Supplementary Materials and Data

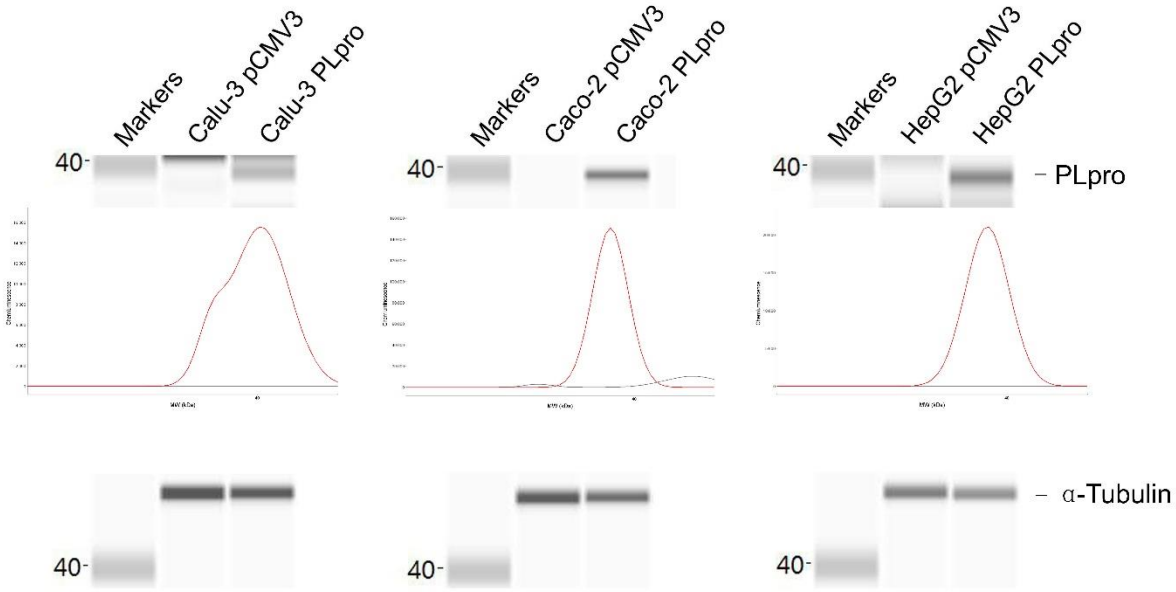

Figure S1. Expression of PLpro protein in epithelial cells. Detection of PLpro in Calu-3, Caco-2, and HepG2 cells by capillary western blot assay. Capillary western blot results for PLpro are shown as gel-like image view in the upper panels, electropherograms in the middle panels, and α-Tubulin as a loading control in the lower panels.

