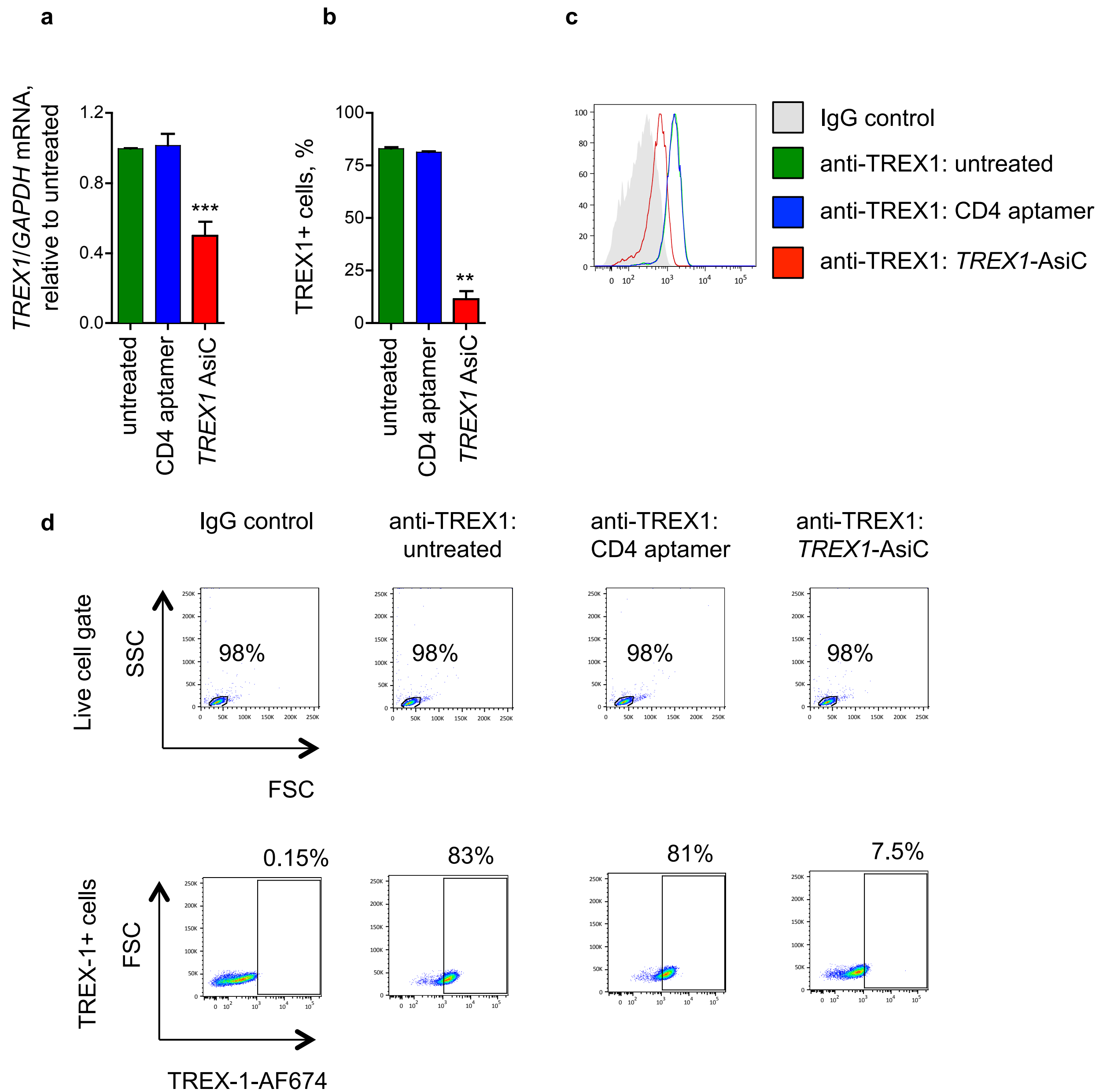


Figure S1. Related to Figure 1: Knockdown of *TREX1* after treatment with *TREX1* CD4-AsiCs.
Expression of *TREX1* mRNA in human primary CD4+ T cells was measured by qRT-PCR relative to *GAPDH* (**a**) and of TREX1 protein was measured by intracellular staining and flow cytometry (**b-d**) 3 d and 7 d, respectively, after treatment with 2 μM *TREX1* AsiC or CD4 aptamer. (**, p< 0.01, ***, p< 0.001). Data are normalized to untreated control and represent mean + S.E.M.

