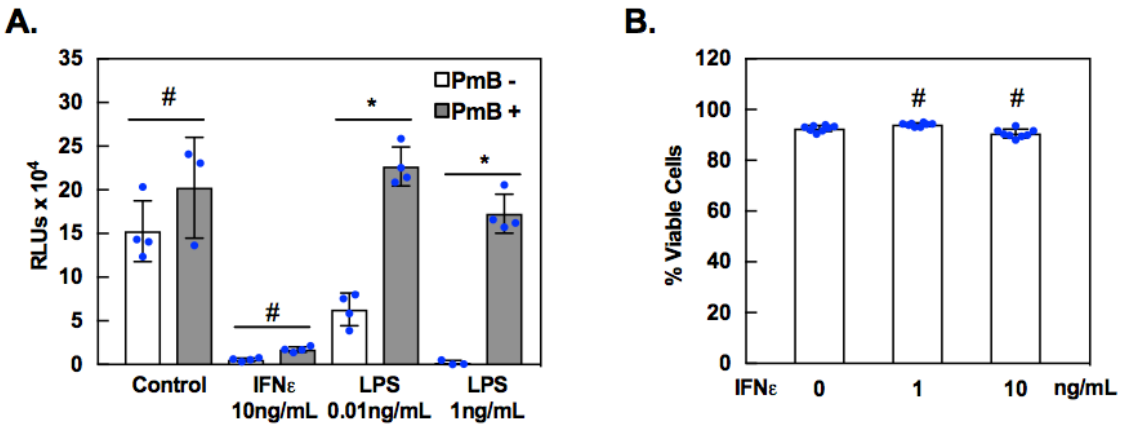
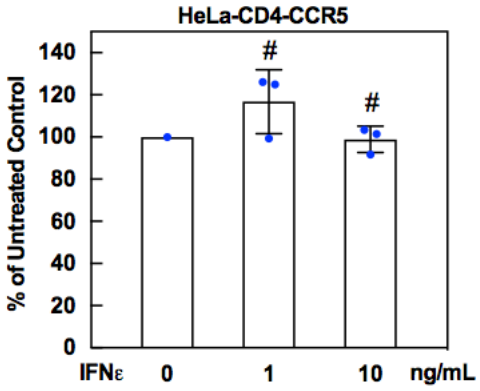
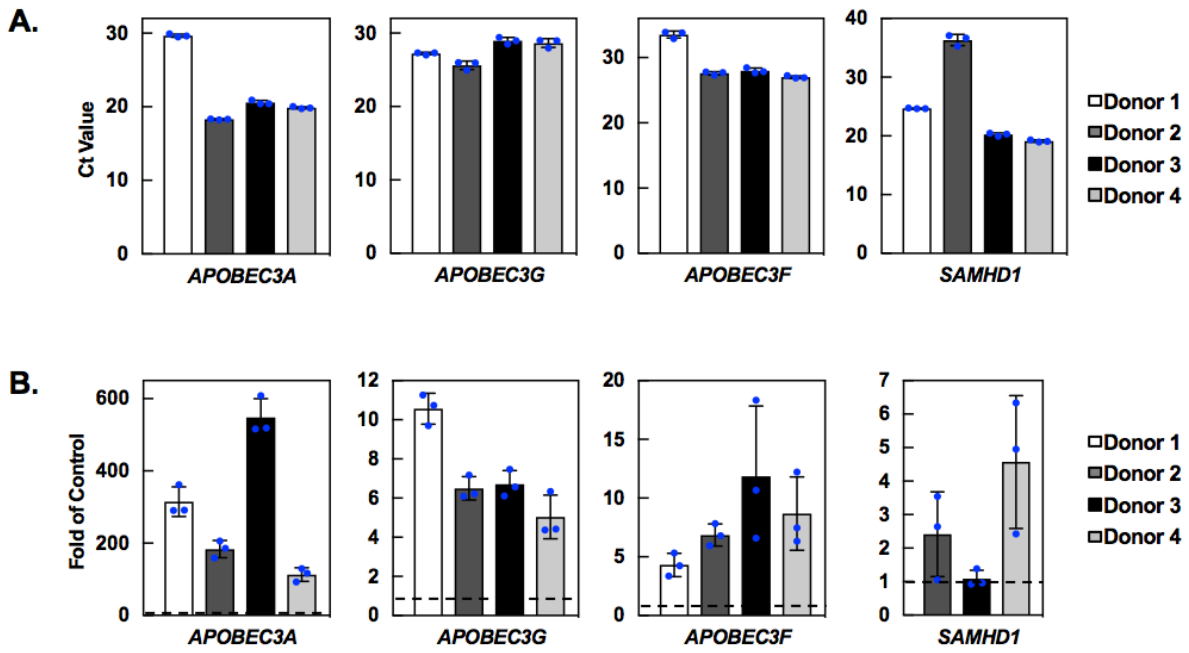


