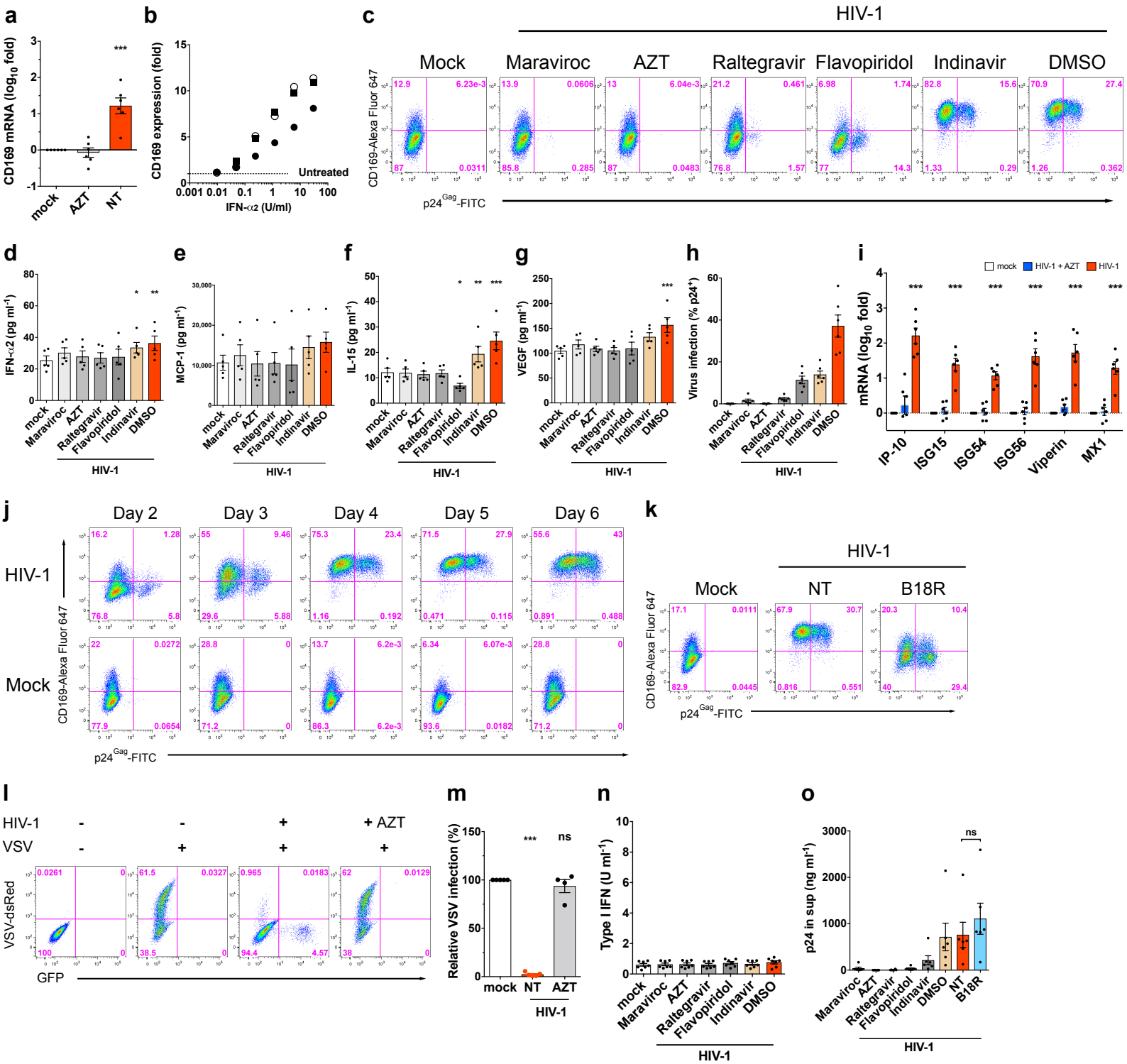
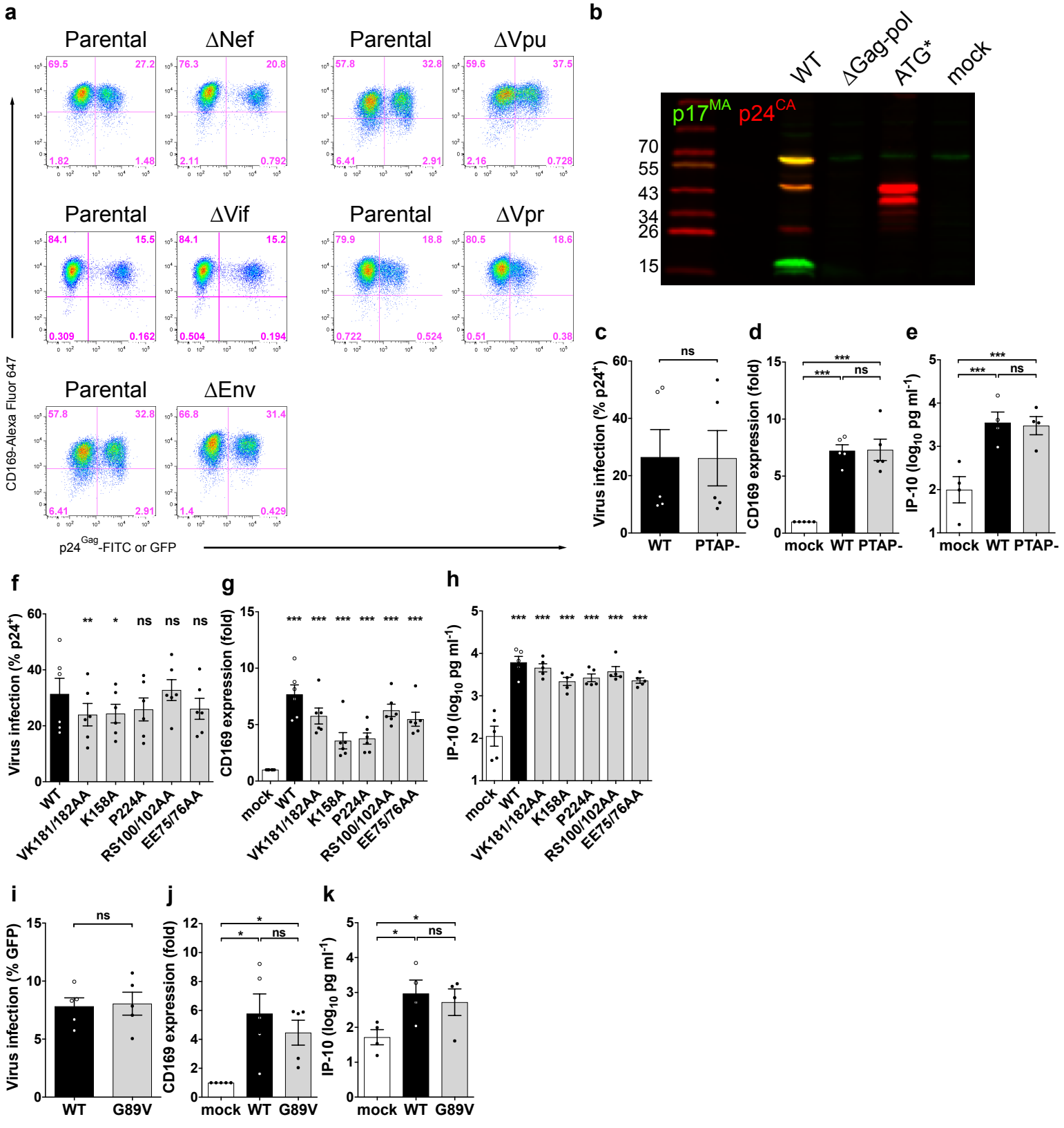
**Supplementary Information**