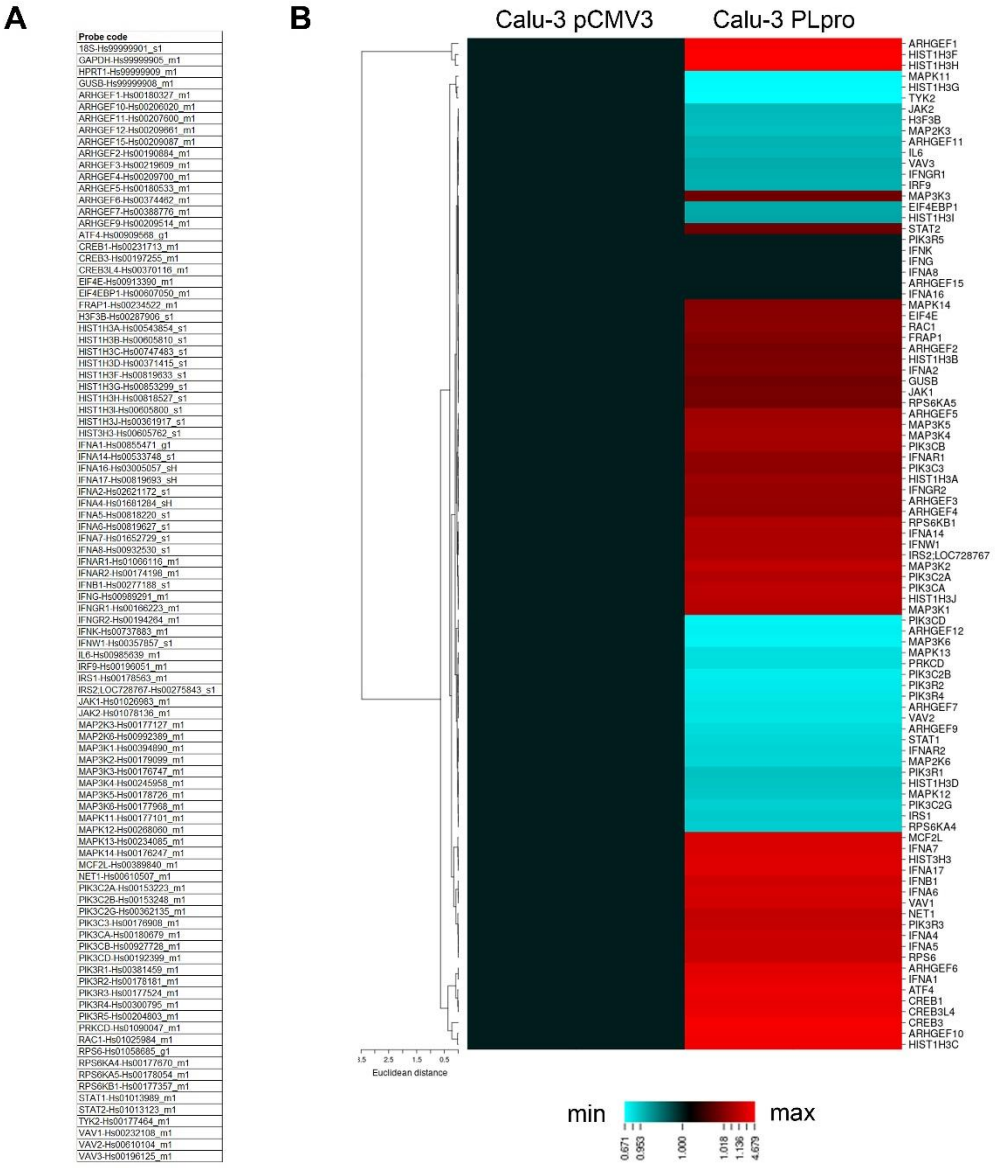

Figure S2. PLpro expression up-regulates genes of IFN pathway. (**A**) List of TaqMan Gene Expression Assays ID for each well on the 96 plate; (**B**) Heatmap representation of interferon-related genes analyzed by TaqMan® Array Human Interferon Pathway that were up-regulated (in red) or down-regulated (in light blue) in Calu-3 cells transfected with plasmid for PLpro (PLpro) or empty vector (pCMV3). Data are the mean ± SD of three independent experiments.

