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Supplemental information

Human NLRP1 is a sensor of pathogenic coronavirus 3CL proteases in lung epithelial cells

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SUPPLEMENTAL INFORMATIONS

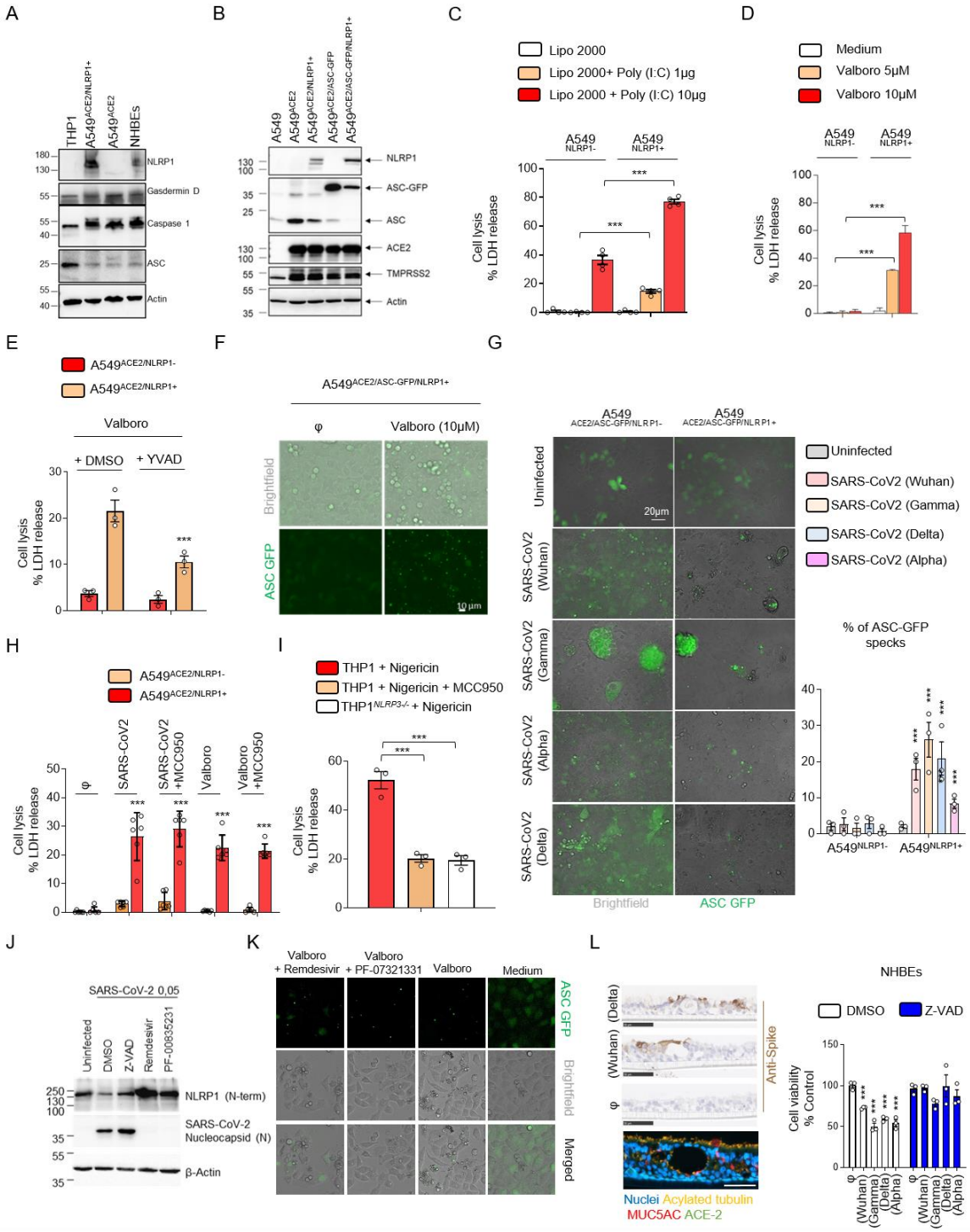


Figure S1 (Related to Figure 1). NLRP1 detects SARS-CoV-2 infection in epithelial cells