# HIV-1 Intron-containing RNA Expression Induces Innate Immune Activation and T Cell Dysfunction

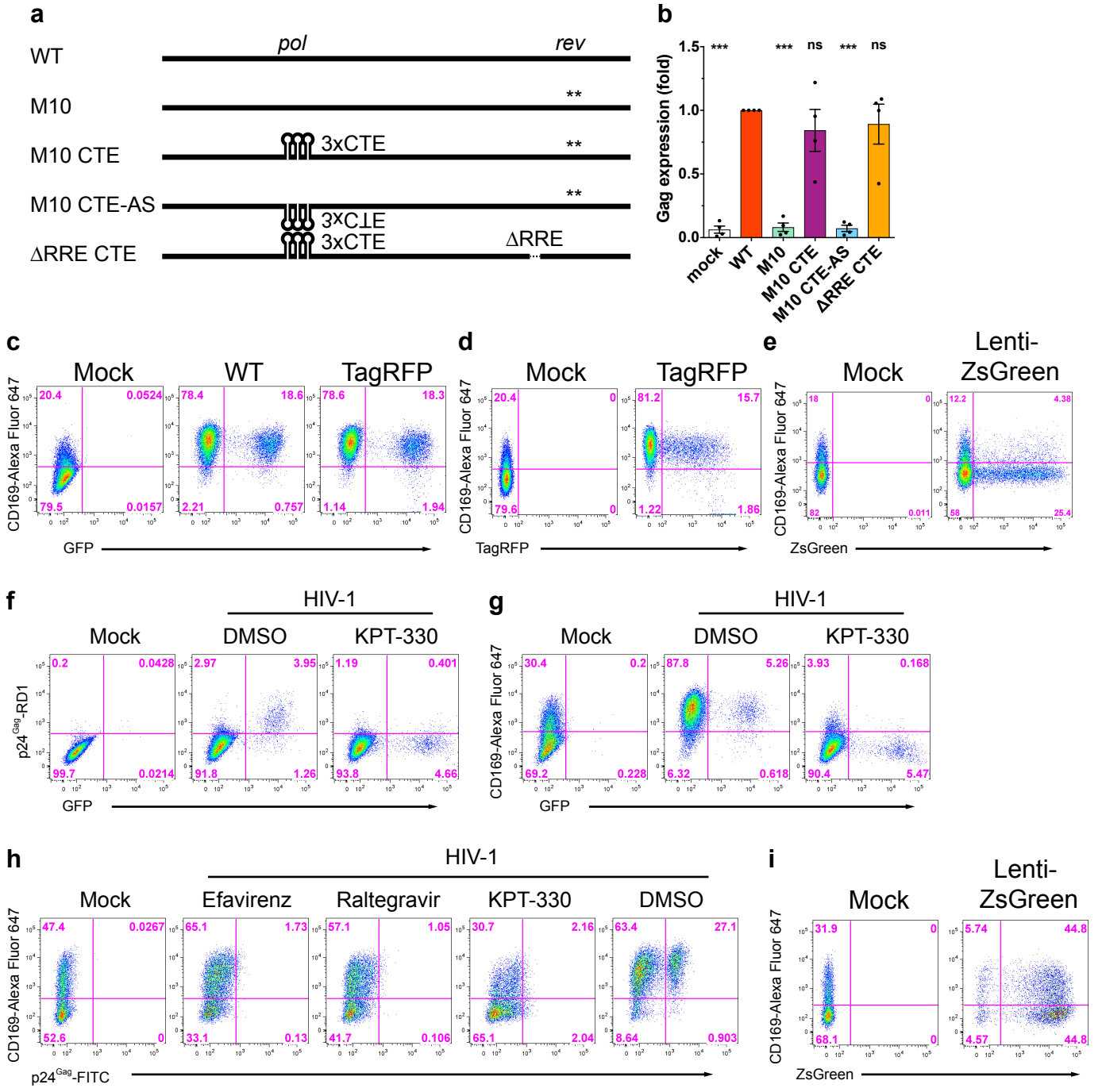
Akiyama et al.