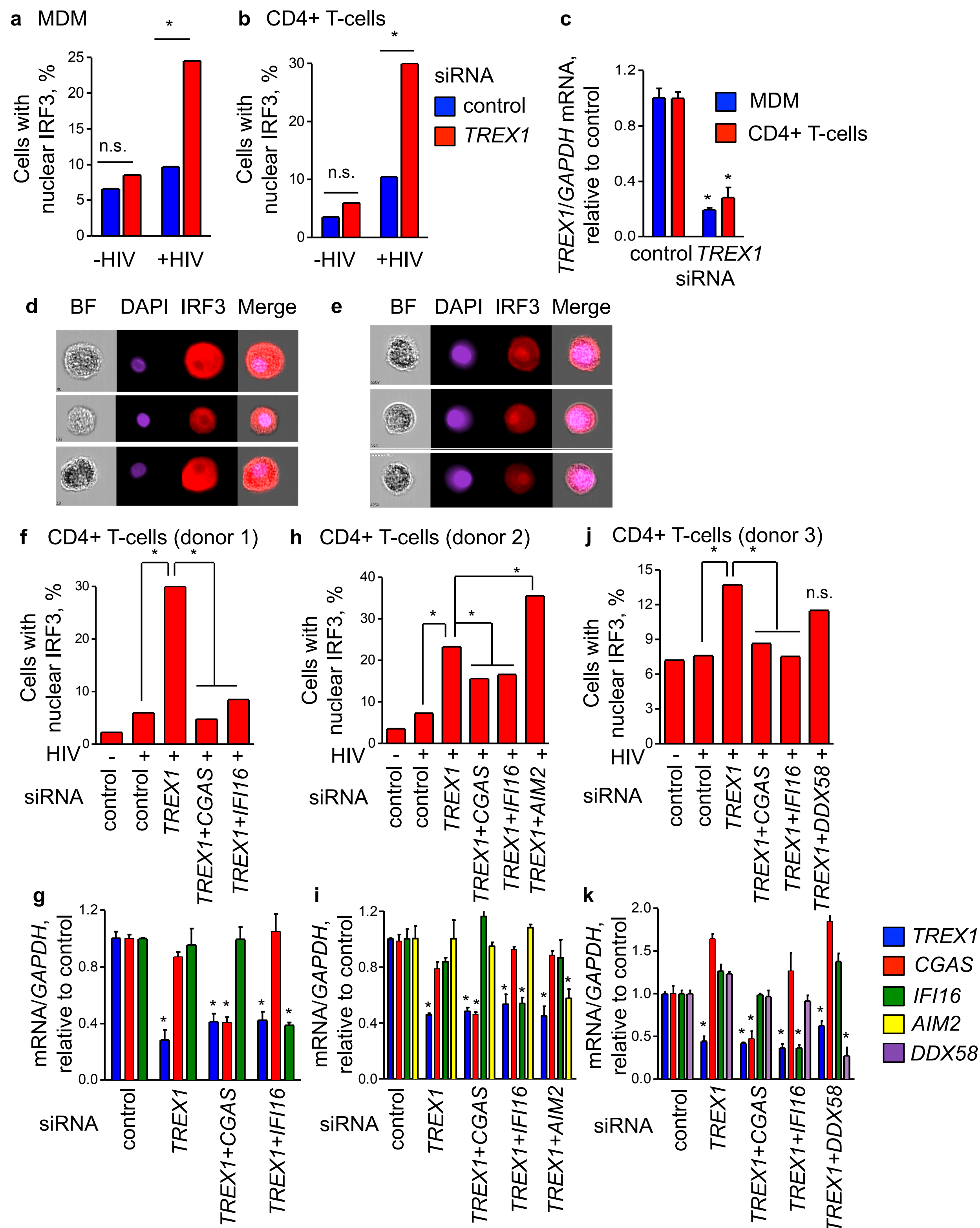


Figure S2. Related to Figure 2: IRF3 nuclear translocation in HIV-infected cells after *TREX1* knockdown.
Nuclear translocation of IRF3 was measured by imaging cytometry performed 16 hrs after HIV infection in primary human MDM (**a**) and CD4+ T-cells (**b**) previously transfected with siRNAs targeting *TREX1* or with non-targeting negative control siRNA. Shown are representative images of MDMs without (**d**) or with (**e**) nuclear IRF3 (BF, brightfield; DAPI, nuclear stain). (**f, h, j**) Nuclear translocation of IRF3 16 hr after HIV infection of primary human CD4+ T cells transfected with siRNAs targeting *TREX1* alone or together with *CGAS*, *IFI16, AIM2 or DDX58*, the gene encoding RIG-I. The data were obtained with PBMCs from three healthy donors and at least 5,000 events were analyzed for each condition. Knockdown was confirmed by qRT-PCR 2 d post transfection (**c** for data in a and b; **g, i, k** for data in f, h and j, respectively). P values in (c,g,i,k) were calculated by Student's t-test (*, p< 0.001 compared to control). P values in (a,b,f,h,j) were calculated by Chi–squared test (*, p< 0.001, n.s., not significant).