(A) Western Blot examination of the expression of NLRP1, ASC, Gasdermin-D (GSDMD), Caspase-1 (CASP1) and ACTIN in THP-1 (monocytes), NHBE (bronchial epithelial cells) or A549 cells engineered for the purpose of this study. Immunoblots

were performed against full-length NLRP1 Nter (p130/110), ASC (p22), GSDMD (p55), CASP1 (p50) and ACTIN (p40).

(B) Western Blot examination of the expression of NLRP1, ASC, ASC-GFP, ACE2, TMRSS2 and ACTIN in A549 cells engineered for the purpose of this study. Immunoblots were performed against full-length NLRP1 Nter (p130/110), ASC (p22), ASC-GFP (p50), TMRSS2 (p54), ACE2 (p130) and ACTIN (p40).

(C-E) Measure of cell lysis (LDH release) in $A549^{NLRP1+}$ or $A549^{NLRP1-}$ transfected with polyI:C (0.1µg or 1µg) (B) or treated with Valboro (5 and 10µM) (C) in presence/absence of the Caspase-1 inhibitor Z-YVAD (25µM) for 10 hours.

(F) Florescence microscopy of ASC-GFP specks in $A549^{ACE2/NLRP1+/ASC-GFP}$ and $A549^{ACE2/NLRP1-/ASC-GFP}$ airway epithelial cell lines treated with 5µM of Valboro for 10 hours. Images shown are from one experiment and are representative of n=3 independent experiments; scale bars 10 µm.

(G) Florescence microscopy and associated quantifications of ASC-GFP specks in $A549^{ACE2/NLRP1+/ASC-GFP}$ airway epithelial cell lines infected for 24 hours with various strains of SARS-CoV-2 (MOI 0.01).

(H) Measure of cell lysis (LDH release) in $A549^{NLRP1+}$ or $A549^{NLRP1-}$ treated with Valboro (5 µM) for 10 hours or infected with SARS-CoV-2 (MOI 0.05) for 24 hours in presence/absence of the NLRP3 inhibitor MCC950 (10µM).

(I) Measure of cell lysis (LDH release) in PMA (100ng/mL)-primed THP1 myeloid cells treated with Nigericin (20µM) for 2 hours in presence/absence of the NLRP3 inhibitor MCC950 (10µM).

(J) Western blot examination of NLRP1 and SARS-CoV-2 Nucleocapsid (N) in $A549^{ACE2/NLRP1+}$ and $A549^{ACE2/NLRP1-}$ airway epithelial cell lines infected with SARS-CoV-2 for 24 hours multiplicity of infection (MOI) of 0.05 in presence /absence of the NSP5 protease inhibitor PF-00835231 (10µM), Remdesivir (5µM), or Z-VAD (25 µM).

(K) Florescence microscopy of ASC-GFP specks in $A549^{ACE2/NLRP1+/ASC-GFP}$ airway epithelial cell lines treated with 5µM of Valboro for 10 hours in presence/absence of the 3CL inhibitor PF-00835231 (10µM) or Remdesivir (5 µM). Images shown are from one experiment and are representative of n=3 independent experiments; scale bars 10 µm.

(L) Microscopy characterization of Air-Liquid Interface-differentiated and infected epithelial cells (ACE2, Mucin 5AC, Acylated tubulin, Nuclei, SARS-CoV-2) and measure of cell lysis (LDH release) in NHBE epithelial cells infected for 36 hours with various strains of SARS-CoV-2 (MOI 1) in presence/absence of the pan Caspase inhibitor Z-VAD (40µM). Images shown are from one experiment and are representative of n=2 independent experiments; scale bars 50 µm.

