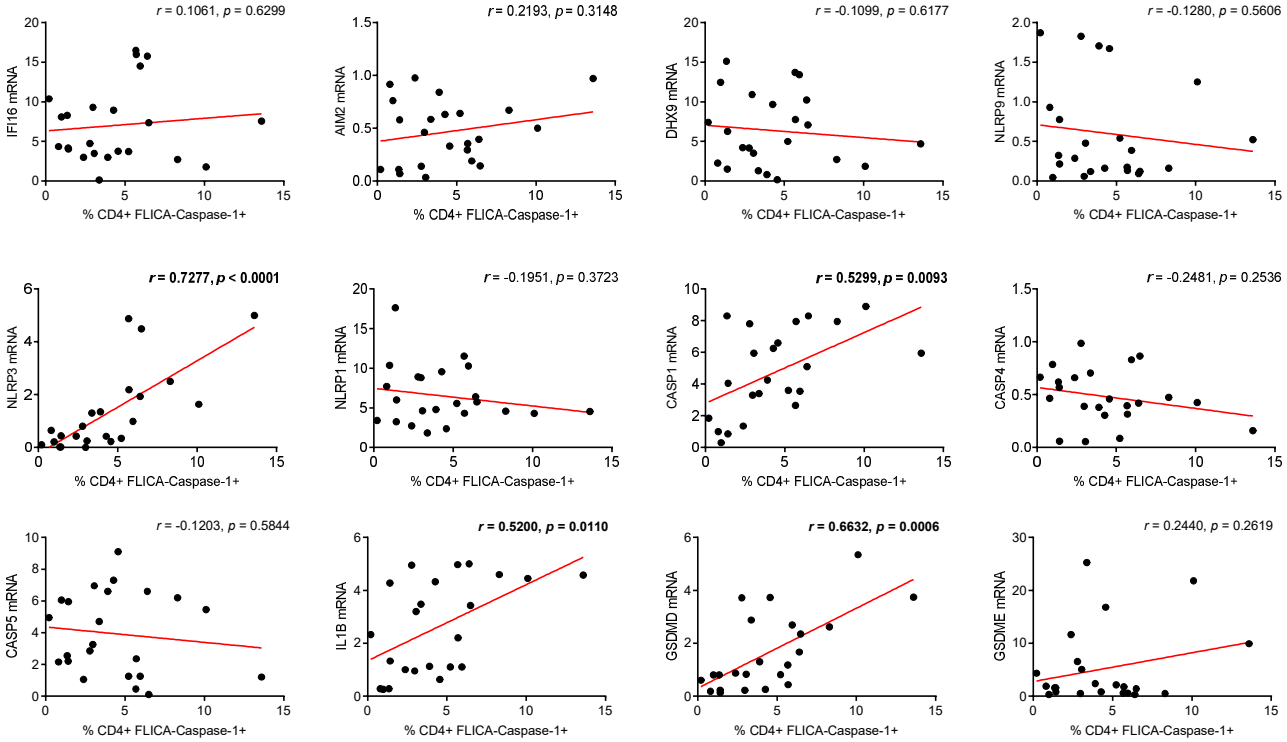
Correlations of FLICA-caspase-1+ CD4+ T cell frequencies with plasma levels of IP-10, MCP-1, IL-6, IL-8, MIP-1β, h-CRP, I-FABP, sCD14 and sCD163 in VIR and ART patients. Associations were evaluated using Spearman correlation test. *p* and Spearman's rho values are presented.

Supplemental Figure 6



Supplemental Figure 6. GSDMD activation in HIV+ LN.
(**A**) Representative immunofluorescence microscopy images of lymph node section from one VIR patient hybridized with HIV-1 viral RNA and stained for CD4 and active GSDMD. Follicles are encircled by the dashed white lines. Scale bar, 80 μm. (**B**) Comparison of different active GSDMD antibodies in HIV+ LN. Representative immunofluorescence microscopy images of LNs sections from VIR individuals stained with anti-active Casp-3 and anti-active GSDMD or anti-active GSDMD (CST) antibodies as indicated. Scale bar, 50 μm.