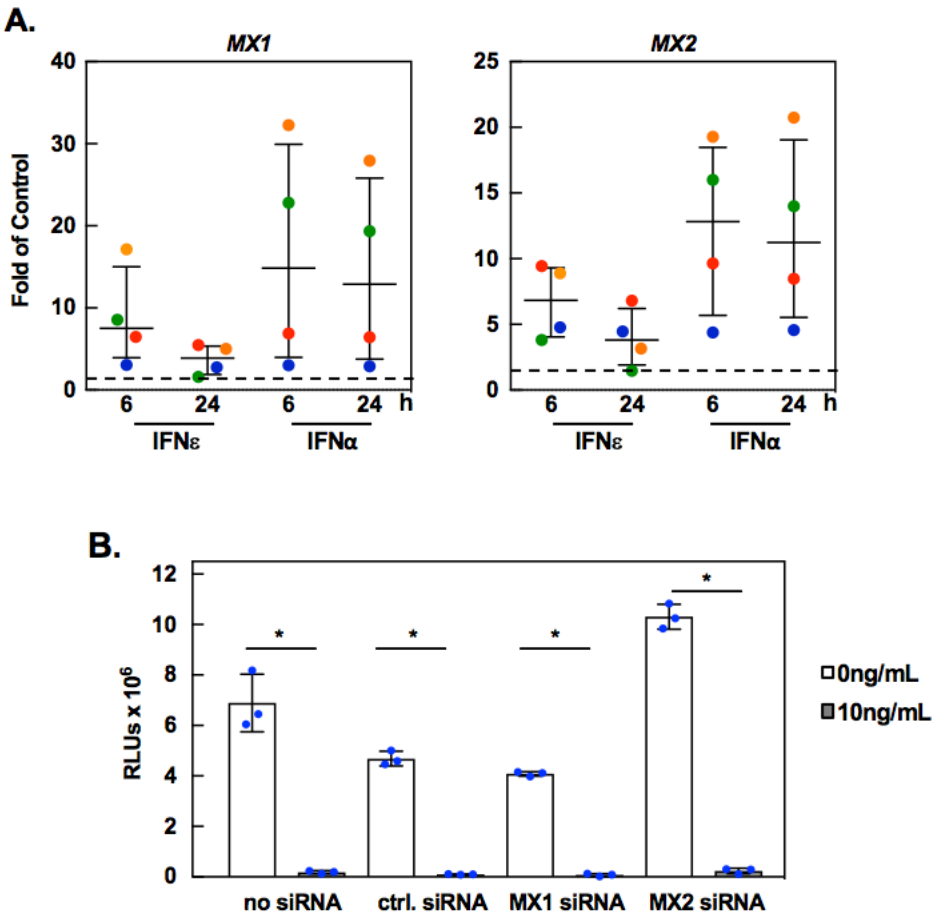
Supplementary Figure 1. Anti-HIV activity of IFNε was not due to LPS contamination or cytotoxicity. (A) Effect of polymyxin B (PmB) on Anti-HIV activity of IFNε. Culture media alone (control) or media with IFNε or LPS were incubated with 10 μg/mL PmB for 1 h at 37°C and then added to MDMs for 24 h followed by HIV-luc (JR-FL) infection; *p<0.5, and #p>0.05. (B) Viability of IFNε-treated cells. MDMs were treated with IFNε for 24 h before determining cell viability. Data are mean ± SD of quadruplicate samples and represent two independent experiments.



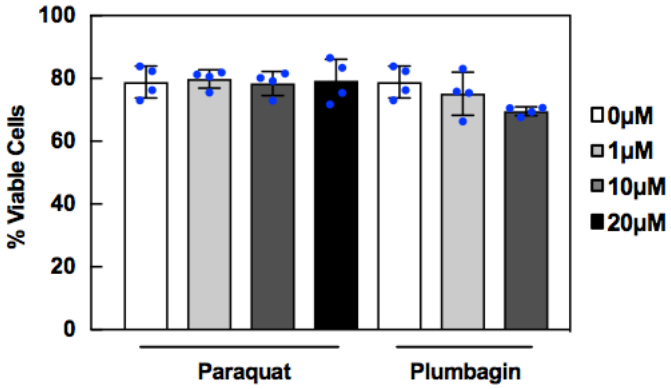
Supplementary Figure 2. IFNε did not exhibit anti-HIV activity in HeLa-CD4-CCR5 cells. HeLa cells expressing CD4 and CCR5 were treated with IFNε for 24 h and then infected with single-cycle HIV-luc (JR-FL) reporter virus; #p>0.05, untreated vs IFNε-treated MDMs. Data are mean ± SD of quadruplicate samples and represent two independent experiments.