Data information:

Western Blot (A, B, J) images are from one experiment performed three times. Graphs C, D, E, H, I, L show data presented as means ± SEM from n=6 (G) or n=3 independent pooled experiments; ***p ≤ 0.001 for the indicated comparisons with t-test. Images (F, G, K) show one experiment performed three times.

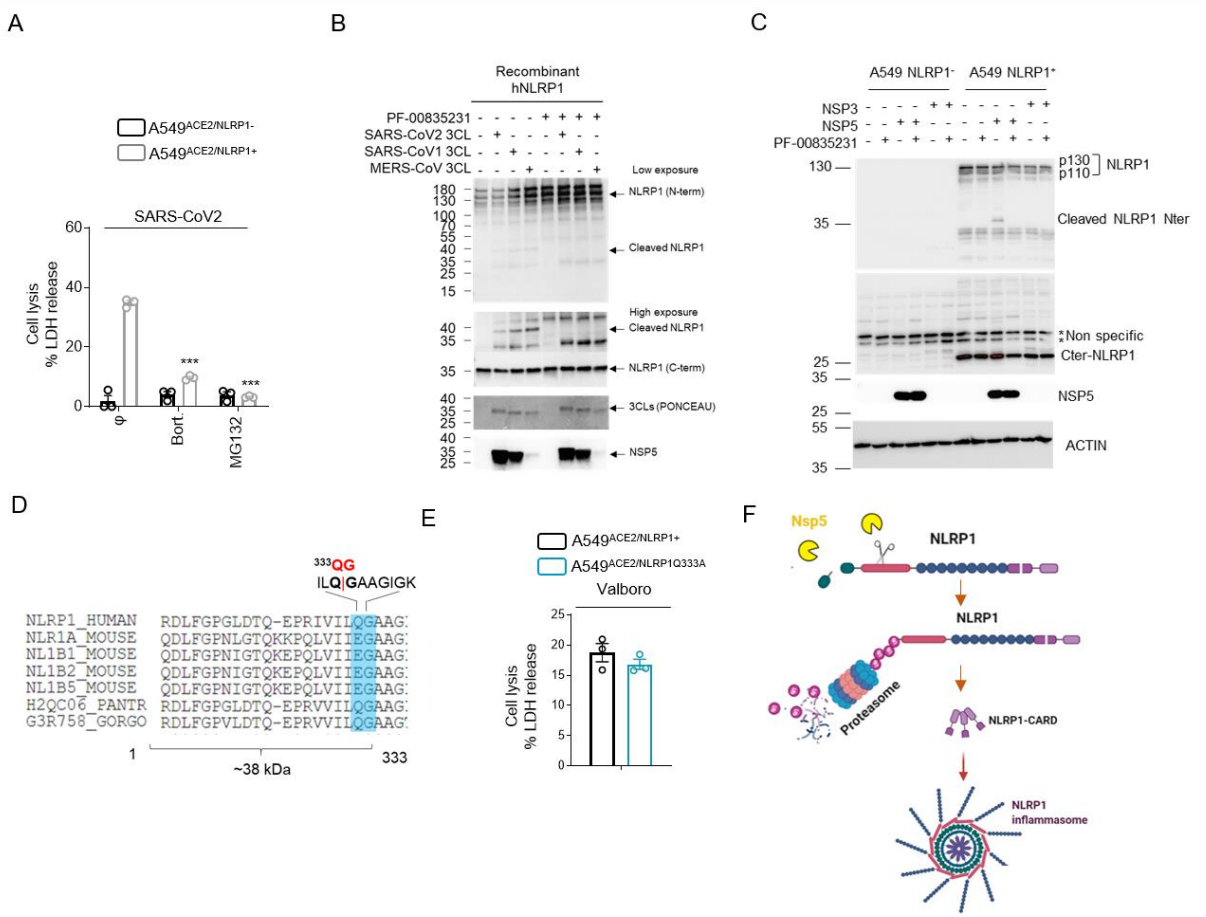


Figure S2 (Related to Figure 2). NSP5-cleaved NLRP1 nucleates inflammasome activation

(A) Measure of cell lysis (LDH release) in A549^{NLRP1+} or A549^{NLRP1-} infected with SARS-CoV-2 (MOI 0.05) for 24 hours in presence/absence of the proteasome inhibitors bortezomib (0.1µM) or MG-132 (0.1µM).