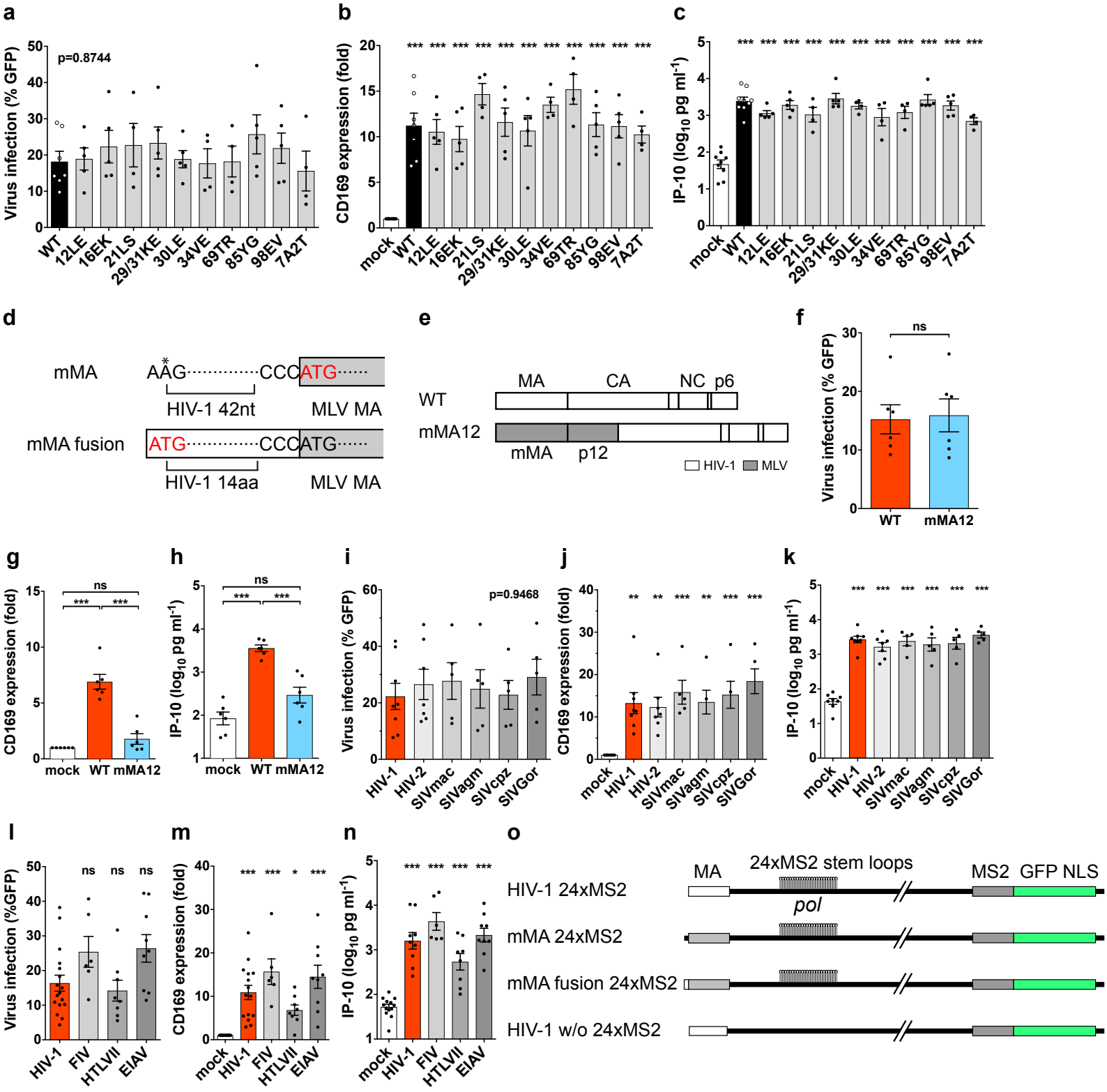

**Supplementary Figure 1. Late step of HIV-1 replication in MDMs triggers immune activation.**
**a**, MDMs were infected with LaiΔenvGFP/G (MOI 2) and harvested for mRNA isolation 3 days post infection (dpi). CD169 mRNA was quantified by qRT-PCR and normalized to that of mock. **b**, MDMs were incubated with the indicated amounts of IFN-$\alpha_2$ for 2 days, and CD169 expression was measured by flow cytometry. **c**, Representative flow cytometry profiles of MDMs infected with Lai/YU-2env (MOI 1) and analyzed for viral infection (intracellular $p24^{Gag}$) and CD169 expression 6 dpi. MDMs were untreated (DMSO) or treated with drugs prior to infection (maraviroc, AZT or raltegravir), or 2-3 hours post infection (flavopiridol or indinavir). **d-g**, Cytokine production in HIV-1-infected MDMs was measured by Luminex (6 dpi). **h**, HIV-1 infection in MDMs was measured by intracellular $p24^{Gag}$ staining. **i**, ISG expression in MDMs infected with LaiΔenvGFP/G (MOI 2) was quantified by qRT-PCR and normalized to that of mock (3 dpi). **j**, Kinetics of CD169 expression. Uninfected MDMs and MDMs infected with Lai/YU-2env (MOI 1) were analyzed 2 to 6 dpi for CD169 and HIV-1 infection (intracellular $p24^{Gag}$). Representative flow cytometry profiles are shown. **k**, Representative flow cytometry profiles of MDMs infected with Lai/YU-2env (MOI 1) in the presence of B18R (6 dpi). **l**, VSV infection in HIV-1-infected MDMs. MDMs were uninfected (mock) or infected with HIV-1 (GFP in place of *nef*) in the absence (NT) or presence of AZT for 6 days, and further infected with VSV-dsRed for 16 hours. **m**, The percentage of VSV infection was normalized to that of mock. **n**, IFN-I secretion from HIV-1-infected MDMs (6 dpi) was measured by a bioassay. **o**, Virus production ($p24^{Gag}$) in HIV-1-infected MDMs culture supernatants was measured by ELISA (6 dpi). The data shown are the means ± SEM and each symbol represents data obtained from cells derived from an independent donor. Two-tailed p values were calculated using one-way ANOVA followed by the Dunnett's post-test (**a, d-h and m**) or the Tukey-Kramer post-test (**o**). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ns: not significant.