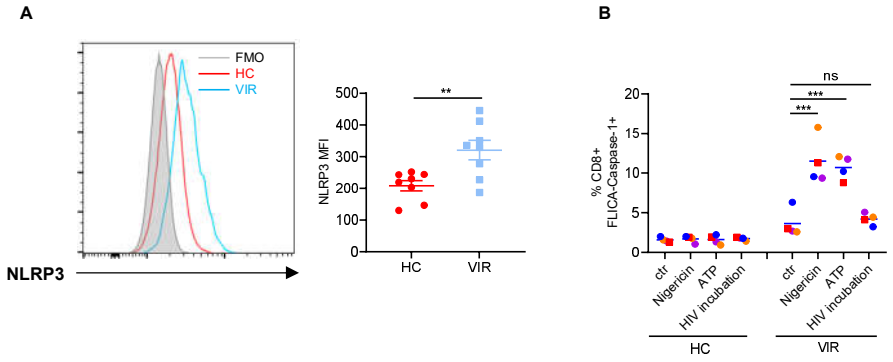
Supplemental Figure 7



Supplemental Figure 7. Association of frequencies of FLICA-caspase-1+CD4+ T cells with inflammasome-associated gene expression.

Correlation of frequencies of FLICA-caspase-1+CD4+ T cells with the expression of *IFI16*, *AIM2*, *DHX9*, *NLRP9*, *NLRP3*, *NLRP1*, *CASP1*, *CASP4*, *CASP5*, *ILIB*, *GSDMD*, and *GSDME*; mRNA expression is normalized to *IPO8*. Associations were evaluated by Spearman correlation test. *p* and Spearman's rho values are presented.

Supplemental Figure 8



Supplemental Figure 8. Induction of pyroptosis of CD8+ T cells from healthy controls. (**A**) Left panel: representative NLRP3 expression in CD8+ T cells of HCs (red) and VIRs (blue). Right panel: MFI of NLRP3 expression in CD8+ T cells from HCs (n = 5) and VIRs (n = 5). (**B**) PBMCs from HCs (n = 4) and VIRs (n = 4) were stimulated with 10 µM nigericin, 5 mM ATP, or HIV-1 viral particles. FLICA-caspase-1+CD8+ T cell frequencies were determined by flow cytometry. A: Mann–Whitney U-test; B: Wilcoxon signed-rank test; $^{**}p < 0.01$, $^{***}p < 0.001$, ns, not significant.

Supplemental Table 1. Characteristics of HIV-1-infected patients enrolled in the study.