(B) Western blot examination of NLRP1 cleavage using an anti NLRP1 Nter antibody (aa1-323) upon co incubation of SARS-CoV-2, SARS-CoV1 or MERS-CoV 3CL (NSP5) proteases with recombinant human NLRP1 in presence or absence of the 3CL inhibitor PF-00835231 (10µM). NLRP1 N-terminal, NLRP1 C-terminal, NSP5 and ACTIN were immunoblotted.

(C) Western blot examination of NLRP1 cleavage using an anti NLRP1 N-terminal antibody (aa1-323) upon co incubation of SARS-CoV-2 NSP5 or SARS-CoV-2 NSP3 proteases with A549^{NLRP1+} cell lysates in presence or absence of the 3CL inhibitor PF-00835231 (10µM). NLRP1 Nter, NLRP1Cetr, NSP5 and ACTIN were immunoblotted.

(D) Sequence alignment of the NSP5-targeted human NLRP1^{Q333} site with mouse and primate NLRP1.

(E) Measure of cell lysis (LDH release) in A549^{NLRP1+} or A549^{NLRP1Q33A} treated with Valboro (10µM) for 10 hours.

(F) Proposed mechanism of human NLRP1 activation by NSP5 protease.

Data informations:

Western Blot (A-C) images are from one experiment performed three times. Graph E show data presented as means ± SEM from n=3 independent pooled experiments; ***p ≤ 0.001 for the indicated comparisons with t-test. G was created using biorender.com