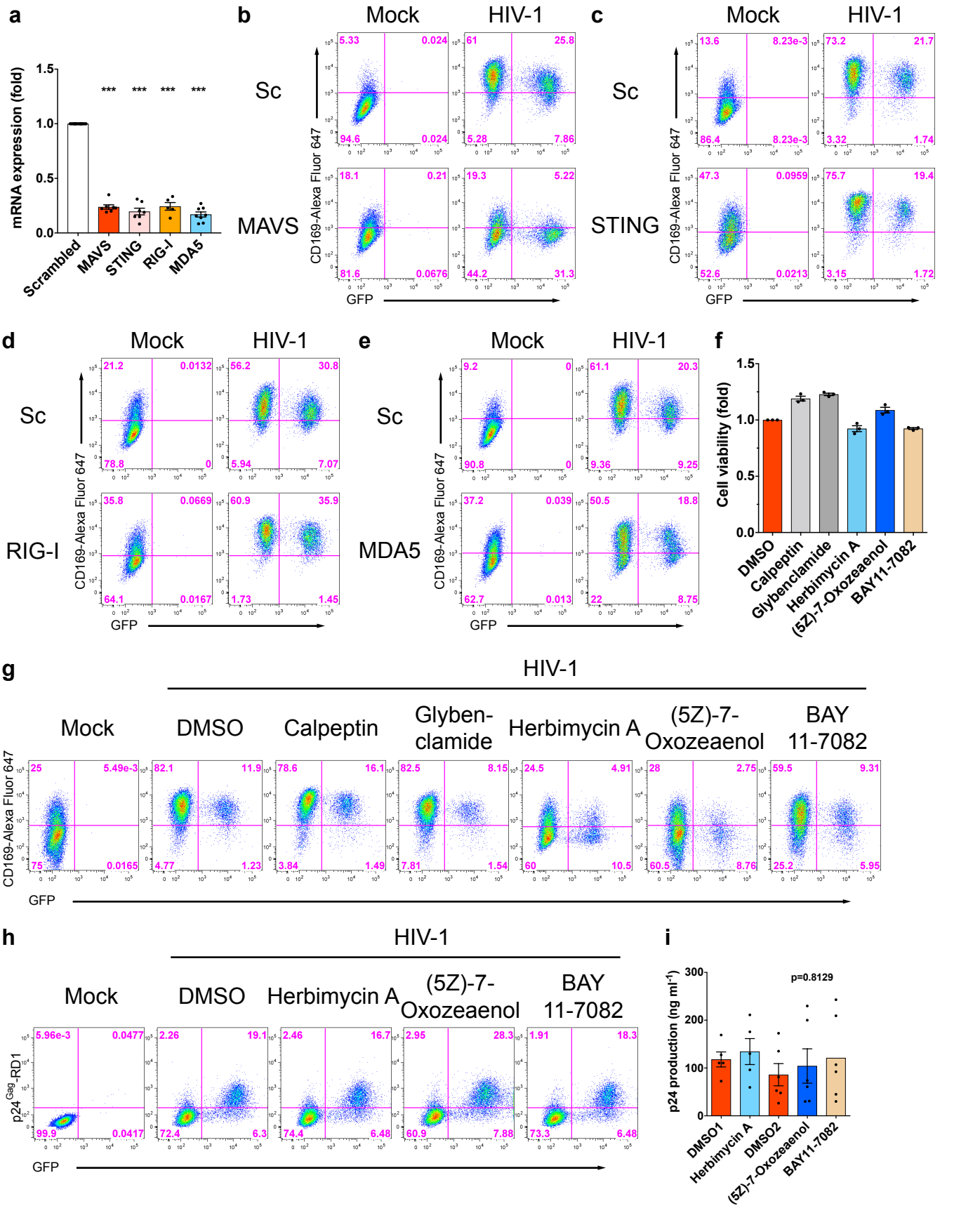

**Supplementary Figure 2. Structural and accessory proteins of HIV-1 do not encode immune-activation determinants.**
**a**, Representative flow cytometry profiles of MDMs infected with HIV-1 mutants and their parental clones at MOI of 1 and analyzed on 6 days post infection for CD169 and viral infection (intracellular $p24^{Gag}$ staining or GFP expression) (6 dpi). **b**, Western blot analysis for MA (p17) and CA (p24) expression in HEK293T cells transfected with LaiΔenvGFP (WT), ΔGag-pol mutant, or ATG* mutant. **c**-**e**, HIV-1 expression (intracellular $p24^{Gag}$) (**c**), CD169 expression (**d**), and IP-10 production (**e**) in MDMs transduced with the wild type (WT) or PTAP- mutant (6 dpi). **f**-**h**, HIV-1 expression (intracellular $p24^{Gag}$) (**f**), CD169 expression (**g**), and IP-10 production (**h**) in MDMs transduced with the wild type (WT) or indicated CA mutants deficient for intra- or inter-hexamer formation (6 dpi). **i**-**k**, HIV-1 expression (GFP) (**i**), CD169 expression (**j**), and IP-10 production (**k**) in MDMs transduced with the wild type (WT) or CyPA-binding-deficient mutant (G89V) (6 dpi). The data shown are the means ± SEM and each symbol represents data obtained from cells derived from an independent donor. Two-tailed p values were calculated using one-way ANOVA followed by the Tukey-Kramer post-test (**d**, **e**, **j**, **k**) or the Dunnett's post-test (**f**, **g**, **h**), or a paired t-test (**c**, **i**). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ns: not significant.