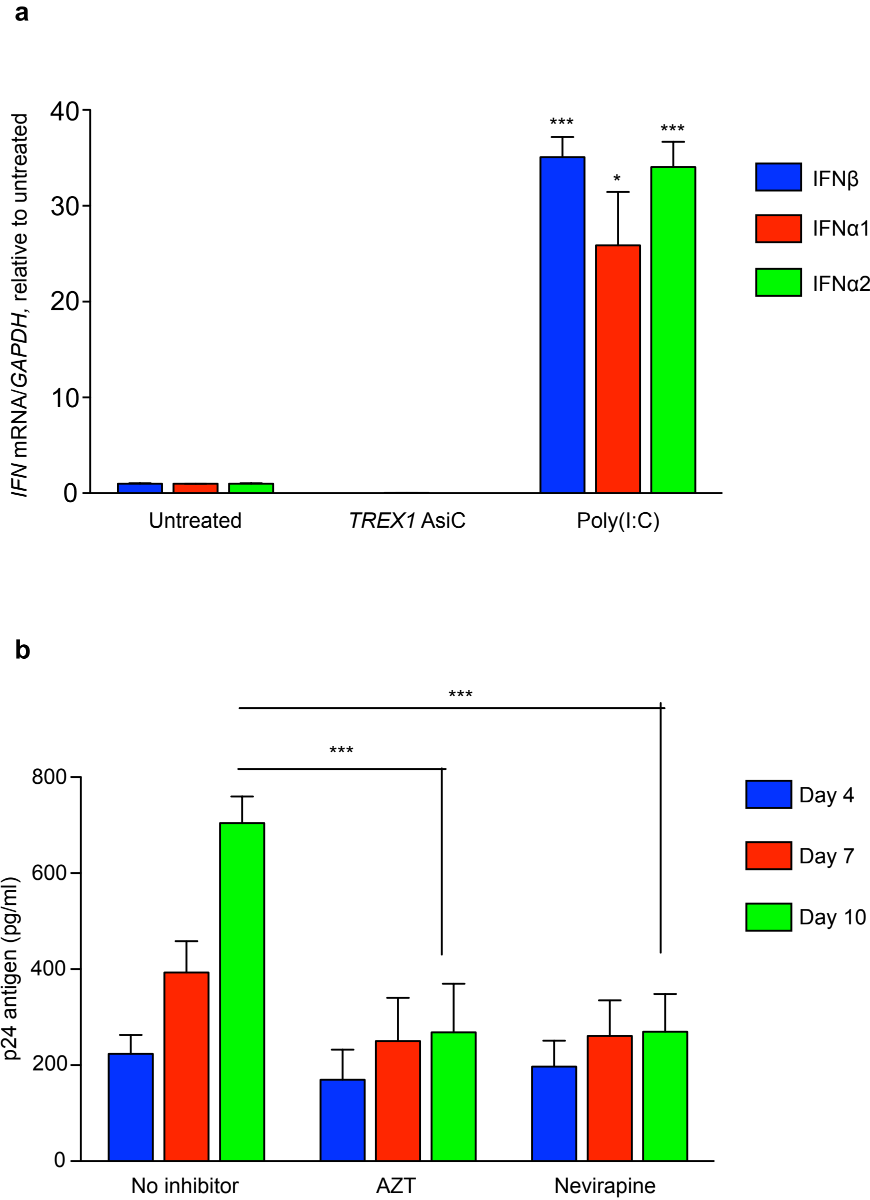
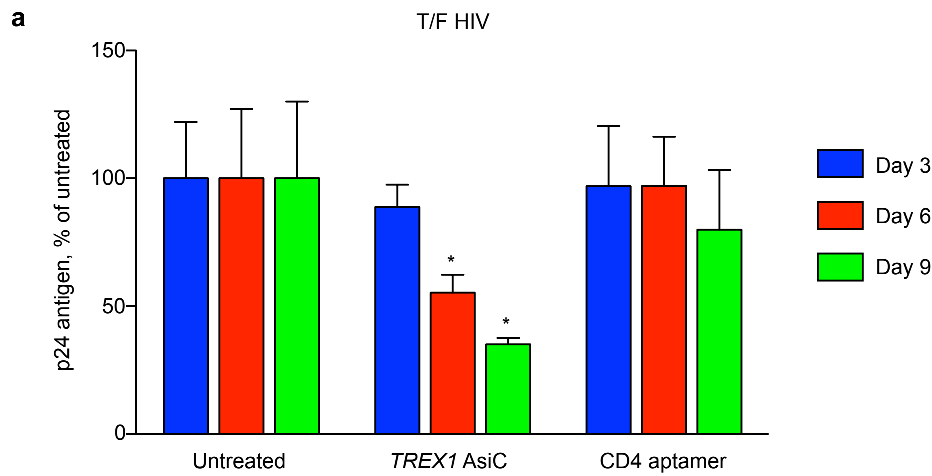


