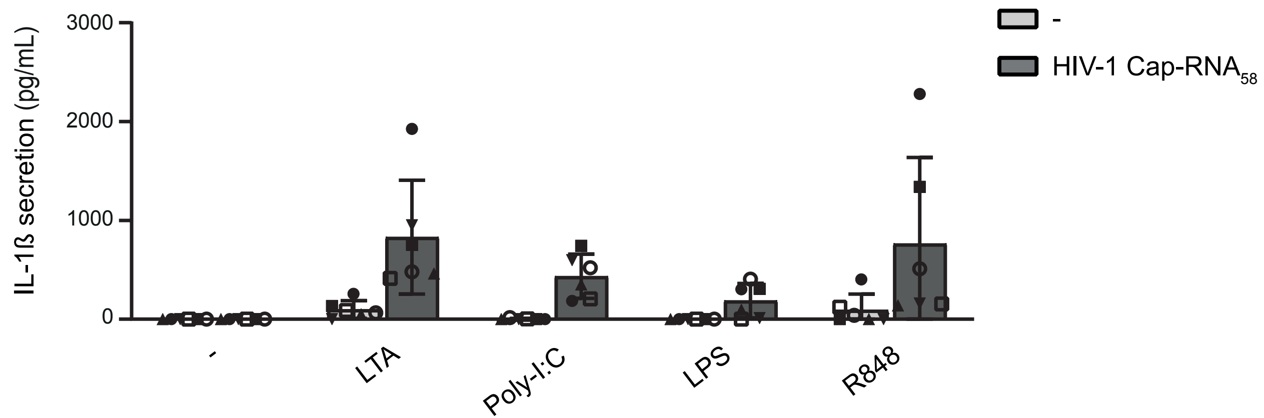
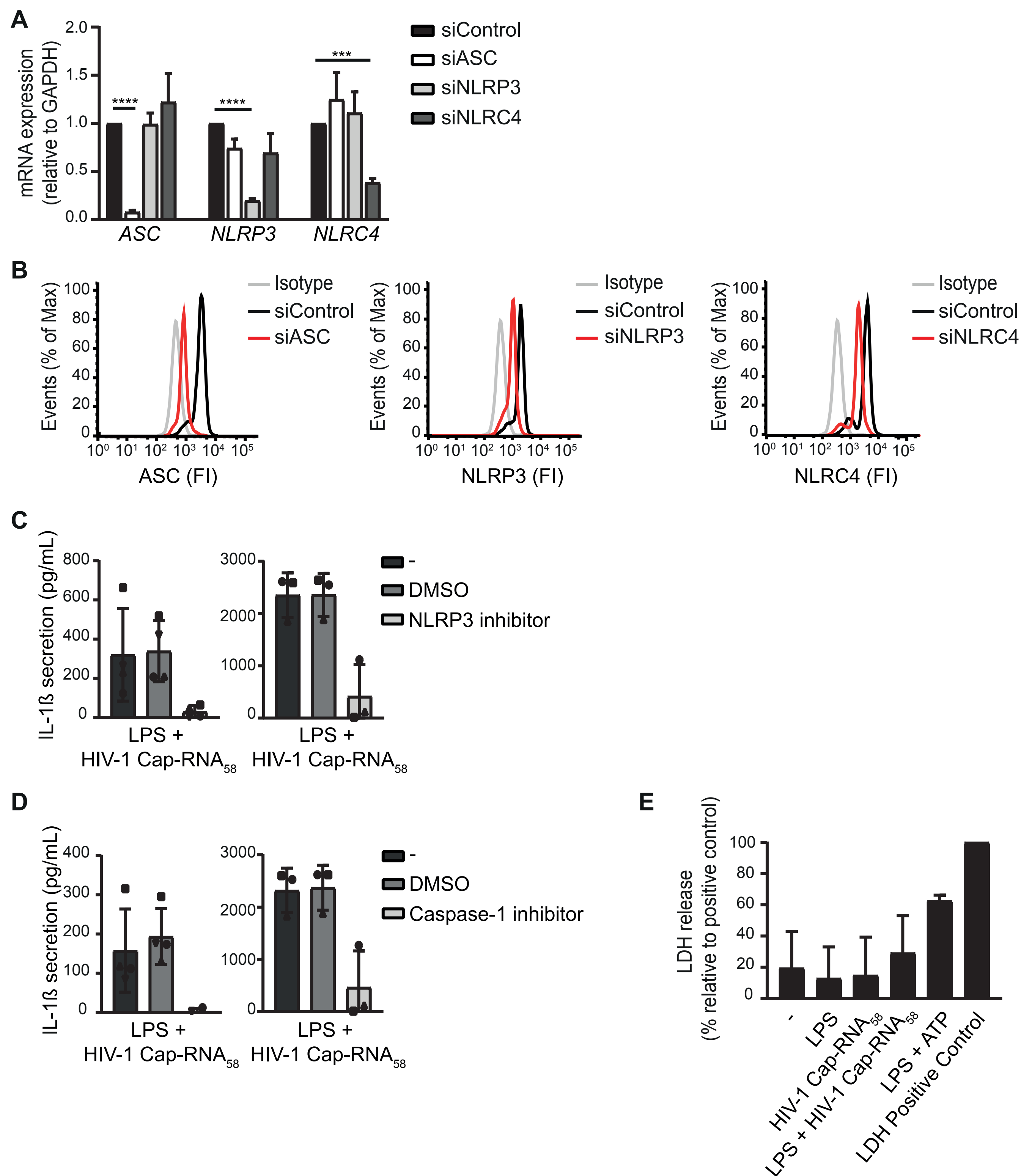
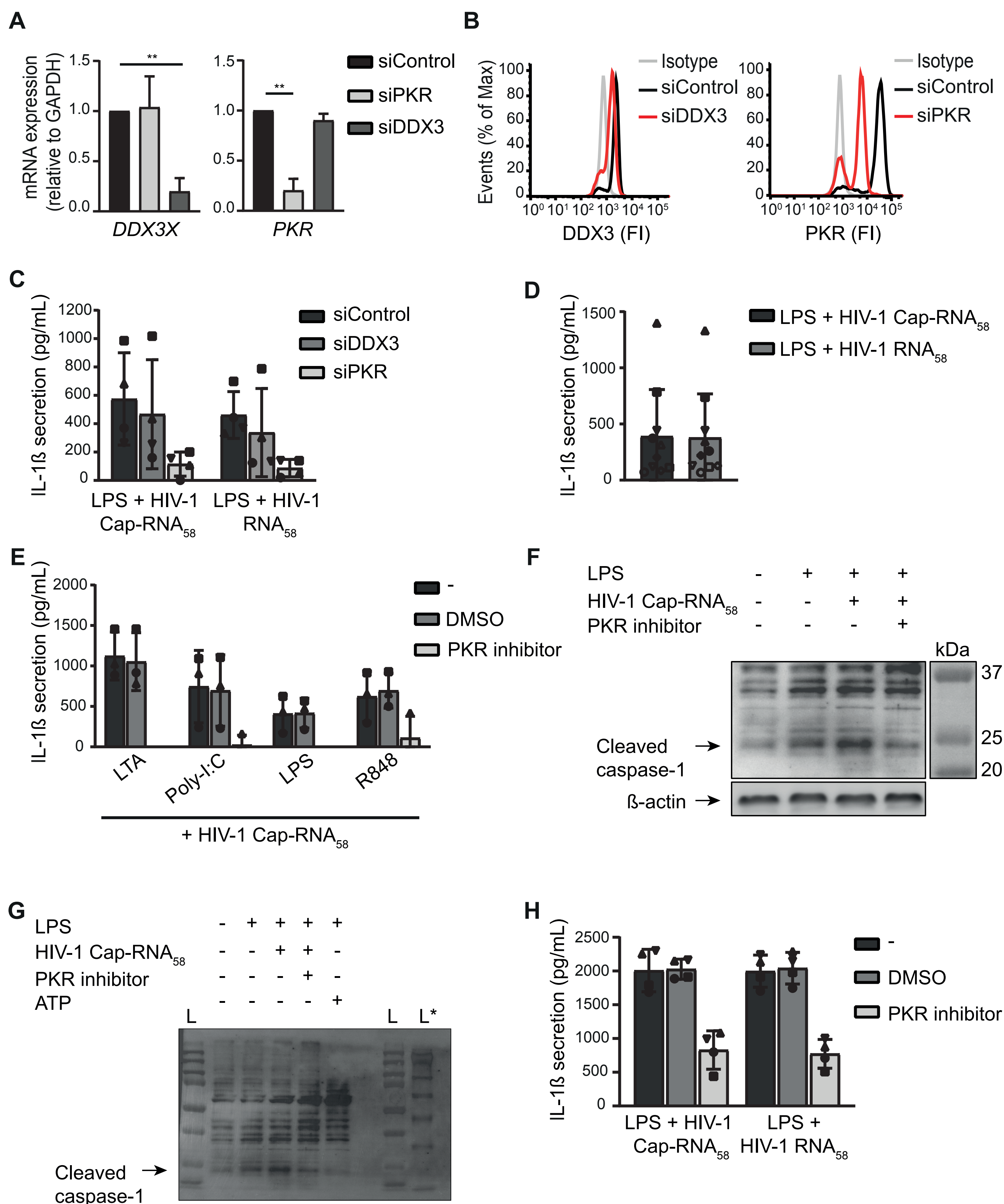


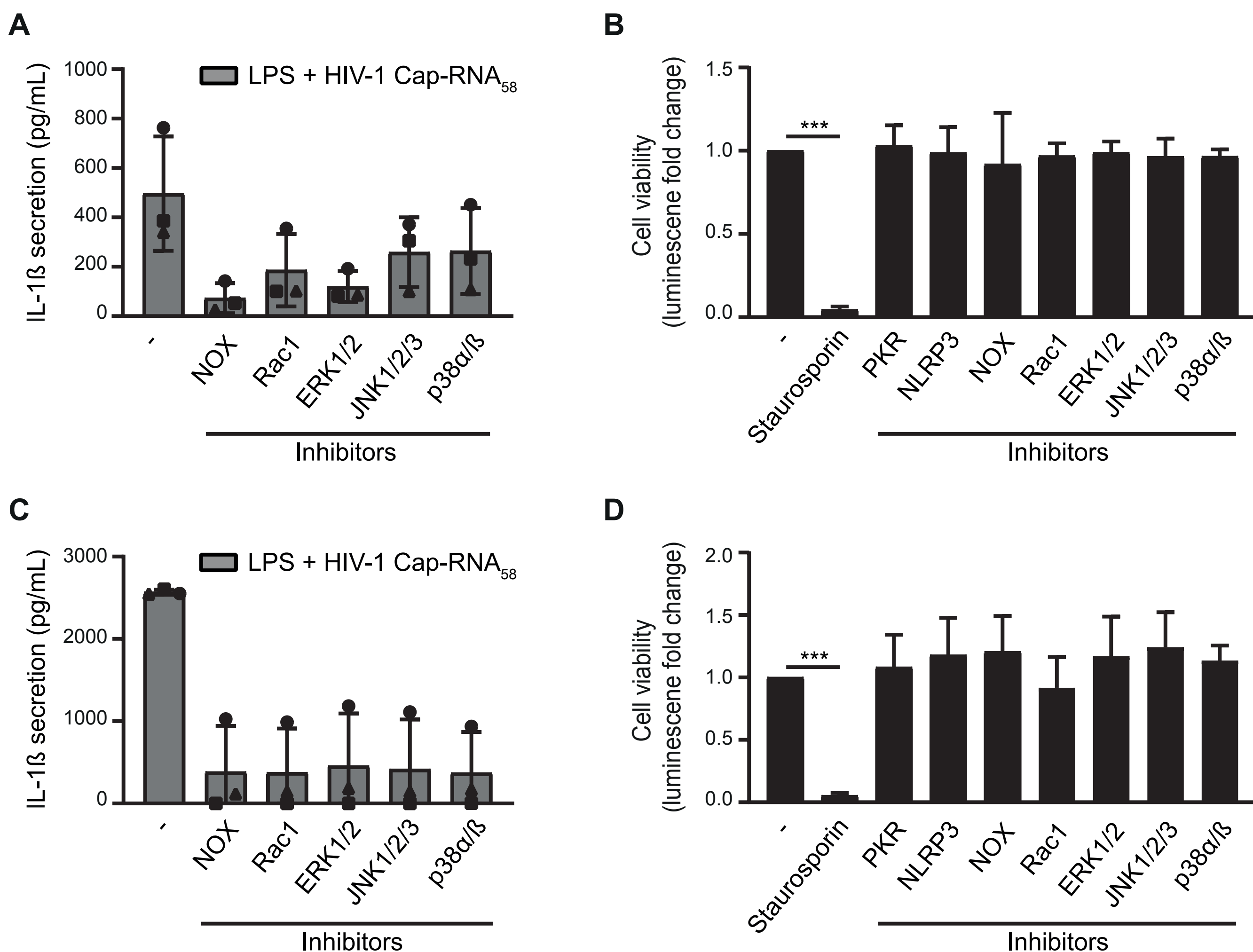
Supplementary Figure S1. Abortive HIV-1 RNA induces pro-IL-1β processing. Monocyte-derived DCs were (co)stimulated with different TLR ligands (LTA, Poly-I:C, LPS or R848) and/or HIV-1 Cap-RNA_{58}. IL-1β secretion (pg/mL) in cell culture supernatant was measured after 24 h by ELISA. Data are representative of collated data of six donors, with each symbol representing a different donor (mean ± s.d.).