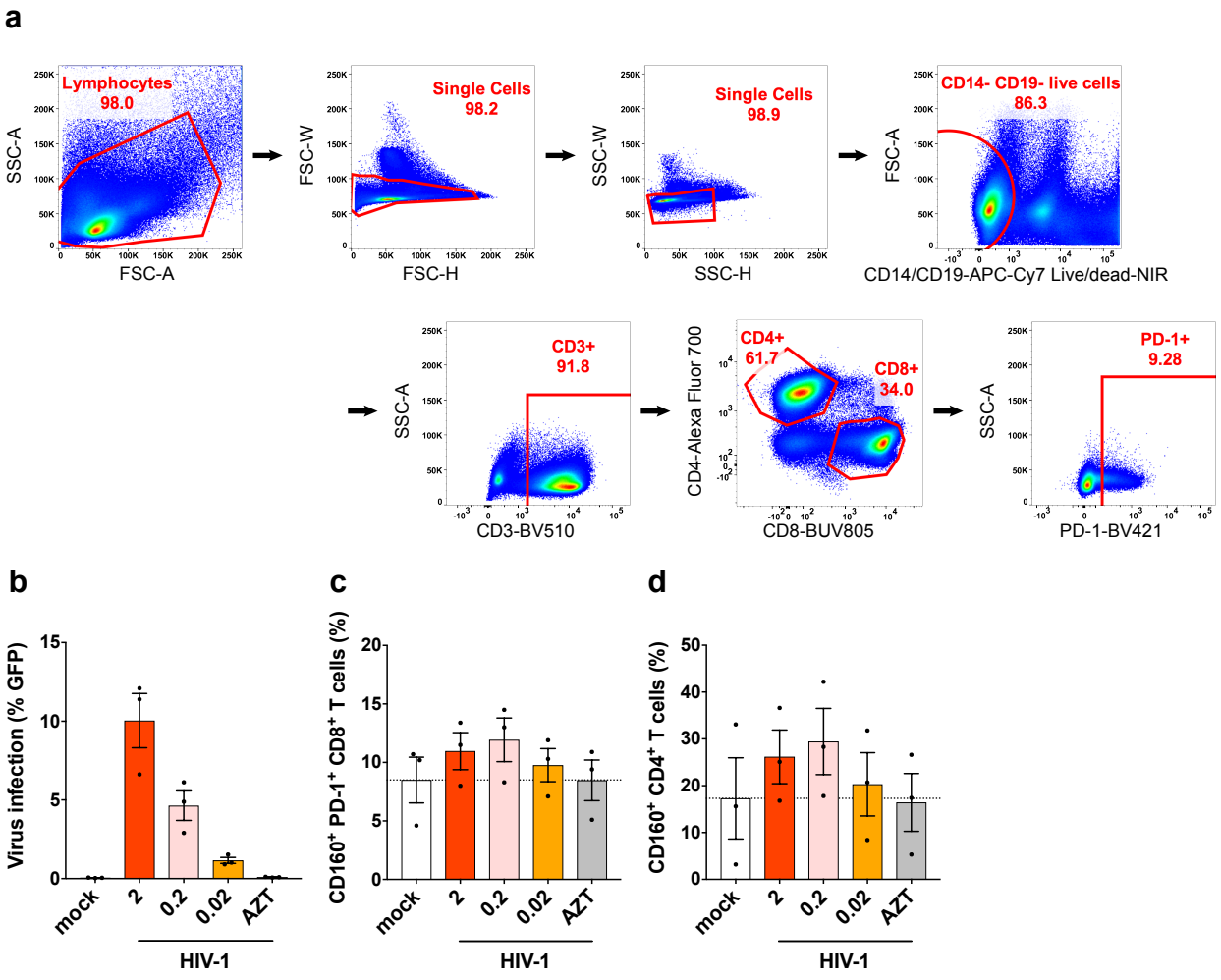

**Supplementary Figure 3. Rev–CRM1 dependent HIV-1 icRNA export is important for sensing.**
**a**, Schematic demonstration of HIV-1 mutants. *: position of mutation, CTE: constitutive transport element, RRE: Rev responsive element. **b**, Gag expression in infected MDMs with WT and Rev mutants (**Fig. 3a**) was quantitated and normalized to WT infection. Two-tailed p values were calculated using one-way ANOVA followed by the Dunnett's post-test to WT. ***: $p < 0.001$, ns: not significant. **c**, **d**, Representative flow cytometry profiles of MDMs transduced with the TagRFP mutant showing CD169 expression and GFP (in place of *nef*) (**c**) or TagRFP expression (in place of MA) (**d**) (6 dpi). **e**, Representative flow cytometry profiles of MDMs transduced with a lentiviral vector expressing ZsGreen driven by the human EF-1α promoter (6 dpi). **f**, Representative flow cytometry profiles of MDMs infected with HIV-1 in the absence (DMSO) or presence of CRM1 inhibitor KPT-330. KPT-330 inhibited $p24^{Gag}$ expression from unspliced RNA but did not inhibit GFP expression (in place of *nef*) from multiply spliced RNA (6 dpi). **g**, Representative flow cytometry profiles of MDMs infected with HIV-1 in the absence (DMSO) or presence of CRM1 inhibitor KPT-330 showing CD169 expression and viral infection (GFP) (6 dpi). **h**, Representative flow cytometry profiles of MDMs infected with HIV-1 in the absence (DMSO) or presence of CRM1 inhibitor KPT-330 showing CD169 expression and viral infection (GFP) (6 dpi). **i**, Representative flow cytometry profiles of MDDCs infected with HIV-1 in the absence (DMSO) or presence of HIV-1 inhibitors (EFZ: efavirenz, Ral: raltegravir) or CRM1 inhibitor KPT-330. MDMs were stained for CD169 and intracelluar $p24^{Gag}$ on day 3 post infection. **j**, Representative flow cytometry profiles of MDDCs infected with a lentiviral vector expressing ZsGreen and stained for CD169 on day 3 post infection.