Figure S3. Related to Figure 2: Interferon response after treatment with *TREX1* AsiCs and HIV replication in polarized human cervical explants.

(a) Lack of IFN induction in human cervical explants treated with *TREX1* AsiC alone. Type I IFN mRNAs were measured by qRT-PCR in polarized cervical explants treated for 6 hrs with 4 µM TREX1 AsiCs. Treatment with 100 µg/ml Poly(I:C) was used as a control (*, $p < 0.05$, ***, $p < 0.001$). Data were normalized to *GAPDH* mRNA. Each bar represents mean + S.E.M of cervical explants from a single donor. **(b)** Inhibition of HIV replication in polarized human cervical explants by reverse transcriptase inhibitors. HIV p24 Ag in conditioned media from polarized human cervical explants infected with HIV_{BaL} in the absence or presence of 50 µM Azidothymidine (AZT) or Nevirapine was measured by ELISA. The increasing levels of released p24 that were inhibited by AZT or Nevirapine (***, $p<0.001$) confirm productive HIV infection in human cervical explants. Data represent mean + S.E.M.

a



b

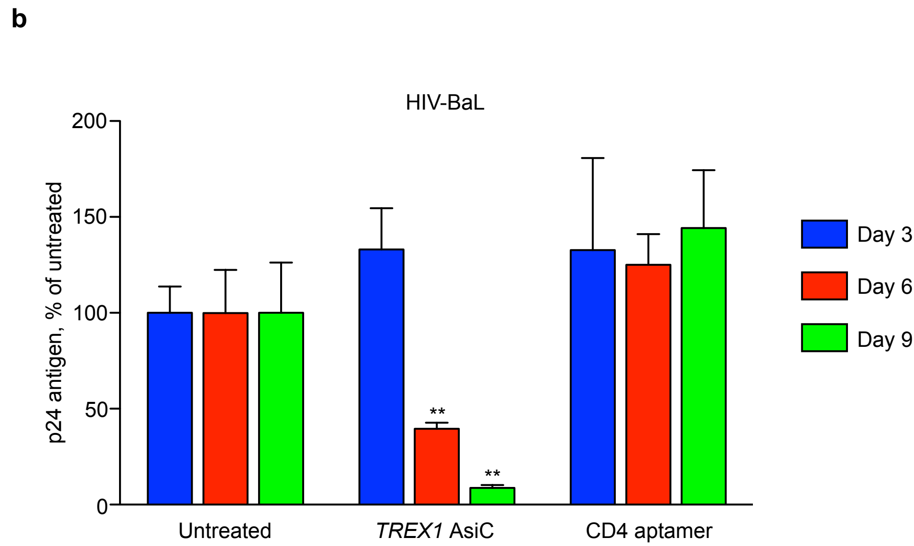


Figure S4. Related to Figure 2: Inhibition of transmitted/founder (T/F) HIV-1 infection in polarized human cervical explants by *TREX1* AsiCs.

HIV p24 Antigen, measured by ELISA, in conditioned media from polarized human cervical explants infected with T/F HIV-1 (pRHPA.c/2635) **(a)** or HIV_{BaL} **(b)** after pre-treatment with 2 μM *TREX1* AsiC or CD4 aptamer 72 and 48 hr prior to infection (*, $p< 0.05$, **, $p< 0.01$). Data represent mean + S.E.M, normalized to the untreated sample on the same day.