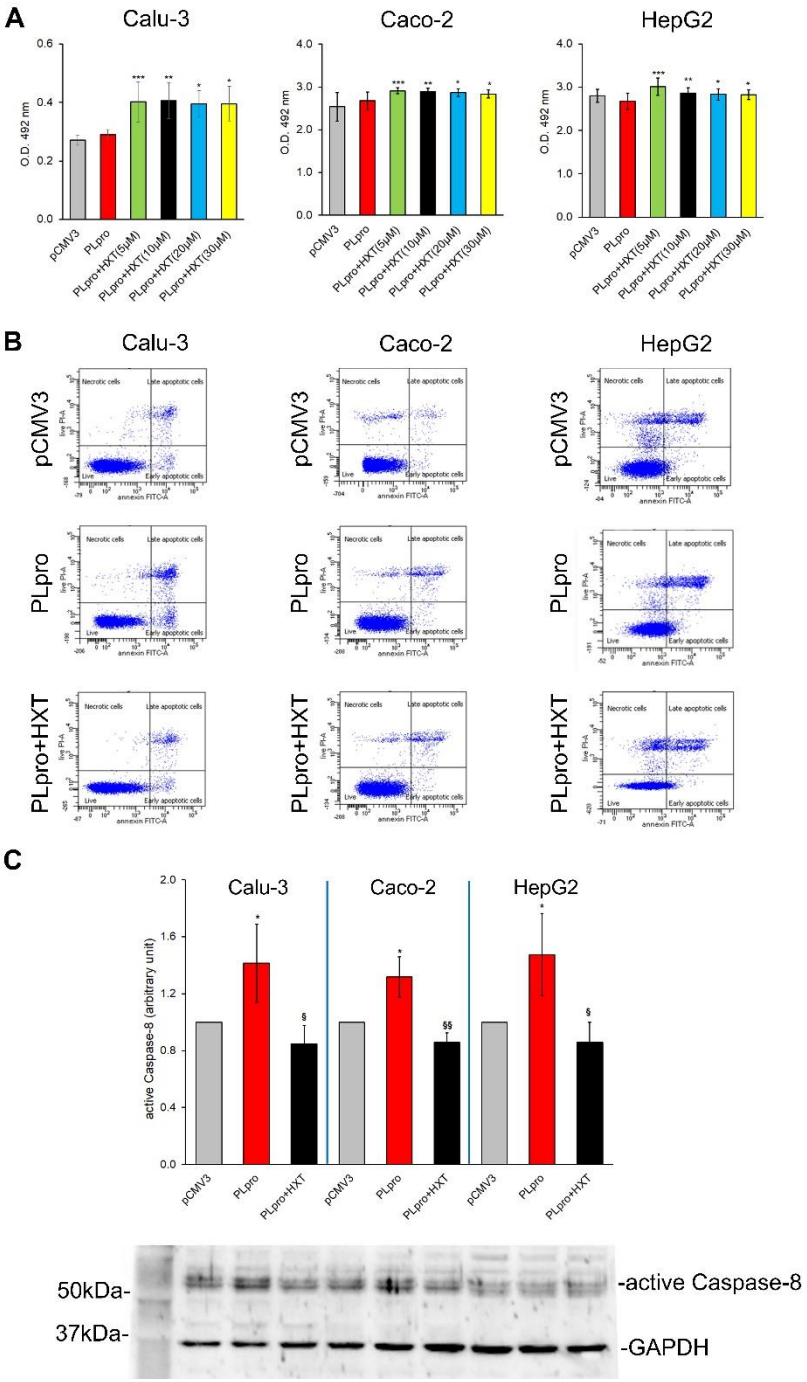

Figure S3. Analysis of apoptosis. (**A**) Cell viability assessed by XTT assay in Calu-3, Caco-2 and HepG2 cells transfected with plasmid for PLpro (PLpro) or empty vector (pCMV3) treated with or without 10 μM HXT for 24 hours. Data refers to mean O.D. of two independent experiments repeated at least in quintupled. Data were analyzed by 2-tailed t tests, **p* <0.05, **p <0.01, ***p <0.001 vs pCMV3. (**B**) Plots show the apoptotic rate of PLpro Calu-3, Caco-2 and HepG2 cells treated with or without 10 μM HXT for 24 hours and compared to untreated (pCMV3) cells measured by FITC Annexin V and flow cytometry. **(C)** Densitometric analysis (*upper panel*) and representative western blotting (*lower panel*) for active Caspase-8 in Calu-3, Caco-2 and HepG2 cells transfected with empty vector (pCMV3), or with plasmid for PLpro (PLpro) subjected or not with 10 μM HXT for 24 hours. GAPDH is reported as loading control. The original image is reported in the supplementary file name blots.

Data were analyzed by 2-tailed *t* tests, *p <0.05 vs pCMV3; § p <0.05, §§ p <0.01 vs PLpro.

A

	A	B	C	D	E	F	G	H	I	J	K	L
1	POS	POS	NEG	NEG	EOTAXIN	EOTAXIN-2	GCSF	GM-CSF	ICAM-1	IFN-γ	I-309	IL-1α
2	POS	POS	NEG	NEG	EOTAXIN	EOTAXIN-2	GCSF	GM-CSF	ICAM-1	IFN-γ	I-309	IL-1α
3	IL-1β	IL-2	IL-3	IL-4	IL-6	IL-6sR	IL-7	IL-8	IL-10	IL-11	IL-12 p40	IL-12 p70
4	IL-1β	IL-2	IL-3	IL-4	IL-6	IL-6sR	IL-7	IL-8	IL-10	IL-11	IL-12 p40	IL-12 p70
5	IL-13	IL-15	IL-16	IL-17	IIP-10	MCP-1	MCP-2	M-CSF	MIG	MIP-1α	MIP-1β	MIP-1δ
6	IL-13	IL-15	IL-16	IL-17	IIP-10	MCP-1	MCP-2	M-CSF	MIG	MIP-1α	MIP-1β	MIP-1δ
7	RANTES	TGF-β1	TNF-α	TNF-β	s TNF RI	s TNF RII	PDGF-BB	TIMP-2	BLANK	BLANK	NEG	POS
8	RANTES	TGF-β1	TNF-α	TNF-β	s TNF RI	s TNF RII	PDGF-BB	TIMP-2	BLANK	BLANK	NEG	POS