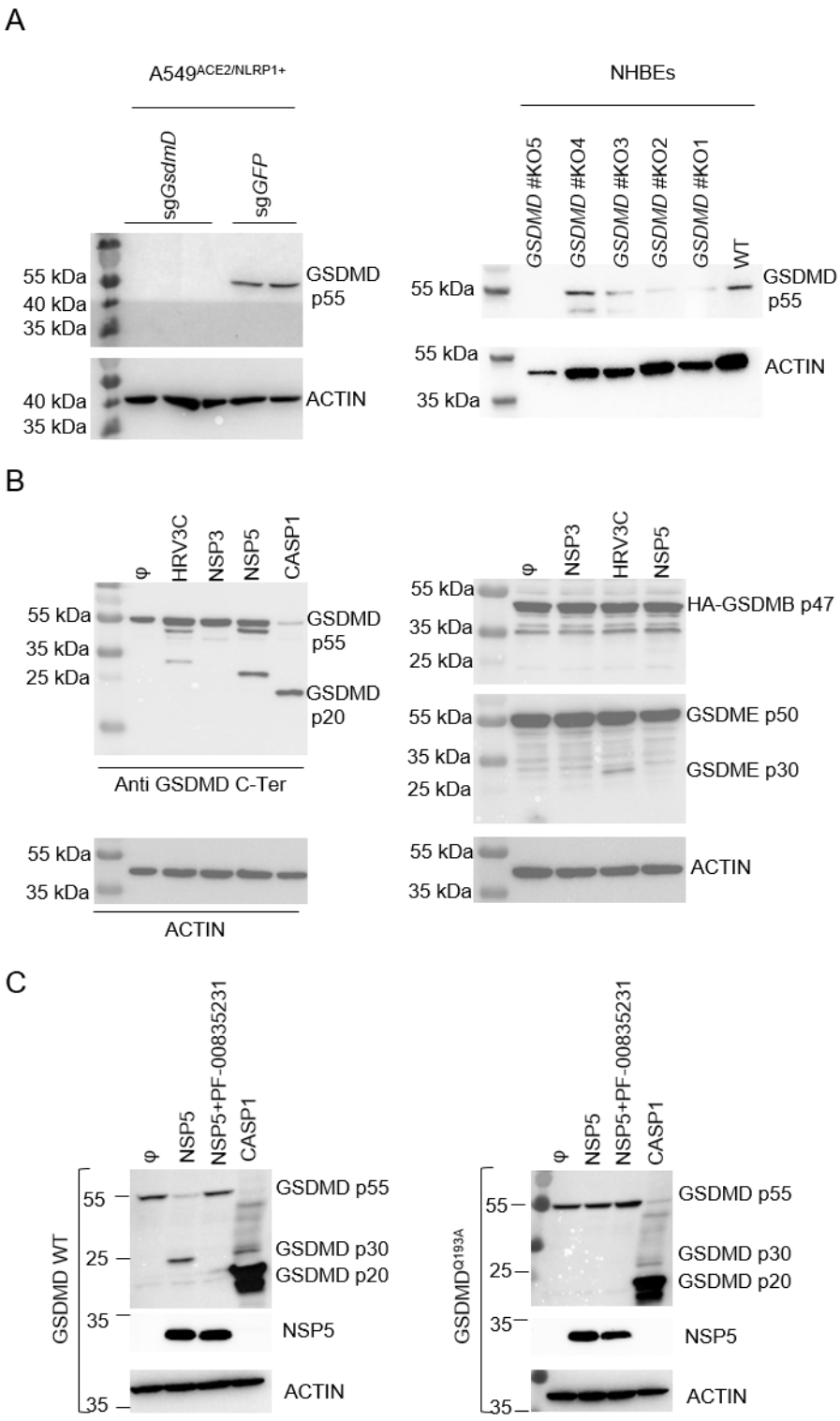


Figure S3 (Related to Figure 3). NSP5 cleaves and inactivates GSDMD

(A) Western Blot examination of genetic invalidation of GSDMD in A549^{NLRP1+} or NHBE cells using CRISPR Cas9 technic.

(B) Western blot examination of Gasdermin cleavages by SARS-CoV-2 NSP5 or NSP3, HRV3C or recombinant human Caspase-1 (CASP1) proteases in cell lysates from A549 expressing GSDMD, GSDMB or GSDME. GSDMD (anti Cter), GSDME, GSDMB, NSP5 and ACTIN were immunoblotted.

(C) Western blot examination of GSDMD cleavage by SARS-CoV-2 3CL (NSP5) or recombinant human Caspase-1 (CASP1) proteases in cell lysates from A549 expressing WT GSDMD or GSDMD^{193A} constructs in presence/absence of the 3CL inhibitor PF-00835231 (10µM). GSDMD (anti Cter), NSP5 and ACTIN were immunoblotted.

Data information:

Western Blot (A-C) images are from one experiment performed three times.