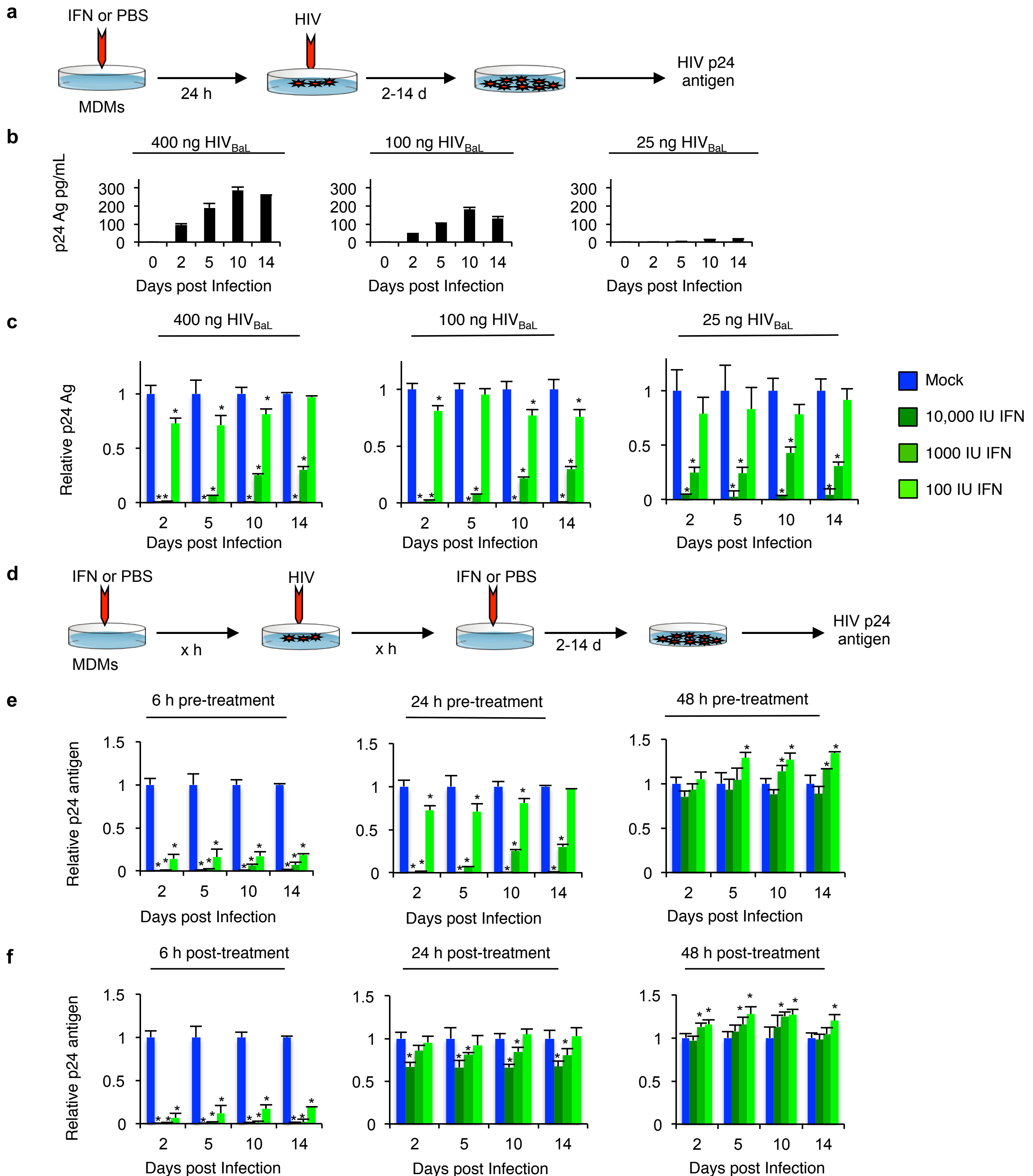


Figure S5. Related to Figure 3 and 4: Treatment with rIFN protects patient-derived MDMs from HIV infection when administered between 24 h prior to infection and 6 h after infection. (a-c) Experimental schema **(a)** MDMs derived from two healthy donors were pretreated with PBS or rIFN at indicated doses and challenged 24 h later with indicated concentrations of HIV_{BaL}. HIV infection was analyzed by p24 Ag ELISA of culture supernatants. (**b**) shows the time course of infection without IFN pretreatment and **(c)** shows the relative p24 Ag produced at each timepoint, corrected for background in uninfected samples and normalized to cultures that did not receive rIFN (mock, blue). **(d-f)** Experimental schema as above, except that rIFN was added to cultures at indicated times before (**e**) and after (**f**) HIV challenge. p24 Ag in culture supernatants was normalized to mock cultures that were infected, but did not receive rIFN. All graphs show mean $\pm$ SEM of 3 independent experiments (*p <0.05, relative to mock-treated sample, by Student's t-test).