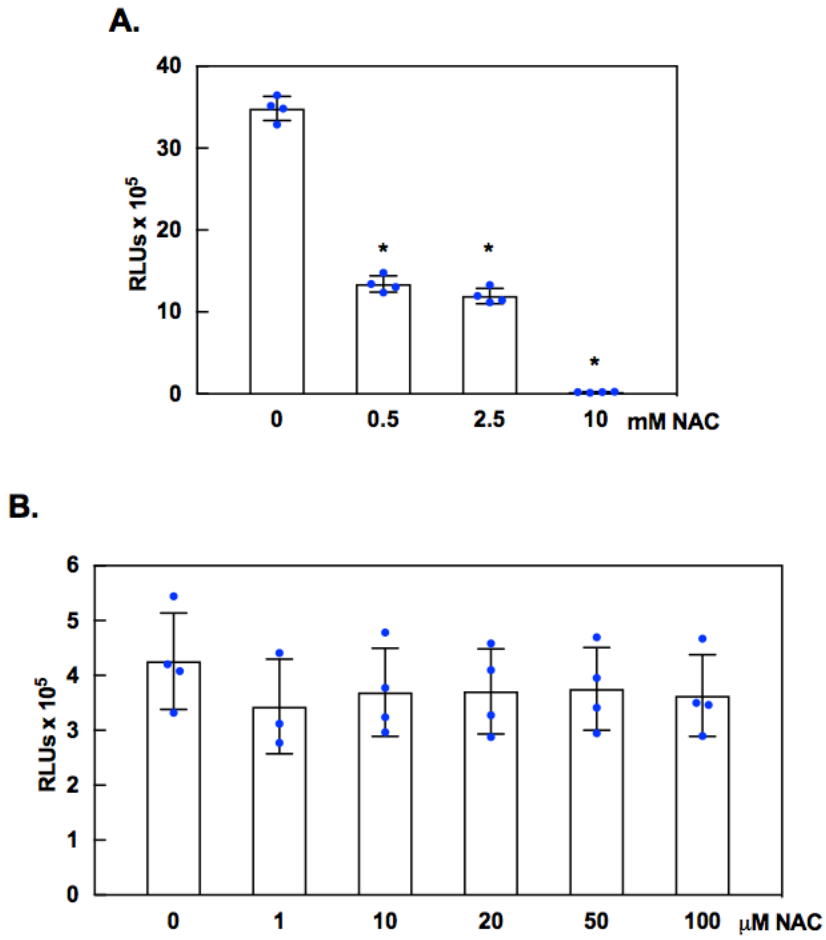
Supplementary Figure 3. Gene expression of HIV host restriction factors in untreated and IFNα-treated MDMs. (A) Baseline gene expression of host restriction factors in MDMs from different donors determined by real-time RT-PCR. Ct values, which are inversely correlated with the abundance of gene expression. Data are mean ± SD of triplicate samples. (B) Induction of HIV restriction factors by IFNα. MDMs were treated with IFNα 10ng/mL for 24 h and the expression of HIV restriction factors was determined by real-time RT-PCR. Each bar represents the fold-change between untreated control and IFNα treated MDMs from different donors. The dashed line indicates the level of gene expression of untreated control cells. Data are mean ± SD of triplicate samples.



Supplementary Figure 4. The effect of IFNε on HIV nuclear import. (A). Gene expression level of MX1 and MX2 in MDMs with IFNε at 10 ng/ml for 6 or 24 h. Donors were the same as Fig. 4C. (B) Effect of IFNε on HIV infection of MX1 or MX2 knockdown MDMs. MDMs were incubated with the transfection reagent alone (no siRNA) or the transfection reagent with 10nM control siRNA, MX1, or MX2 siRNA for 10 h followed by IFNε stimulation for 14 h before exposure to HIV-luc (JR-FL) luciferase reporter virus. Data are mean ± SD of triplicate samples. *p<0.05, IFNε-treated vs untreated controls.



Supplementary Figure 5. ROS inducers did not cause cytotoxicity. MDMs were treated with ROS inducers paraquat or plumbagin at various concentrations for 24 h before determining cell viability. Data are mean ±SD of quadruplicate samples and represent two independent experiments.



Supplementary Figure 6. NAC at high concentrations blocked HIV infection of MDMs. MDMs were treated with NAC at various concentrations for 24 h and washed off before exposure to HIV-luc (JR-FL). HIV infection was determined by a single-cycle infection assay. Pretreatment of MDMS with high (panel A) but not low (panel B) concentrations inhibited HIV infection. *p<0.05, untreated vs NAC-treated MDMs. Data are mean ± SD of triplicate samples and represent three independent experiments.