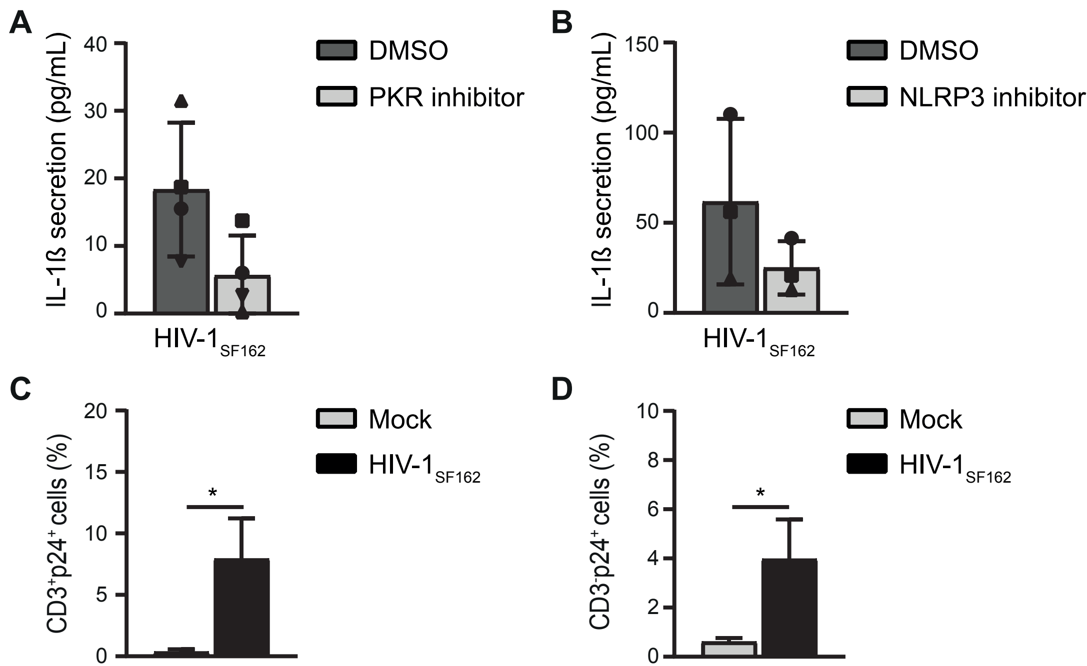
Supplementary Figure S2. Silencing efficiency and NLRP3 inflammasome activation. (A, B) DCs were silenced for ASC, NLRP3 and NLRC4 and silencing efficiency was examined using quantitative real-time PCR, relative to GAPDH, or using flow cytometry to determine fluorescent intensity (FI). Responses induced by cells treated with siControl were set at 1. **(C, D)** DCs (left bar graph) or PBMCs (right bar graph) were incubated with DMSO control or with NLRP3 inhibitor MCC950 or caspase-1 inhibitor Ac-YVAD-cmk for 2 h, followed by costimulation with LPS and HIV-1 Cap-RNA_{58}. IL-1β secretion (pg/mL) in the supernatant was measured after 24 h by ELISA. **(E)** DCs were stimulated with LPS, HIV-1 Cap-RNA_{58} or costimulated with LPS and HIV-1 Cap-RNA_{58} for 24 h. ATP was added to the LPS alone condition for the final 4 h of stimulation. The percentage (%) LDH release was assessed relative to the LDH Positive Control of the LDH assay, which was set at 100%. Data are representative of collated data of three donors (A, C-E) or representative of three donors (B), with each symbol representing a different donor (C, D) (mean ± s.d.). ***$P < 0.001$, ****$P < 0.0001$, Student's t-test.