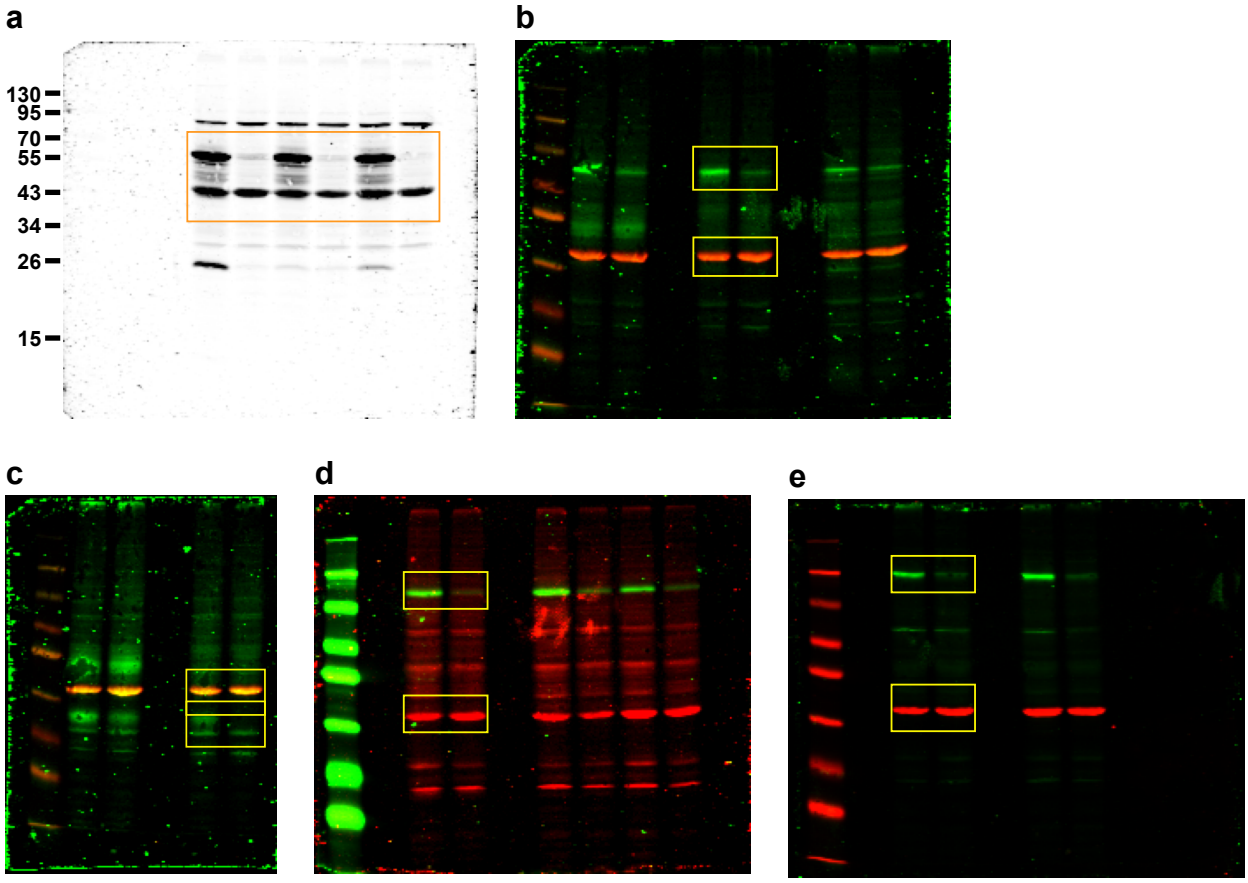

**Supplementary Figure 4. Membrane targeting of viral icRNA is required for MDM activation.**
**a-c,** Virus infection (GFP) (**a**), CD169 expression normalized to mock (**b**), and IP-10 production (**c**) in MDMs transduced with WT or indicated MA mutants lacking various MA functions (**Supplementary Table 1**) (6 dpi). **d**, Schematic demonstration of HIV-1 mutants encoding MLV MA. Both mutants contain HIV-1 MA sequences (42 nt) prior to the start codon for MLV MA. However, one (mMA) does not lead to expression of any of HIV-1 MA amino acids (aa) due to inactivation of the HIV-1 MA start codon (*), while the other (mMA-fusion) results in translation of first 14 aa of HIV-1 MA in frame with the MLV MA protein. **e**, Schematic demonstration of MLV/HIV-1 chimera, mMA12. **f-h**, Viral gene expression (GFP) (**f**), normalized CD169 expression (**g**), and IP-10 production (**h**) in MDMs infected (at MOI of 2) with WT or mMA12 (6 dpi). **i-k**, Viral gene expression (GFP) (**i**), CD169 expression normalized to mock (**j**), and IP-10 production (**k**) in MDMs transduced with WT or indicated chimeric viruses encoding MA from various primate lentiviruses (6 dpi). **l-n**, Viral gene expression (GFP) (**l**), CD169 expression normalized to mock (**m**), and IP-10 production (**n**) in MDMs transduced with WT or indicated chimeric viruses encoding MA from various lentiviruses and retroviruses (6 dpi). **o**, Schematic demonstration of HIV-1 mutants containing 24xMS2 stem loops in the *pol* orf and MS2-GFP-NLS fusion protein in place of the *nef* orf. The data shown are the means ± SEM and each symbol represents data obtained from cells derived from an independent donor. Two-tailed p values were calculated using one-way ANOVA followed by the Tukey-Kramer post-test (**g**, **h**) or the Dunnett's post-test (**b**, **c**, **j**, **k**, **l**, **m**, **n**), or a paired t-test (**f**), *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ns: not significant.