Patient no.	Gender	Age (y)	Group	Possible transmission route	Viral load (copies/mL)	CD4+T cells/μL	CD8+T cells/μL	CD4/CD8 ratio
CD4>500 (n=15)								
1	M	22	Viremic	Heterosexual	20,330	768	1,152	0.67
2	M	19	Viremic	MSM	51,765	702	2,329	0.3
3	M	21	Viremic	Bisexual	8,236	688	1,424	0.48
4	M	21	Viremic	MSM	54,679	674	1,332	0.51
5	M	34	Viremic	MSM	3,189	640	688	0.93
6	M	51	Viremic	MSM	123,024	598	1,059	0.56
7	M	26	Viremic	MSM	44,341	569	2,156	0.26
8	M	35	Viremic	MSM	19,321	551	1,554	0.35
9	M	28	Viremic	MSM	16,020	550	873	0.63
10	M	24	Viremic	NA	12,409	543	1,042	0.52
11	M	24	Viremic	MSM	34,277	533	1,365	0.39
12	M	51	Viremic	MSM	294,209	533	612	0.87
13	M	27	Viremic	MSM	363,548	511	945	0.54
14	M	27	Viremic	MSM	13,105	506	1,267	0.4
15	M	35	Viremic	MSM	14,420	501	663	0.76
350<CD4≤500 (n=23)								
16	M	29	Viremic	MSM	26,244	480	1,243	0.39
17	M	39	Viremic	MSM	337,060	477	2,121	0.22
18	M	32	Viremic	MSM	28,985	469	837	0.56
19	M	23	Viremic	MSM	226,712	465	1,862	0.25
20	M	27	Viremic	MSM	36,007	460	1,954	0.24
21	M	45	Viremic	MSM	14,453	453	916	0.49
22	M	25	Viremic	MSM	9,364	452	2,093	0.22
23	M	26	Viremic	MSM	94,752	434	1,014	0.43
24	M	53	Viremic	MSM	35,751	431	1,265	0.34
25	M	36	Viremic	MSM	195,555	427	1,487	0.29
26	M	46	Viremic	MSM	45,213	417	936	0.45
27	M	30	Viremic	MSM	969,625	416	1,027	0.41
28	M	30	Viremic	MSM	43,805	411	1,434	0.29
29	M	21	Viremic	NA	82,228	404	1,275	0.32
30	F	18	Viremic	Heterosexual	78,602	393	620	0.63
31	M	25	Viremic	MSM	16,256	390	862	0.45
32	M	30	Viremic	MSM	31,064	365	652	0.56
33	M	26	Viremic	MSM	7,479	361	1,212	0.3
34	M	30	Viremic	MSM	15,394	359	949	0.38
35	M	25	Viremic	MSM	16,955	358	1,755	0.2
36	M	30	Viremic	MSM	66,665	357	1,391	0.26
37	M	18	Viremic	MSM	53,314	351	856	0.41
38	M	30	Viremic	MSM	263,326	351	1,370	0.26
200<CD4≤350 (n=28)								
39	M	28	Viremic	MSM	144,524	340	1,337	0.25
40	M	22	Viremic	MSM	60,779	334	434	0.77
41	M	29	Viremic	MSM	32,421	334	1,057	0.32
42	M	39	Viremic	MSM	87,363	326	1,052	0.31
43	M	58	Viremic	MSM	1,474,085	326	1,837	0.18
44	M	47	Viremic	MSM	497,328	314	1,475	0.21
45	M	22	Viremic	MSM	38,353	303	866	0.35
46	M	33	Viremic	Heterosexual	11,993	302	917	0.33
47	M	32	Viremic	MSM	136,603	301	1,372	0.22
48	M	24	Viremic	MSM	63,354	298	582	0.51
49	M	35	Viremic	Heterosexual	123,066	287	1,351	0.21
50	M	31	Viremic	MSM	1,244,084	279	1,675	0.17
51	F	63	Viremic	Heterosexual	13,920	274	1,140	0.24
52	M	31	Viremic	NA	82,632	267	889	0.3
53	M	49	Viremic	NA	185,653	260	361	0.72
54	M	39	Viremic	MSM	14,869	250	710	0.35
55	M	35	Viremic	MSM	4,039	250	685	0.36
56	M	37	Viremic	MSM	8,762	249	1,497	0.17
57	M	57	Viremic	MSM	817,815	247	1,063	0.23
58	M	35	Viremic	MSM	458,189	235	999	0.24
59	M	42	Viremic	MSM	489,953	228	774	0.29
60	M	18	Viremic	MSM	47,549	228	1,538	0.15
61	M	33	Viremic	MSM	218,611	223	1,367	0.16
62	M	48	Viremic	MSM	99,634	214	958	0.22
63	M	25	Viremic	MSM	60,996	209	410	0.51
64	M	61	Viremic	Heterosexual	32,415	208	592	0.35
65	M	23	Viremic	MSM	14,560	202	494	0.41
66	M	45	Viremic	MSM	896,609	201	1,633	0.12
CD4≤200 (n=14)								
67	M	32	Viremic	MSM	134,564	193	1,194	0.16
68	M	29	Viremic	MSM	68,635	187	1,057	0.18
69	M	24	Viremic	MSM	5,489	178	801	0.22
70	M	32	Viremic	MSM	139,365	152	514	0.3
71	M	27	Viremic	MSM	1,727,737	152	3,016	0.05
72	M	30	Viremic	MSM	133,515	110	706	0.16
73	M	20	Viremic	MSM	225,106	101	199	0.51
74	M	26	Viremic	MSM	513,973	99	634	0.16
75	M	28	Viremic	MSM	1,222,585	59	854	0.07
76	M	36	Viremic	MSM	328,419	58	1,909	0.03
77	M	39	Viremic	MSM	876,546	57	353	0.16
78	F	42	Viremic	Heterosexual	1,813,274	51	491	0.1
79	M	32	Viremic	MSM	1,826,860	7	1,326	0.01
80	M	59	Viremic	NA	362,177	3	281	0.01