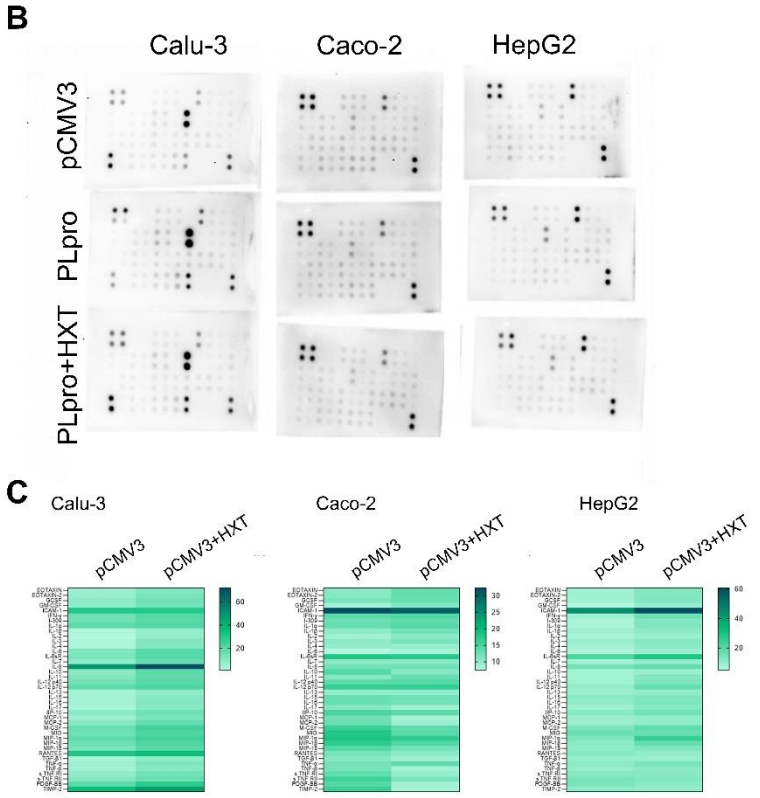

Figure S4. Inflammatory pattern by antibody array. (A) Layout of antibodies as spotted on the membrane (upper panel). The abbreviated names for 40 different cytokine probes are reported. **(B)** Representative photographs of cytokine arrays in which each cytokine is represented by duplicate spots on a single membrane (lower panel). **(C)** Heatmap representation of semi-quantitative expression of 40 pro-inflammatory cytokines and chemokines by antibody array in Calu-3, Caco-2, and HepG2 cells expressing pCMV3 treated or not with HXT.

Table S1. Primers sequences for SYBR Green qRT-PCR.

Genes	Accession number	Sequence 5′ - 3′
SarsCoV2 PLpro/NSP3	NC_045512.2(4955-5905nt)	Forward: CAAGGGCTGGTGAAGCTGCTA Reverse: CTTAAGGGTTGTCTGCTGTTGTCC
IFNA1	NM_024013.3	Forward: GGAGTTTGATGGCAACCAGT Reverse: CTCTCCTCCTGCATCACACA
IFNA7	NM_021057.2	Forward: CAGACCCACAGCCTGCGT Reverse: AAACTCCTCCTCTGGGAATCTG
ATF4	NM_182810.3	Forward: TCAAACCTCATGGGTTCTCC Reverse: GTGTCATCCAACGTGGTCAG
CREB3	NM_006368.5	Forward: TTGCTAGGCTAGTACTGACAGATGA Reverse: ATTTCAAGACCCTGCTCTCTAAACC
CREB3L4	NM_001255978.2	Forward: CTCAAGAAGGTCAGGAGGAAAATCC Reverse: CTGAGCTACCAAGGAGATGTTGTG
HIST3H3	NM_003493.3	Forward: GAGCTGCTAATCCGCAAGTT Reverse: TGGATGACACACAGGTTGGT
HIST1H3F	NM_021018.3	Forward: AGCTACTGATTCGCAAGCTACC Reverse: ACAGGTTGGTGTCCTCAAAGAG
HIST1H3H	NM_003536.3	Forward: GATCAGAAAGCTGCCTTTTCAG Reverse: GATGTCCTTGGGCATGATAGTC
MCF2L	NM_015205.3	Forward: ATGAAATCATGCACCAGGACAT Reverse: CATGACATTCTGGAACTCCTTG
TNF-α	NM_000594.4	Forward: CCGAGTGACAAGCCTGTAGC Reverse: AGGAGGTTGACCTTGGTCTG
IL-6	NM_000600.5	Forward: AGACAGCCACTCACCTCTTCAG Reverse: TTCTGCCAGTGCCTCTTTGCTG
IL1-β	NM_000576.3	Forward: GCTGAGGAAGATGCTGGTTC Reverse: TCCATATCCTGTCCCTGGAG
GAPDH	NM_002046.7	Forward: TCCAAAATCAAGTGGGGCGA Reverse: TGATGACCCTTTTTGGCTCCC