**Supplementary Figure 5. MDM immune activation is initiated via a MAVS dependent pathway.**
**a**, MDMs were transduced with lentiviral vectors expressing shRNA against indicated genes and mRNA levels of the target genes were quantitated with qRT-PCR. Each mRNA level was normalized to that of GAPDH and then normalized to that of scrambled-shRNA-transduced MDMs. **b, c**, Representative flow cytometry profiles of CD169 expression in MDMs transduced with either scrambled shRNA (Sc), shRNA against MAVS (**b**) or STING (**c**) and infected with LaiΔenvGFP/G (6 dpi). **d, e**, Representative CD169 expression profiles of MDMs transduced with scrambled shRNA (Sc), shRNA against RIG-I (**d**) or MDA5 (**e**) and infected with LaiΔenvGFP/G (6 dpi). **f**, MDMs were cultured for 6 days in the presence of the indicated inhibitors and cell viability was measured by MTT assay. The values were normalized to that of untreated (DMSO). **g**, Representative CD169 expression profiles of MDMs infected with HIV-1 in the presence of indicated inhibitors for 6 days. **h**, Representative flow cytometry profiles of MDMs infected with HIV-1 (GFP in place of *nef*) in the presence or absence (DMSO) of drugs showing intracellular $p24^{Gag}$ (y-axis) and GFP (x-axis) (6 dpi). **i**, MDMs were infected with HIV-1 and cultured with indicated drugs. The $p24^{Gag}$ contents in the supernatants harvested 6 dpi was measured. DMSO1: 0.1% DMSO, DMSO2: 0.01% DMSO. The data shown are the means ± SEM and each symbol represents data obtained from cells derived from an independent donor. Two-tailed p values were calculated using one-way ANOVA followed by the Dunnett's post-test. ***: $p < 0.001$.




**Supplementary Figure 6. HIV-1-infection-induced MDM activation results in expression of IRs on T cells and their functional impairment.** **a**, Representative flow cytometry profiles to show the gating strategy for inhibitory receptor expression profiling and intracellular cytokine staining. **b**, MDMs were infected at various MOIs or in the presence of AZT, and GFP expression was measured with flow cytometry 6 days post infection. **c, d**, MDMs infected with HIV-1 at different MOIs were co-cultured with PBMCs, and the percentage of $CD160^+$ $PD\text{-}1^+$ $CD8^+$ T cells (**c**) and $CD160^+$ $CD4^+$ T cells (**d**) on day 5 post initiation of co-culture was quantitated by flow cytometry. The dotted lines indicate the background levels (mock).




**Supplementary Figure 7. Uncropped immunoblot images.**
Uncropped immunoblot images for (**a**) Figure 3a, (**b**) Figure 5a, MAVS, (**c**) Figure 5a, STING, (**d**) Figure 5f, RIG-I and (**e**) Figure 5f, MDA5. Orange and yellow rectangles indicate where images were cropped.