Continued

Patient no.	Gender	Age (y)	Group	CD4+T cells/μL	CD8+T cells/μL	CD4/CD8 ratio	Possible transmission route	CD4+T cells before ART /μL	Viral load before ART (copies/mL)	ART regimen
CD4>500 (n=22)										
1	M	29	ART	1,029	588	1.75	MSM	748	61,300	3TC/TDF/EFV
2	M	38	ART	974	927	1.05	MSM	619	168,000	3TC/TDF/EFV
3	M	43	ART	946	1,465	0.65	MSM	892	66,900	3TC/TDF/EFV
4	M	30	ART	901	533	1.69	Heterosexual	598	69,200	3TC/TDF/EFV
5	M	29	ART	845	1,000	0.85	MSM	537	13,800	3TC/TDF/EFV
6	M	29	ART	798	1,183	0.67	MSM	596	239,000	3TC/TDF/EFV
7	M	37	ART	783	896	0.87	MSM	693	20,400	3TC/TDF/EFV
8	M	28	ART	778	929	0.84	MSM	435	28,300	3TC/TDF/EFV
9	M	26	ART	770	1,400	0.55	MSM	479	34,500	3TC/TDF/EFV
10	M	31	ART	700	1,088	0.64	MSM	691	566,000	3TC/TDF/EFV
11	M	32	ART	671	3,082	0.22	MSM	396	431,000	3TC/TDF/EFV
12	M	33	ART	657	1,290	0.51	MSM	483	715	3TC/TDF/EFV
13	M	30	ART	652	1,788	0.36	Heterosexual	545	174,000	3TC/TDF/EFV
14	M	41	ART	645	783	0.82	MSM	540	167,000	3TC/TDF/EFV
15	M	21	ART	617	995	0.62	MSM	545	444,000	3TC/TDF/EFV
16	M	42	ART	573	589	0.97	MSM	326	72,000	3TC/TDF/EFV
17	M	45	ART	572	600	0.95	MSM	440	30,600	3TC/TDF/EFV
18	M	23	ART	524	950	0.55	MSM	594	49,000	3TC/TDF/EFV
19	M	39	ART	522	1,386	0.38	MSM	326	87,400	3TC/TDF/EFV
20	M	23	ART	512	1,833	0.28	MSM	456	227,000	3TC/TDF/EFV
21	M	41	ART	508	871	0.58	MSM	351	191,000	3TC/AZT/EFV
350<CD4≤500 (n=9)										
22	M	28	ART	500	763	0.66	MSM	647	12,800	3TC/TDF/EFV
23	M	34	ART	485	1,385	0.35	MSM	206	165,000	3TC/TDF/EFV
24	M	29	ART	476	1,422	0.33	MSM	399	381,000	3TC/TDF/EFV
25	M	15	ART	469	1,458	0.32	MSM	413	17,200	3TC/TDF/EFV
26	M	37	ART	463	938	0.49	MSM	275	80,700	3TC/TDF/EFV
27	M	50	ART	452	513	0.88	MSM	299	274,000	3TC/TDF/EFV
28	M	25	ART	433	1,234	0.35	MSM	307	74,900	3TC/TDF/EFV
29	M	21	ART	419	874	0.48	MSM	269	590,000	3TC/TDF/EFV
30	M	22	ART	416	837	0.5	MSM	343	30,100	3TC/TDF/EFV
31	M	56	ART	413	723	0.57	MSM	221	98,400	3TC/TDF/EFV
200<CD4≤350 (n=4)										
32	M	48	ART	342	619	0.55	MSM	220	19,200	3TC/TDF/EFV
33	M	51	ART	281	455	0.62	MSM	195	85,900	3TC/TDF/EFV
34	M	23	ART	273	1,308	0.21	MSM	266	46,200	3TC/TDF/EFV
35	M	62	ART	212	804	0.26	MSM	157	137,000	3TC/TDF/EFV
200<CD4≤350 (n=6)										
36	M	45	ART	170	395	0.43	NA	11	1,010,000	3TC/TDF/EFV
37	M	28	ART	161	560	0.29	Heterosexual	167	56,500	3TC/TDF/EFV
38	M	22	ART	145	392	0.37	MSM	127	239,000	3TC/TDF/EFV
39	M	21	ART	142	535	0.27	MSM	52	1,130,000	3TC/TDF/EFV
40	M	64	ART	129	939	0.14	NA	78	119,000	3TC/TDF/EFV
41	M	23	ART	51	597	0.09	MSM	4	141,000	3TC/TDF/EFV