Table S2. Means of raw data for pro-inflammatory protein array.

	Calu-3		Caco-2		HepG2	
	Mean pCMV3	*Mean PLpro*	*Mean pCMV3*	*Mean PLpro*	*Mean pCMV3*	*Mean PLpro*
EOTAXIN	6.232	11.203	10.520	12.958	8.328	12.806
EOTAXIN-2	6.358	11.680	10.674	13.040	8.251	12.455
GCSF	8.695	11.774	11.557	12.774	7.906	13.015
GM-CSF	8.172	9.235	8.592	10.860	5.087	9.869
ICAM-1	30.616	30.528	32.398	30.451	44.419	60.744
IFN-γ	14.789	12.654	13.708	14.407	8.337	16.236
I-309	15.390	12.532	12.577	13.506	6.886	13.066
IL-1α	18.798	15.320	13.039	13.963	6.218	13.119
IL-1β	1.978	7.938	10.089	12.23	8.619	11.243
IL-2	1.210	5.603	8.571	10.264	6.630	9.157
IL-3	2.441	7.7	10.235	11.622	8.994	12.125
IL-4	2.335	7.097	9.839	9.564	7.684	11.313
IL-6	10.919	16.342	7.458	8.4525	5.787	9.021
IL-6sR	12.284	13.358	17.948	17.364	21.323	28.872
IL-7	8.194	8.4845	10.295	10.411	7.710	10.213
IL-8	48.953	62.727	11.916	12.169	15.038	17.671
IL-10	9.219	7.134	14.072	10.619	7.349	9.097
IL-11	11.290	9.686	10.578	12.756	7.832	12.647
IL-12 p40	14.259	12.007	13.758	14.306	8.320	13.711
IL-12 p70	18.657	16.880	16.392	16.477	10.266	17.258
IL-13	3.856	5.502	9.795	9.739	9.233	10.246
IL-15	4.407	8.15	12.739	12.084	11.495	12.967
IL-16	4.536	6.231	12.824	11.925	10.792	13.378
IL-17	2.992	5.276	10.615	8.815	8.181	11.502
IIP-10	9.344	8.464	14.604	13.633	12.038	15.110
MCP-1	5.839	8.721	12.289	8.309	8.957	9.938
MCP-2	10.208	8.763	12.587	8.974	9.051	10.534
M-CSF	15.744	13.903	16.016	13.320	13.537	18.214
MIG	14.966	13.864	17.596	13.847	16.297	16.691
MIP-1α	18.111	16.136	18.356	16.909	13.076	25.018
MIP-1β	15.373	13.014	16.865	14.398	11.443	17.175
MIP-1δ	12.546	11.107	14.182	12.015	10.643	13.496
RANTES	30.834	61.17	11.591	10.209	12.084	10.323
TGF-β1	3.378	5.151	10.541	8.5385	9.406	9.423
TNF-α	5.166	7.374	11.857	13.610	11.286	14.594
TNF-β	4.620	6.196	11.708	8.5425	9.961	11.357
s TNF RI	8.007	8.527	13.194	9.473	11.862	11.812
s TNF RII	10.866	9.305	15.526	11.441	14.356	12.311
PDGF-BB	18.442	13.364	14.0605	8.544	13.820	11.504
TIMP-2	35.236	43.716	16.01125	8.485	10.991	10.332