Supplemental Data

Figures

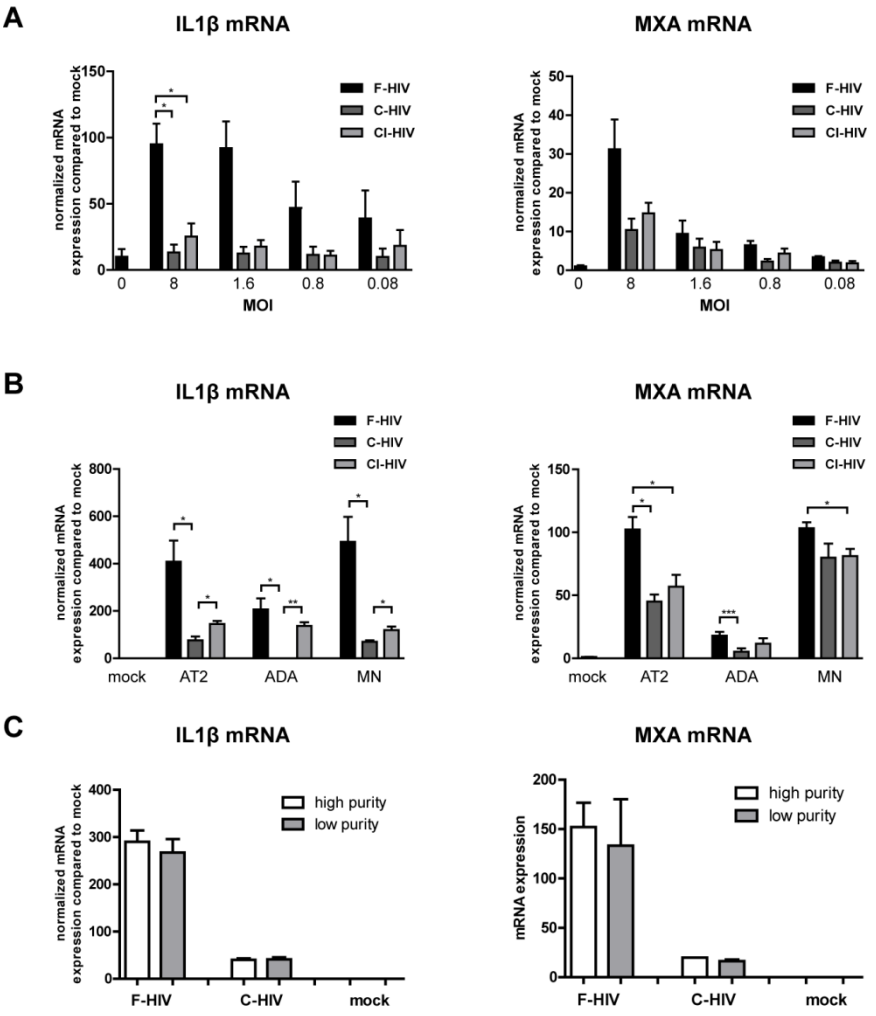

Figure S1. Complement opsonization of HIV-1 decreased inflammatory and antiviral responses in IDCs using different viral strains, concentrations and purities. (A) DCs ($1 \cdot 10^6$/ml) were exposed to 0.08-8 MOI free HIV_{Bal} (F-HIV), complement opsonized HIV_{Bal} (C-HIV), HIV_{Bal} opsonized with both complement and antibodies (CI-HIV) or mock treated for 6h and mRNA expression of IL1β and MXA was determined by qPCR. **(B)** DCs ($1 \cdot 10^6$/ml) were exposed to 8 MOI of AT2-inactivated HIV-1_{Bal}, HIV-Ada, HIV-MN or mock treated for 6h and mRNA expression of IL1β and MXA was determined by qPCR. **(C)** DCs ($1 \cdot 10^6$/ml) were exposed to 8 MOI highly purified HIV-1_{Bal} or virus diluted in preparations containing microvesicles and other virus production "byproducts" for 6h, followed by qPCR analysis of IL1β and MXA mRNA expression. Values have been normalized with mock values set to 1 and data are shown as mean +SEM of 2-4 independent experiments. *p<0.05.