Table S1: Primers for AsiC Synthesis

5' Primers	
5' T7 Primer CD4	5' - TAA TAC GAC TCA CTA TAG GGA GAC AAG AAT AAA CGC - 3'
Template DNA	
CD4 Aptamer	5' - GGG AGA CAA GAA TAA ACG CTC AAT GAC GTC CTT AGA ATT GCG CAT TCC TCA CAC AGG ATC TTT TCG ACA GGA GGC TCA CAA CAG GC - 3'
3' Primers	
3' *TREX* Primer "Sequence a"	5' – TCG TAG CGG TCA CCA TTG TGC CTG TTG TGA GCC TCC TGT CGA A - 3'
3' *TREX* Primer "Sequence b"	5' – TGA CAG CAG ATG GTC TTG GGC CTG TTG TGA GCC TCC TGT CGA A - 3'
3' *CCR5* Primer CD4	5' - AAT TTC GAC ACC GAA GCA GAG GCC TGT TGT GAG CCT CCT GTC GAA - 3'
3' *gag* Primer CD4	5' - AAC CTG TCT CTC AGT ACA ATC GCC TGT TGT GAG CCT CCT GTC GAA - 3'
3' *vif* Primer CD4	5' - AAG GGA TGT GTA CTT CTG AAC GCC TGT TGT GAG CCT CCT GTC GAA - 3'

Table S2: siRNAs

CCR5	Sense: 5’ P-CUC UGC UUC GGU GUC GAA A dTdT - 3'
	Antisense: 5’ P-UUU CGA CAC CGA AGC AGA G dTdT - 3’
gag	Sense: 5' P-GAU UGU ACU GAG AGA CAG GCU-dTdT – 3’
	Antisense: 5' P-CCU GUC UCU CUC AGU ACA AUC dTdT-3'
vif	Sense: 5' P-GTT CAG AAG TAC ACA TCC C-dTdT
	Antisense: 5' P-GGG AUG UGU ACU UCU GAA CdTdT-3'
TREX1 Sequence a	Sense: 5’ P- ACA AUG GUG ACC GCU ACG AdTdT -3’
	Antisense: 5’ P-TCG TAG CGG TCA CCA TTG TdTdT -3’
TREX1 Sequence a	Sense: 5’ P- ACA AUG GUG ACC GCU ACG AdTdT -3’
	Antisense: 5’ P-TCG TAG CGG TCA CCA TTG TdTdT -3’

Table S3: Primers for RT-PCR

GAPDH For	5' - AGC CAC ATC GCT CAG ACA C - 3'
GAPDH Rev	5' - GCC CAA TAC GAC CAA ATC C - 3'
IL-8 For	5' - AGA CAG CAG AGC ACA CAA GC - 3'
IL-8 Rev	5' - ATG GTT CCT TCC GGT GGT - 3'
IL-6 For	5' - GAT GAG TAC AAA AGT CCT GAT CCA - 3'
IL-6 Rev	5' - CTG CAG CCA CTG GTT CTG T - 3'
IFNα For	5’ – GCT TTA CTG ATG GTC CTG GTG GTG – 3’
IFNα Rev	5’ – GAG ATT CTG CTC ATT TGT GCC AG - 3’
IFNβ For	5' - TTG CTC TGG CAC AAC AGG TA - 3'
IFNβ Rev	5' - TGG AGA AGC AAC CAG GAG A - 3'
IFNγ For	5' - GGC ATT TTG AAG AAT TGG AAA G - 3'
IFNγ Rev	5' - TTT GGA TGC TCT GGT CAT CTT - 3'
OAS-1 For	5' - GGT GGA GTT CGA TGT GCT G - 3'
OAS-1 Rev	5' - AGG TTT ATA GCC GCC AGT CA - 3'
IP-10 For	5' - GAA AGC AGT TAG CAA GGA AAG GT - 3'
IP-10 Rev	5' - GAC ATA TAC TCC ATG TAG GGA AGT GA - 3'
STAT1 For	5' - TTG GCA CCT AAC GTG CTG - 3'
STAT1 Rev	5' - TTC GTA CCA CTG AGA CAT CCT G - 3'
IL-12 For	5' - CAC TCC CAA AAC CTG CTG CTG AG - 3'
IL-12 Rev	5' - TCT CTT CAG AAG TGC AAG GGT A - 3'
HIV-gag For	5’ – AGT GGG GGG ACA TCA AGC AGC CAT GCA AAT – 3’
HIV-gag Rev	5’ – TGC TAT GTC ACT TCC CCT TGG TTC TCT – 3’
TREX1 For	5’ – GCA TCT GTC AGT GGA GAC CA – 3’
TREX1 Rev	5’ – AGA TCC TTG GTA CCC CTG CT – 3’
IL1-β For	5’ – GTG AAA TGA TGG CTT ATT ACA GTG – 3’
IL1-β Rev	5’ – CTG ACG CGG CCT GCC TGA AGC CCT – 3’
TNFα For/Rev	IDT Prime Time Primer Sequence Hs.PT.58.45380900
CCR5 For/Rev	IDT Prime Time Primer Sequence Hs.PT.58.3437570
cGAS For	5’ – GGG AGC CCT GCT GTA ACA CTT CTT AT – 3’
cGAS Rev	5’ – CCT TTG CAT GCT TGG GTA CAA GGT – 3’
IFI16 For	5’ – GGT CTG CGA TCC TGA ATG GG – 3’
IFI16 Rev	5’ – TCA CTA TCG AGA TAC TTG TGG GT – 3’
AIM2 For	5’ – TGG CAA AAC GTC TTC AGG AGG– 3’
AIM2 Rev	5’ – AGC TTG ACT TAG TGG CTT TGG– 3’
DDX58 For	5’ – GTG CAA AGC CTT GGC ATG T – 3’
DDX58 Rev	5’ – TGG CTT GGG ATG TGG TCT ACT C – 3’