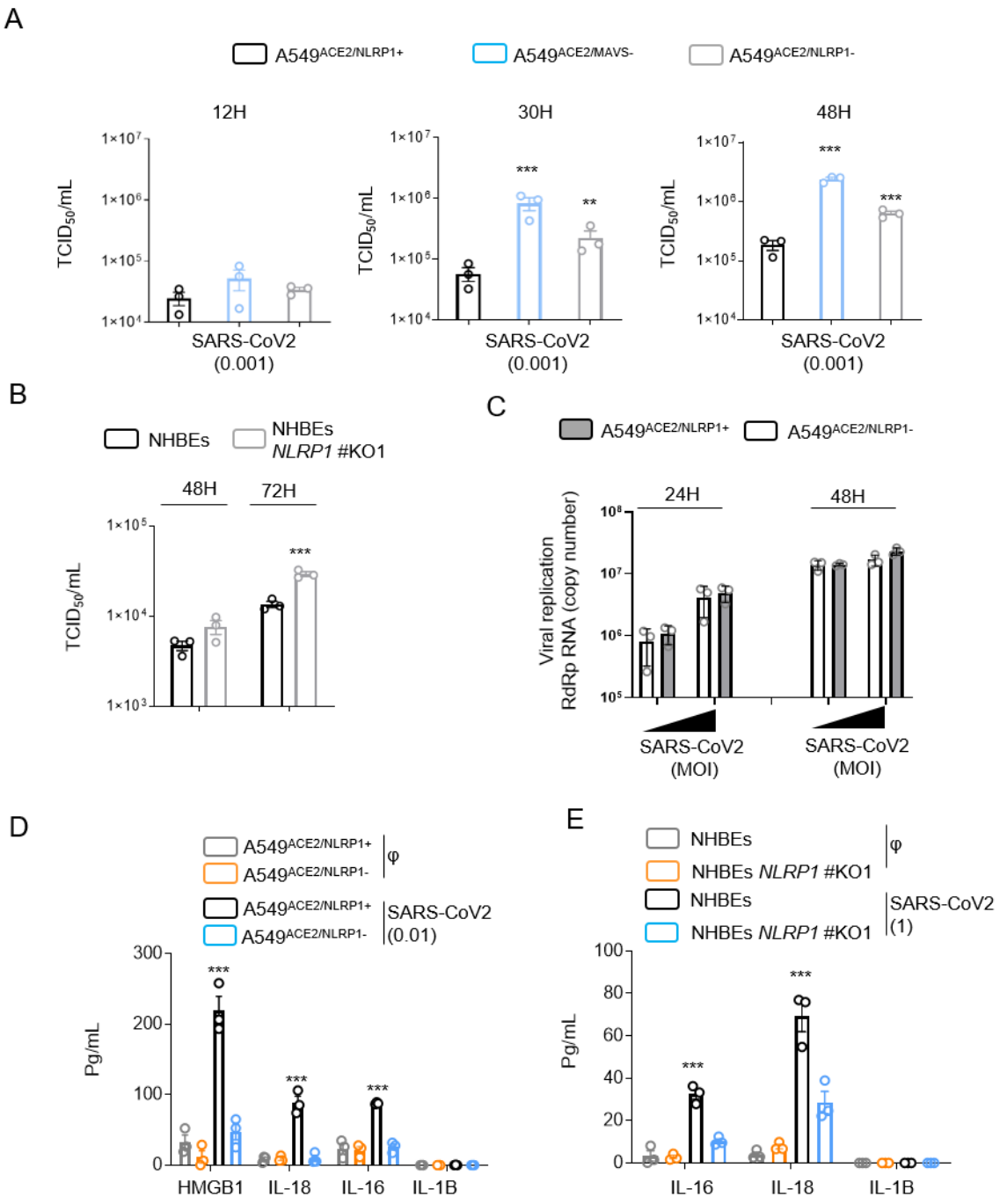


Figure S4 (Related to Figures 1-4). NLRP1-dependent pyroptosis both limits the production of infectious particles and promotes alarmin/DAMP release

(A) TCID50 measure of production of infectious viral particles in A549$^{ACE2/NLRP1+}$, A549$^{ACE2/NLRP1-}$ and A549$^{ACE2/MAVS-}$ cells infected for various times with SARS-CoV-2.