Supplementary Figure S3. Silencing efficiency and PKR-dependent processing of pro-IL-1β. (A, B) DCs were silenced for DDX3 and PKR and silencing efficiency was examined using quantitative real-time PCR, relative to GAPDH (A) or using flow cytometry to determine fluorescent intensity (FI) (B). Responses induced by cells treated with control siRNA (siControl) were set at 1 (A). **(C, D)** DDX3 and PKR-silenced DCs (C) or unsilenced DCs (D) were costimulated with LPS and either HIV-1 Cap-RNA$_{58}$ or HIV-1 RNA$_{58}$. IL-1β secretion (pg/mL) was measured in the supernatant by ELISA. **(E)** DCs were incubated with DMSO or PKR inhibitor C16 for 2 h, followed by costimulation with HIV-1 Cap-RNA$_{58}$ and different TLR ligands. IL-1β secretion (pg/mL) was measured in the supernatant after 24 h by ELISA. **(F, G)** Cropped (F) and original uncropped (G) western blot image of cleaved caspase-1 in cell lysates of untreated DCs or DCs treated with LPS or LPS and HIV-1 Cap-RNA$_{58}$in the presence and absence of PKR inhibitor C16, or LPS + ATP, harvested after 8 h. Molecular weight in kilo dalton (kDa) was shown (F). Ladders are indicated by L (Precision plus dual color, Bio-Rad) or L* (Thermo Fisher) (G). **(H)** PBMCs were incubated with DMSO or PKR inhibitor C16 for 2 h, followed by costimulation with with LPS and either HIV-1 Cap-RNA$_{58}$ or HIV-1 RNA$_{58}$. IL-1β secretion (pg/mL) was measured in the supernatant after 24 h by ELISA. Data are representative of collated data of three (E), four (A, C, H) or ten (D) donors, or representative of three donors (B, F, G), with each symbol representing a different donor (C-E, H). (mean ± s.d.). $^{**}P < 0.01$, Student's t-test.



Supplementary Figure S4. IL-1β processing via ROS and MAP kinases. (A-D) DCs (A, B) or PBMCs (C, D) were treated with inhibitors C16 (PKR), diphenyleneiodonium chloride (NAPDH oxidase (NOX)), NSC23766 (Rac1), FR180204 (ERK1/2), SP600125 (JNK1/2/3) and SB203580 (p38α/β) for 2 hours, followed by costimulation with LPS and HIV-1 Cap-RNA_{58} (A, C), or without costimulation for 24 hours to assess cell viability (B, D). IL-1β secretion (pg/mL) was measured in the supernatant after 24 h by ELISA (A, C). Cell viability was assessed by measuring luminescence proportional to ATP production (B, D). Cells were treated with staurosporin for 4 h as the control for cell death. Measurements in untreated DCs were set at 1. Data are collated data of three (A, C, D) or four (B) donors, with each symbol representing a different donor (A,C) (mean ± s.d.). ***P < 0.001, Student's t-test.



Supplementary figure S5. HIV-1_{SF162} infection induces IL-1β secretion via PKR and inflammasome activation. (A, B) PBMCs were incubated with IL-2 for 3 days, treated with PKR inhibitor C16 or NLRP3 inhibitor MCC950 for 2 h and infected with CCR5 (R5)-tropic virus HIV-1_{SF162} with MOI 0.1. IL-1β was measured in the supernatant after 48 h by ELISA. **(C, D)** Similarly, IL-2-treated PBMCs were infected with HIV-1_{SF162} and the percentage of intracellular $p24^+$ cells was measured after 4 days by flow cytometry in the $CD3^+$ (C) and $CD3^-$ (D) cell population. Data are representative of collated data of four (A, C, D) or three (B) donors, with each symbol representing a different donor (A, B) (mean ± s.d.). *$P < 0.05$, Student's t-test.

Table S1. Quantitative real-time PCR primers to analyze mRNA expression

Gene	Forward primer	Reverse primer
GAPDH	CCATGTTCGTCATGGGTGTG	GGTGCTAAGCAGTTGGTGGTG
DDX3X	AGTGATTACGATGGCATTGGC	AGCGACTGTTTCCACCACG
PKR	TGGCCGCTAAACTTGCATATC	CTGGTCACTAAAGAGTTGCTTTGG
IL1B	TTTGAGTCTGCCCAGTTCCC	TCAGTTATATCCTGGCCGCC
ASC	TGCACTTTATAGACCAGCACCG	AGCCACTCAACGTTTGTGACC
NLRP3	TTCTTTGCCGCCATGTACTACC	AAGCTTCAAACGACTCCCTGG
NLRC4	TACCTGGCACAATCAGGAAGC	GAAAAGAACCCTCTGCCGC