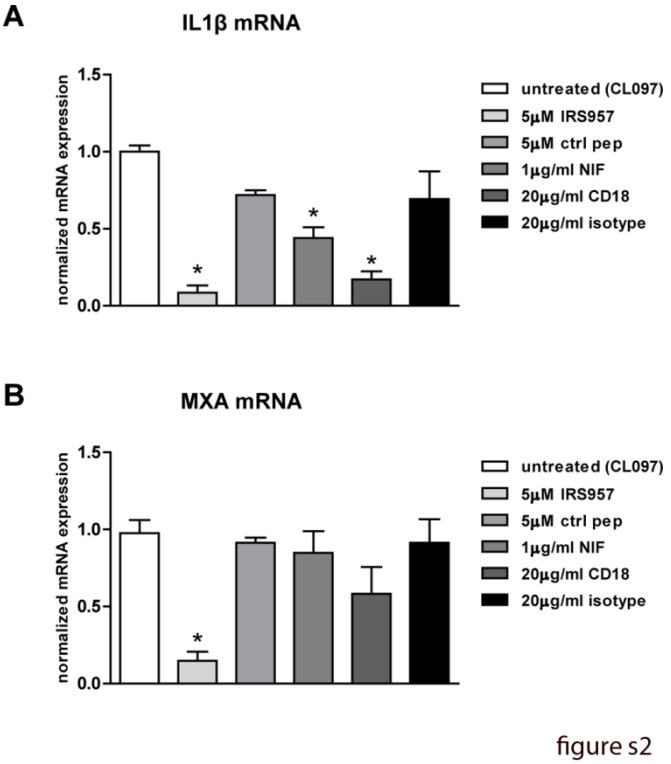

figure s2

Figure S2. Induction of TLR8 signaling using a synthetic agonist induced similar response as F-HIV. DCs ($1 \cdot 10^6$/ml) were pretreated with the TLR8 inhibitory oligonucleotide IRS957, control oligonucleotide, CR3 activating factor NIF, CR3 activating mAb CD18, or isotype control mAb and exposed to 5μg/ml TLR8 ligand CL097 for 6h. mRNA levels of IL1β **(A)** and MXA **(B)** were assessed by qPCR. Values have been normalized with untreated CL097 values set to 1 and data are shown as mean +SEM of 3-4 independent experiments. *$p<0.05$.

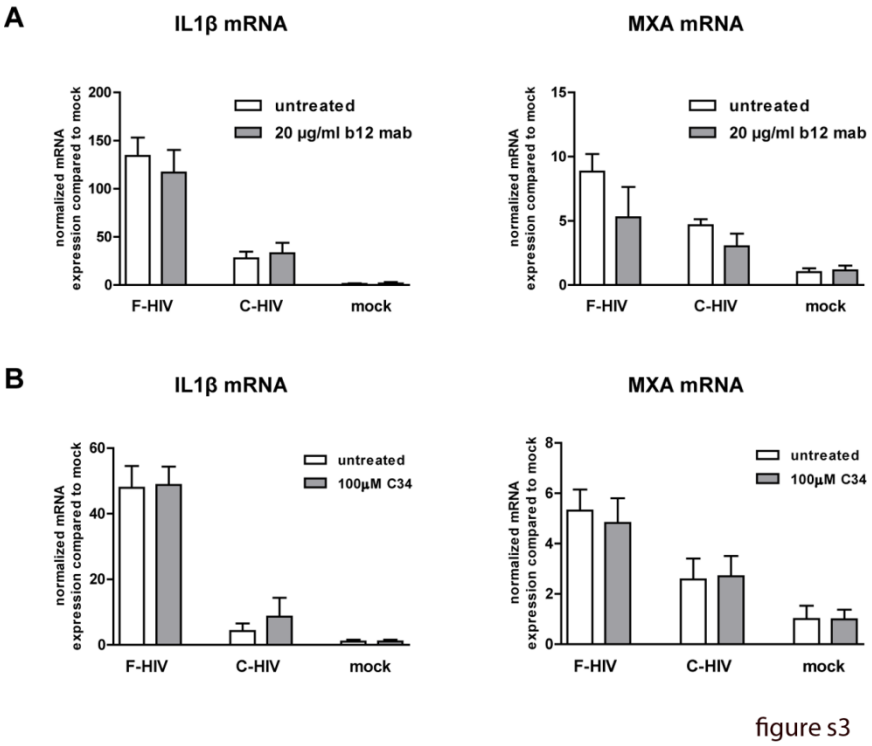

Figure S3. The inflammatory and antiviral responses induced by F-HIV were not dependent on viral fusion or assess to the cell cytosol. DCs ($1 \cdot 10^6$/ml) were pretreated with the neutralizing mAb b12 **(A)** or fusion inhibitor C34 **(B)** before exposure to 8 MOI free HIV_{Bal} (F-HIV), complement opsonized HIV_{Bal} (C-HIV) or mock treated for 6h to assess involvement of intracellular sensors such as NOD2. mRNA levels for IL1β and MxA were assessed by qPCR. Values have been normalized with mock values set to 1 and data are shown as mean +SEM of 3-6 independent experiments. *$p<0.05$.