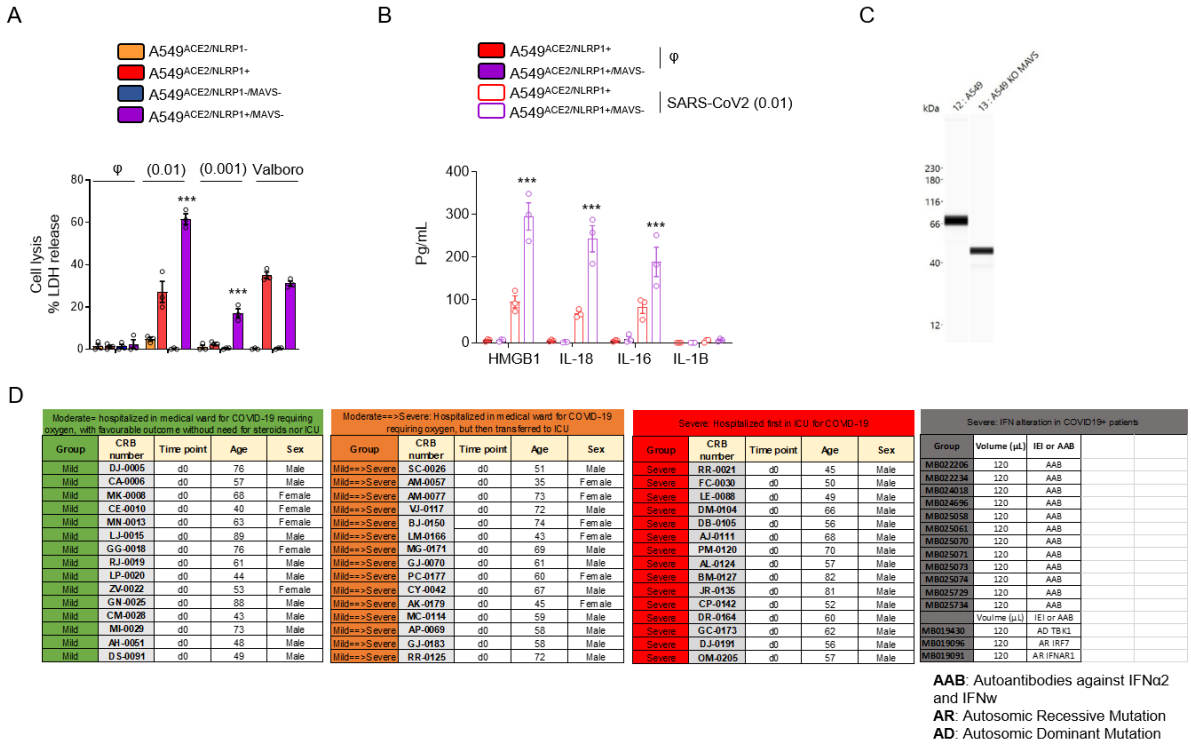
(B) TCID50 measure of production of infectious viral particles in NHBE^{WT} and NHBE$^{NLRP1-/-}$ cells infected for various times with SARS-CoV-2 (MOI 0.5).

(C) Q-RT PCR evaluation of SARS-CoV-2 RdRp mRNA levels in $A549^{ACE2/NLRP1+}$ or $A549^{ACE2/NLRP1-}$ cells after 24 and 48 hours of SARS-CoV-2 infection (MOIs 0.05 and 0.005).

(D, E) Measure of the release of various Alarmins/Cytokines after 24 (D) or 36 (E) hours of infection with SARS-CoV-2 (MOI 0.05 and 1 respectively) by $A549^{ACE2/NLRP1+}$, $A549^{ACE2/NLRP1-}$, $NHBE^{WT}$ and $NHBE^{NLRP1-/-}$ cells.

Data informations:

Graphs A-E show data presented as means ± SEM from n=3 independent pooled experiments; **p ≤ 0.01, ***p ≤ 0.001 for the indicated comparisons with t-test.