Supplemental Experimental procedures

Cells. Human PBMCs were isolated by Ficoll (GE) density centrifugation from whole blood obtained from the Kraft Family Blood Donor Center and from the Brigham and Women's Hospital Specimen Bank, Boston, MA with Institutional Review Board approval. Mononuclear cells were cultured in H10 medium (RPMI 1640 (Cellgro) containing 10% Human AB serum (GemCell), 100 U/mL penicillin and 100 µg /mL streptomycin sulfate). CD4+ cells were separated using immunomagnetic beads (Miltenyi) and CD4+ T cells and MDMs were prepared as previously described (Song et al., 2003). T cells were cultured in H10 containing 60 IU/mL IL-2 (Proleukin from Chiron Corporation, Emeryville, CA) and were activated using 4 µg/mL PHA (Difco). Resting PBMCs were cultured in H10 containing 4 µg/mL IL-15 (R&D Systems).

Quantitative RT-PCR (qRT-PCR) was performed as previously described (Palliser et al., 2006) using primers in **Table S3**. mRNA expression was normalized to *GAPDH* expression, and then calculated as a percentage relative to mock-treated controls.

Flow cytometry. Direct immunostaining of CD3, CD4, CD8, CD14, CD45, and CD19 was performed using 1/20 dilutions of fluorescently conjugated murine mAb (BioLegend) for 30-60 min at 4 ºC. Cells were stained in PBS containing 0.5% FCS, 1 mM EDTA, and 25 mM HEPES. Samples were washed twice in the same buffer. Intracellular flow cytometry for TREX1 was performed using a rabbit monoclonal antibody (Abcam, Cambridge, MA) with a donkey anti-rabbit AF647 secondary antibody (Life Technologies). Data were acquired for one and two-color experiments using a FACSCalibur (BD Biosciences), and for multi-color and cell sorting experiments using a FACSAria II (BD Biosciences). All data were analyzed using FlowJo (Treestar, Inc.) software.

Viruses. HIV_{BaL} was used to infect cells and cervicovaginal explants *in vitro* unless otherwise specified. HIV_{IIIB} was used to infect primary CD4+ T cells. HIV_{BaL} and HIV_{IIIB} were obtained from the NIH AIDS Research and Reference Reagent Program and viral stocks were generated as previously described (Wheeler et al., 2011; Wheeler et al., 2013). For mouse experiments $HIV\text{-}1_{JR\text{-}CSF}$ stocks were produced by the Virology Core of the Ragon Institute as previously described (Wheeler et al., 2013). T/F HIV-1 Infectious Molecular Clone (pRHPA.c/2635) was obtained from the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (cat#11744, from Dr. John Kappes). T/F HIV-1 and HIV_{BaL} (200 µL of $1x10^5$ TCID50/mL virus) were used to infect polarized human cervical explants.