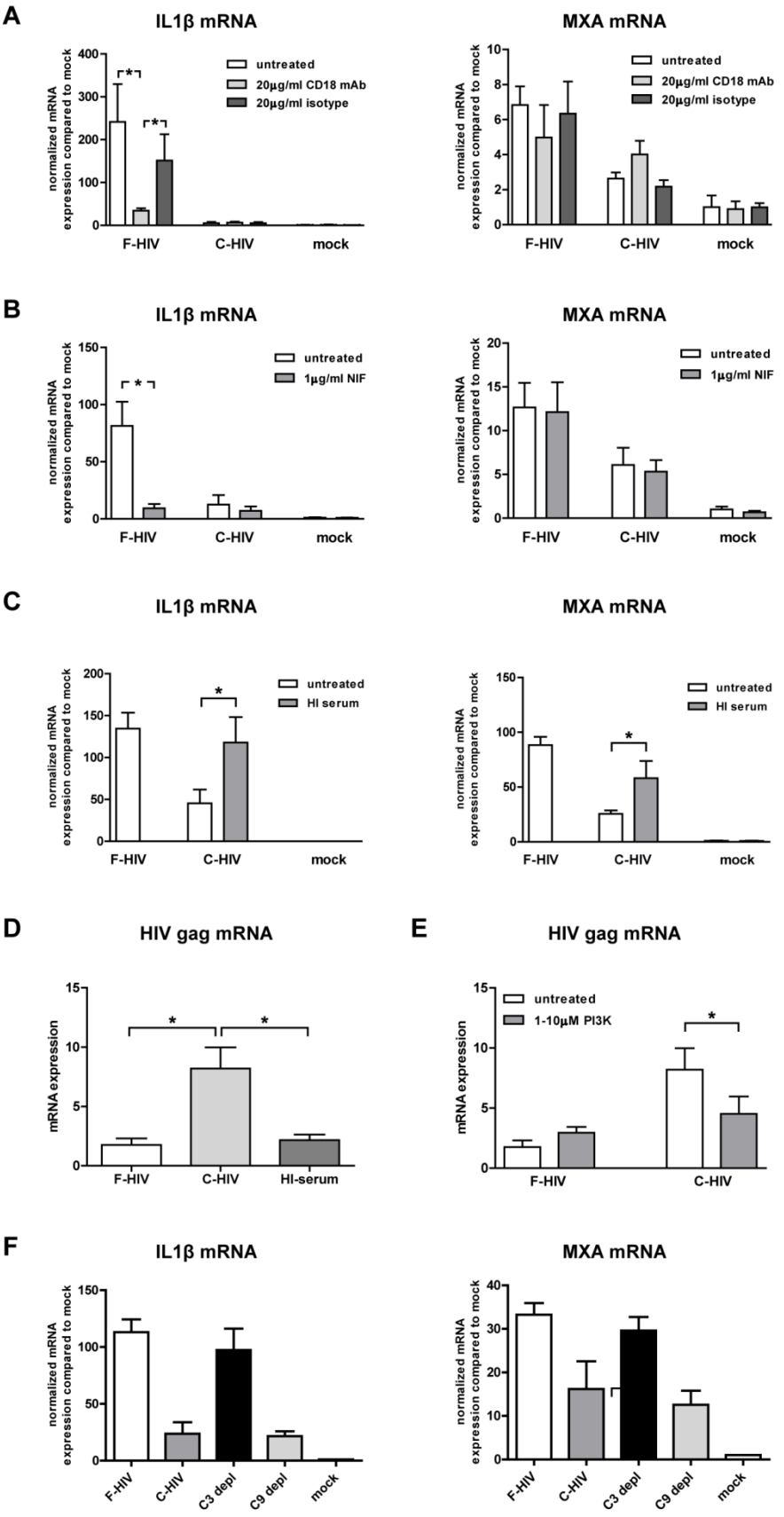

Figure S4. Inhibition of inflammatory and antiviral responses and enhancement of infection with complement opsonized HIV was dependent on CR3 activation and P13K signaling. DCs ($1 \cdot 10^6$/ml) were preexposed to CD18 neutralizing mAb

KIM185, isotype control mAb, the CR3 activator NIF or P13K inhibitor and exposed to 8 MOI of free HIV_{Bal} (F-HIV), complement opsonized HIV_{Bal} (C-HIV), HIV_{Bal} opsonized in HI serum, C3 depleted serum, C9 depleted serum or mock treated for 6h. mRNA levels for IL1β, MXA and early HIV transcript gag were assessed by qPCR to evaluate involvement of complement – CR3 interactions **(A,B,C,F)** and P13K **(D)** signaling in inflammatory and antiviral responses and infection. Values have been normalized with mock values set to 1 and data are shown as mean +SEM of 2-5 independent experiments. *$p<0.05$.