Abbreviations: NA, not applicable; M, male; F, female; MSM, men who have sex with men.

Supplemental Table 2. Characteristics of study participants for lymph node biopsies.

Patient ID	Gender	HIV status	Years ART at Biopsy	Biopsy Date	Age at biopsy	CD4 count at Biopsy (cells/mm^3)	pVL at Biopsy (copies/mL)
LN414	M	Negative	NA	20120414	27	NA	NA
LN317	M	Negative	NA	20181221	5	NA	NA
LN437	M	Negative	NA	20130114	49	NA	NA
LN9	M	Positive	NA	20111113	31	497	2512
LN62	M	Positive	NA	20110922	26	382	1259
LN82	M	Positive	NA	20110727	26	57	25119
LN21	M	Positive	NA	20110824	35	209	398107
LN202	M	Positive	NA	20181225	55	86	251189
LN403	M	Positive	NA	20120312	48	314	37660
LN81	F	Positive	NA	20131114	42	151	113002
LN57	F	Positive	2	20110916	29	61	LDL
LN64	F	Positive	0.5	20110824	28	375	LDL
LN69	F	Positive	4	20110928	26	139	LDL
LN77	M	Positive	0.5	20181130	20	698	LDL

Abbreviations: NA, not applicable; M, male; F, female; LDL, Lower Detection Limit; pVL, plasma viral load.

Supplemental Table 3. Reagent or Resource.