Moderate= hospitalized in medical ward for COVID-19 requiring oxygen, with favourable outcome without need for steroids nor ICU

Group	CRB number	Time point	Age	Sex
Mild	DJ-0005	d0	76	Male
Mild	CA-0006	d0	57	Male
Mild	MK-0008	d0	68	Female
Mild	CE-0010	d0	40	Female
Mild	MN-0013	d0	63	Female
Mild	LJ-0015	d0	89	Male
Mild	GG-0018	d0	76	Female
Mild	RJ-0019	d0	61	Male
Mild	LP-0020	d0	44	Male
Mild	ZV-0022	d0	53	Female
Mild	GN-0025	d0	88	Male
Mild	CM-0028	d0	43	Male
Mild	MI-0029	d0	73	Male
Mild	AH-0051	d0	48	Male
Mild	DS-0091	d0	49	Male

Moderate==>Severe: Hospitalized in medical ward for COVID-19 requiring oxygen, but then transferred to ICU

Group	CRB number	Time point	Age	Sex
Mild==>Severe	SC-0026	d0	51	Male
Mild==>Severe	AM-0057	d0	35	Female
Mild==>Severe	AM-0077	d0	73	Female
Mild==>Severe	VJ-0117	d0	72	Male
Mild==>Severe	BJ-0150	d0	74	Female
Mild==>Severe	LM-0166	d0	43	Female
Mild==>Severe	MG-0171	d0	69	Male
Mild==>Severe	GJ-0070	d0	61	Male
Mild==>Severe	PC-0177	d0	60	Female
Mild==>Severe	CY-0042	d0	67	Male
Mild==>Severe	AK-0179	d0	45	Female
Mild==>Severe	MC-0114	d0	59	Male
Mild==>Severe	AP-0069	d0	58	Male
Mild==>Severe	GJ-0183	d0	58	Male
Mild==>Severe	RR-0125	d0	72	Male

Severe: Hospitalized first in ICU for COVID-19

Group	CRB number	Time point	Age	Sex
Severe	RR-0021	d0	45	Male
Severe	FC-0030	d0	50	Male
Severe	LE-0088	d0	49	Male
Severe	DM-0104	d0	66	Male
Severe	DB-0105	d0	56	Male
Severe	AJ-0111	d0	68	Male
Severe	PM-0120	d0	70	Male
Severe	AL-0124	d0	57	Male
Severe	BM-0127	d0	82	Male
Severe	JR-0135	d0	81	Male
Severe	CP-0142	d0	52	Male
Severe	DR-0164	d0	60	Male
Severe	GC-0173	d0	62	Male
Severe	DJ-0191	d0	56	Male
Severe	OM-0205	d0	57	Male

Severe: IFN alteration in COVID19+ patients

Group	Volume (µL)	IEI or AAB
MB022206	120	AAB
MB022234	120	AAB
MB024018	120	AAB
MB024696	120	AAB
MB025058	120	AAB
MB025061	120	AAB
MB025070	120	AAB
MB025071	120	AAB
MB025073	120	AAB
MB025074	120	AAB
MB025729	120	AAB
MB025734	120	AAB
	Voulme (µL)	IEI or AAB
MB019430	120	AD TBK1
MB019096	120	AR IRF7
MB019091	120	AR IFNAR1

AAB: Autoantibodies against IFNα2 and IFNw
AR: Autosomic Recessive Mutation
AD: Autosomic Dominant Mutation

Figure S5 (Related to Figure 4). Plasmas from COVID19 patients show enrichments in (IL)-16/-18/GSDME and CASP3.

(A) Measure of cell lysis (LDH release) in A549^{NLRP1+}, A549^{NLRP1-}, A549$^{NLRP1+/MAVS-}$ and A549$^{NLRP1-/MAVS-}$ upon SARS-CoV-2 infection or Valboro (5 µM) treatment for 24 hours.

(B) Measure of cytokine/alarmin release in A549^{NLRP1+} or A549$^{NLRP1+/MAVS-}$ upon SARS-CoV-2 infection for 24 hours.

(C) Western Blot examination of genetic invalidation of MAVS in A549 cells. MAVS appears at the 66kDa.

(D) Information on COVID-19^{+} patients hospitalized and COVID-19^{+} patients exhibiting Interferon alterations used in this study

Data information:

Graphs A, B show data presented as means ± SEM from n=3 independent pooled experiments; ***p ≤ 0.001 for the indicated comparisons with t-test. Western Blot (C) image is from one experiment.

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