# Supplementary Table 1

| Plasmid | | Primer | Ref. |
|---|---|---|---|
| **M10** | F | GCCTCTTCAGCTACCACCGGATCTGAGACTTACTCTTGATTGTAAC | 1 |
| | R | GTTACAATCAAGAGTAAGTCTCAGATCCGGTGGTAGCTGAAGAGGC | |
| **CTE** | F | TTTTTGGATCCACTATAGGGCGAATTGAATTTAGCG | 2 |
| | R | GGATAACAAT TTCACACAGG AAACAGCTAT GAC | |
| **CTE AS** | F | TTTTTGCTAGCACTATAGGGCGAATTGAATTTAGCG | 2 |
| | R | TTTTTGGATCCCAGAATTAACCCTCACTAAAGGGAC | |
| **ΔMA** | F | TTTAAAGCTA GCCAGGTCAG CCAAAATTAC CCTATA | |
| | R | AAATTTGCTA GCTCTCGCAC CCATCTCTCT CCTT | |
| **ATG*** | F | GCTAGAAGGAGAGAGAAGGGTGCGAGAGCGT | |
| | R | ACGCTCTCGCACCCTTCTCTCTCCTTCTAGC | |
| **HIV-1 + AatII** | F | AAAAAAGACGTCAGCCAAAATTACCCTATAGTGCAGAAC | |
| | R | CTGGGTTCGCATTTTGGACC | |
| **HIV-1-vec** | F | AGGCTAGAAGGAGAGCCATGGGTGCGAGAGCG | |
| | R | CGCTCTCGCACCCATGGCTCTCCTTCTAGCCT | |
| **HIV-1-vec-plus** | F | AGAGGAGCTC TCTCGACG | 3 |
| | R | AAAAAACCATGGGATCTAATTCTCCCCCGCTTAATAC | |
| **HIV-1 MA** | F | AAAAACCATGGGTGCGAGAGCGTC | |
| | R | AAAACCGGTCTTCCGACGTCGTAATTTTGGCTGACCTGGC | |
| **HIV-2 MA** | F | AAAAACCATGGGCGCGAGAAACTC | |
| | R | AAAACCGGTCTTCCGACGTCGTAATTTCCTCCCTTCTCGC | |
| **MLV MA** | F | AAAAACCATGGGCCAGACTGTTACC | 4 |
| | R | AAAACCGGTCTTCCGACGTCATAAAGGGAGGATCGAGGCG | |
| **SIVmac MA** | F | AAAAACCATGGGCGTGAGAAACTC | |
| | R | AAAACCGGTCTTCCGACGTCGTAATTTCCTCCTCTGCC | |
| **SIVagm MA** | F | AAAAACCATGGGTGCGAGTAACTCAG | |
| | R | AAAACCGGTCTTCCGACGTCGTAATTTTGTGATCCACCAC | |
| **SIVcpz MA** | F | AAAAACCATGGGTGCGAGAGC | |
| | R | AAACCGGTCTTCCGACGTCGTATAATCTACTTCCGCTAGG | |
| **SIVgor MA** | F | AAAAACCATGGGTGCGAGAGC | |
| | R | AAACCGGTCTTCCGACGTCATAGTTCTGACTTGTTTCAGG | |
| **FIV MA** | F | AAAAACCATGGGGAATGGACAGGG | |
| | R | AAAAAAGACGTCATATGCCTGTGGAGGGCCTTC | |
| **HTLVII MA** | F | AAAAACCATGGGACAAATCCACGGG | 5 |
| | R | AAAAAAGACGTCGAAGCATTGCGTGGTGG | |
| **EIAV MA** | F | AAAAACCATGGGAGACCCTTTGACATG | 6 |
| | R | AAAAAAGACGTCATATTCTTCAGAGGGCTCAGACTG | |
| **TagRFP** | F | AAAAACCATGGTGTCTAAGGGCGAAGAGC | |
| | R | AAAAAAGACGTCTCAATTAAGTTTGTGCCCCAGTTTGC | |
| **12LE** | F | GTATTAAGCGGGGGAGAAGAAGATCGATGGGAAAAAATTCG | 7 |
| | R | CGAATTTTTTCCCATCGATCTTCTTCTCCCCCGCTTAATAC | |
| **16EK** | F | GGGGAGAATTAGATCGATGGAAGAAAATTCGGTTAAGGCC | 8 |
| | R | GGCCTTAACCGAATTTTCTTCCATCGATCTAATTCTCCCC | |
| **21LS** | F | GATGGGAAAAAATTCGGTCAAGGCCAGGGGGAAAG | 9 |
| | R | CTTTCCCCCTGGCCTTGACCGAATTTTTTCCCATC | |
| **29/31KE** | F | GGGGGAAAGA AAAAATATGA ATTAGAACAT ATAGTATGGG C | 10 |
| | R | GCCCATACTA TATGTTCTAA TTCATATTTT TTCTTTCCCC C | |
| **30LE** | F | GGCCAGGGGGAAAGAAAAAATATAAAGAAAAACATATAGTATGGGC | 7 |
| | R | GCCCATACTATATGTTTTTCTTTATATTTTTTCTTTCCCCCTGGCC | |
| **34VE** | F | AAATTAAAACATATAGAATGGGCAAGCAGGGAGCTAGAAC | 11 |
| | R | GTTCTAGCTCCCTGCTTGCCCATTCTATATGTTTTAATTT | |
| **69TR** | F | CTACAACCATCCCTTCAGAGAGGATCAGAAGAACTTAG | 12 |
| | R | CTAAGTTCTTCTGATCCTCTCTGAAGGGATGGTTGTAG | |
| **85YG** | F | ATACAGTAGCAACCCTCGGTTGTGTGCATCAAAGGATAG | 13 |
| | R | CTATCCTTTGATGCACACAACCGAGGGTTGCTACTGTAT | |
| **98EV** | F | GATAGAGATAAAAGACACCAAGGTTGCTTTAGACAAGATAGAGG | 12 |
| | R | CCTCTATCTTGTCTAAAGCAACCTTGGTGTCTTTTATCTCTATC | |
| **7A2T** | F | GTTAGCGCCAGGGGGAGCGGCAGCATATACATTAACACATATAGTATGGGCAAGCAGGGAGC | 14 |
| | R | CCGCTCCCCCTGGCGCTAACGCAATTGCTTCCCATGCATCTAATTCTCCCCCGCTTAATACTGACG | |
| **MS2-GFP 5'** | F | TTTTTCCATGGCTTCTAACTTTACTCAGTTCGTTC | 15 |
| | R | CTCACCATGGTGGCGACCGGTGGGTCCGCGTAGATGCCG | |
| **MS2-GFP 3'** | F | TTCCCACCGGTCGCCACC | 15 |
| | R | TTTTTCTCGAGTTATACCTTTCTCTTCTTTTTTGGCTTG | |

## Supplementary Table 2

| Gene | Sequence | Ref. |
|---|---|---|
| **MAVS** | ATGTGGATGTTGTAGAGATTC | Sigma (TRCN0000236031) |
| **STING** | GCCCGGATTCGAACTTACAAT | Sigma (TRCN0000163296) |
| **RIG-I** | CCAGAATTATCCCAACCGATA | Sigma (TRCN0000153712) |
| **MDA5** | CCAACAAAGAAGCAGTGTATA | Sigma (TRCN0000050849) |

## Supplementary Table 3

| Gene | Forward | Reverse |
|---|---|---|
| **HIV-1 RNA** | TGTGTGCCCGTCTGTTGTGT | CTCTCCTTCTAGCCTCCGCT |
| **GAPDH** | CAAGATCATCAGCAATGCCT | AGGGATGATGTTCTGGAGAG |
| **IFNβ** | ATTCTAACTGCAACCTTTCG | GTTGTAGCTCATGGAAAGAG |
| **CD169** | GGCTGTTACGATGGTTTATGATG | AATCAAAGGCATCATTTTAGGGATA |
| **IP-10** | AAAGCAGTTAGCAAGGAAAG | TCATTGGTCACCTTTTAGTG |
| **ISG15** | TCCTGGTGAGGAATAACAAGGG | GTCAGCCAGAACAGGTCGTC |
| **ISG54** | GGTCTCTTCAGCATTTATTGGTG | TGCCGTAGGCTGCTCTCCA |
| **ISG56** | TAGCCAACATGTCCTCACAGAC | TCTTCTACCACTGGTTTCATGC |
| **Viperin** | TGGGTGCTTACACCTGCTG | GAAGTGATAGTTGACGCTGGTT |
| **Mx1** | GTTTCCGAAGTGGACATCGCA | CTGCACAGGTTGTTCTCAGC |
| **MAVS** | GTACCCGAGTCTCGTTTC | GCAGAATCTCTACAACATCC |
| **STING** | ACTGTGGGGTGCCTGATAAC | TGGCAAACAAAGTCTGCAAG |
| **RIG-I** | ATCCCAGTGTATGAACAGCAG | GCCTGTAACTCTATACCCATGTC |
| **MDA5** | GGCATGGAGAATAACTCATCAG | CTCTTCATCTGAATCACTTCCC |

## Supplementary References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