REAGENT or RESOURCE	SOURCE	IDENTIFIER	Clone
Antibodies			
FAM FLICA Casepase-1 Kit	Bio-rad	Cat# ICT097	
active caspase-3 AF647	BD	560626	C92-605
CD3-APC/cy7	BD	557832	SK7
CD4-BV510	BD	562970	SK3
CD8a-FITC	Biolegend	344704	SK1
CCR7-PerCP/cy5.5	BD	561144	150503
CD45RA-BV510	BD	563031	HI100
CD27-PE/cy7	Biolegend	302838	O323
HLA-DR-BV605	Biolegend	307640	L243
CD38-AF700	Biolegend	303524	HIT2
CXCR4-PE	Biolegend	306506	12G5
CCR5-BV421	Biolegend	359118	J418F1
CD25-BV421	BD	562442	M-A251
Foxp3-PE	ebioscience	12-4777-42	236A/E7
CXCR5-PE-cy7	Biolegend	356923	J252D4
PD-1-BV510	Biolegend	329932	EH12.2H7
CXCR3-BV605	Biolegend	353728	G025H7
CCR4-PE/cy7	Biolegend	359409	L291H4
CCR6-PE	Biolegend	353410	G034E3
NLRP3-PE	Miltenyi	130-111-209	REA668
CD4	Zsbio	ZM-0418	UMAB64
active-GSDMD	National Institute of Biological Sciences, Beijing, China.	Dr. Feng Shao	
active-GSDMD (CST)	Cell Signaling Tech	36425S	E7H9G
Chemicals, Peptides, and Recombinant Proteins			
PMA	sigma	P1585	
Nigericin	MCE	HY-100381	
staurosporine	MCE	HY-15141	
VX765	MCE	HY-13205	
MCC950 sodium	MCE	HY-12815A	
ATP	sigma	FLAAS-1VL	
Mito-SOX	Thermo	M36008	
NAC	MCE	HY-B0215	
mito-TEMPO	Sigma	SML0737-5mg	
Hoechst	Thermo	H3569	
Experimental Models: Cell Lines			
THP-1	ATCC	Cat#TIB-202	
Critical Commercial Assays			
EasySep™ Human CD4+ T Cell Enrichment Kit	Stem Cell	Cat# 19052	
PE / R-Phycoerythrin Conjugation Kit	abcam	Cat# ab102918	
TransStart Probe qPCR SuperMix	TransGen Biotech	Cat# AQ401-01	
TransScript Probe One-Step qRT-PCR SuperMix	TransGen Biotech	Cat# AQ221-01	
BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution	BD Biosciences	554722	
RNAscope® Target Retrieval Reagents	ACD	32200	
RNAscope® H202 and Protease Reagents	ACD	322381	
RNAscope® Mutiplex Fluorescent Detection Reagents	ACD	323110	
Software and Algorithms			
BD FACSDiva™ Version 8.0	BD Biosciences	https://www.bdbiosciences.com	
GraphPad Prism Version 8	GraphPad Software, Inc.	https://www.graphpad.com	
R Version 3.6.1	Open source	https://www.r-project.org/	
ImageJ	Open source	http://imagej.nih.gov/ij	

Supplemental Table 4. Primers used in this study.

Primers	Sequences (5'-3')	Product length (bp)
hIL-1B_F445	GGACAGGATATGGAGCAACAAG	124
hIL-1B_R568	TCAACACGCAGGACAGGTA	
hCASP1_F308	CCACATCCTCAGGCTCAGA	113
hCASP1_R4220	GCGGCTTGACTTGTCCATTA	
hIPO8_F2768	AAGATGAGGAGGAGGAAGATG	91
hIPO8_R2858	TTGTCAAGGTCAAGTGGAGTA	
hDHX9_F1607	AAGTTCGCATTGTTCTTATGTCT	199
hDHX9_R1805	TCACCACCATCATCATCCTTAT	
hGSDMD_F339	CTCCAGCACCTCAATGAATG	178
hGSDMD_R516	GACTTCCACCTCCTTCTGT	
hGSDME_F1259	TTCGTGCTCTGTCTGATGAT	82
hGSDME_R1340	ATCCCAAACCTTTCTGTATCTTTC	
hNLRP3_F2427	CCTCGGTGACTTCGGAAT	139
hNLRP3_R2565	ATGGCTGGTGCTCAATACT	
hNLRP9_F2342	GCTGTCTCACCTCTGTCTC	111
hNLRP9_R2452	ATGCCACTCCATTATCTTCCA	
hIFI16_F1844	GTGCCAGCGTAACTCCTA	151
hIFI16_R1994	ACCACCACTTCCATCTTCC	
hCASP4_F577	ACCAGACCAGAGCACAAGT	85
hCASP4_R661	GCACAGTTCCGCAGATTCC	
hCASP5_F748	GCCAGACCAGAGCACAAG	84
hCASP5_R831	CGCAGTTCCGCAGATTCC	
hNLRP1_F2890	AGTGATGAGATGAGGCAGGAA	147
hNLRP1_R3036	CCGCTTGAGTGAGGATGTG	
hAIM2_F507	CCAAGAAGGCAAGCAGGAG	195
hAIM2_R701	TGGTCAGATTCAGCATCTAACAC	