Human cervical polarized tissue explants. AsiCs or aptamers in 50 µL Optimem (Invitrogen) were applied on 2 consecutive days to the apical surface of polarized explants, and the explants were then incubated at 37 °C for 4-6 h before adding 150 µL H10 medium to each well. In some experiments 15 µg of neutralizing antibodies against both IFNα and IFNβ (Cell Sciences and BioLegend, respectively) were added to the culture medium at the same time as the second topical AsiC administration and at the time of HIV infection. At sequential time points, 100 µL of medium from the lower chamber was removed for HIV and IFNβ ELISA assays. Explants were cultured for not more than 10 d to reduce confounding problems due to loss of viability during extended culture. Cervicovaginal tissue was digested with collagenase as previously described (Wheeler et al., 2011; Wheeler et al., 2013). Cell subsets were obtained by fluorescence-activated cell sorting after staining for CD4, CD14, CD3 and CD19. RNA from separated monocyte, CD4 T cell and B cell subsets were analyzed by qRT-PCR.

ELISA assays. p24 antigen levels were measured using the HIV-1 p24 ELISA Kit (Perkin Elmer). IFNβ protein was measured by assay of culture supernatants using the Verikine Human IFN beta ELISA kit (R&D). rhIFN Type I was measured in mouse serum samples using Human IFN-alpha Serum Sample ELISA Kit (R & D Systems).

rIFN. A universal Type I IFN (R&D System) was used for both *in vitro* and *in vivo* experiments.

Analysis of HIV infection. Blood was obtained by venipuncture of the facial vein at weekly intervals for 12 wks following HIV challenge. Analysis of HIV infection was as previously described (Wheeler et al., 2011; Wheeler et al., 2013). Cells were pelleted by centrifugation and plasma was stored at -80 ºC until analysis. Cell pellets were twice treated with red blood cell (RBC) lysis buffer (Sigma), washed with flow cytometry buffer described above and stained using a 1/20 dilution of CD3, CD4, and CD8 mAb (BioLegend). Viral RNA was extracted from 75 µL of plasma using Trizol (Invitrogen) according to the manufacturer's instructions. cDNA was reverse transcribed

using SuperscriptIII (Invitrogen) and HIV gag mRNA was assessed by qRT-PCR using *gag*-specific primers (**Table S3)**. The remaining serum was aliquoted for p24 Ag ELISA (Perkin Elmer).

Imaging cytometry. MDMs and human primary CD4+ T cells were knocked down for *TREX1, cGAS, IFI16, AIM2, RIG-I* as indicated, by siRNA nucleofection as previously described (Yan et al., 2010), and infected 3 days later with HIV_{BaL} and HIV_{IIIB}, respectively. siRNAs targeting *TREX1* were pro-siRNAs produced in bacteria as described (Huang and Lieberman, 2013). siRNAs targeting *cGAS, IFI16, AIM2* and *DDX58* were from Dharmacon (siGENOME™ SMARTpool, except *AIM2* siRNA which were ON-TARGET plus). Non-targeting negative control siRNA was from Ambion (Silencer® Select Negative Control No. 1 siRNA, cat#4390843). Knockdown was confirmed by qRT-PCR 2 d post transfection. Cells were stained 16 hr post infection with DAPI (Sigma) and IRF3 rabbit monoclonal antibody (AbCAM) or rabbit IgG (AbCAM) as control and then donkey anti-rabbit AF647 secondary antibody (Life Technologies) using BD Cytofix/Cytoperm kit (BD Biosciences). Nuclear translocation of IRF3 was assessed using a 5-laser ImageStream X Mark II imaging cytometer (Amnis-Millipore) as described (Fasler-Kan et al, 2016). Cell populations were sequentially gated on single cells positive for DAPI and IRF3 staining. Image analysis was performed using a nuclear mask and Imagestream Data Exploration and Analysis Softare (IDEAS) 6.1 (Amnis-Millipore). The similarity score parameter, a log-transformed Pearson correlation coefficient of the pixel values of two images, was used to correlate the location of IRF3 staining and the nuclear dye (DAPI) to identify cells with nuclear IRF3.

Statistical analysis. Data for most experiments were analyzed by Student's t-test. All P-values are for two-tailed significance tests. For analysis of data based on independent experiments using samples from multiple donors, one-way analysis of variance (ANOVA) with Dunnett multiple comparison test was performed using GraphPad Prism (GraphPad Software). P values for the imaging cytometry experiment were calculated by Chi–squared test. P values below 0.05 were considered significant. The limit of detection of HIV infection was calculated for each assay using the method of (Armbruster and Pry, 2008).

Supplemental References

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