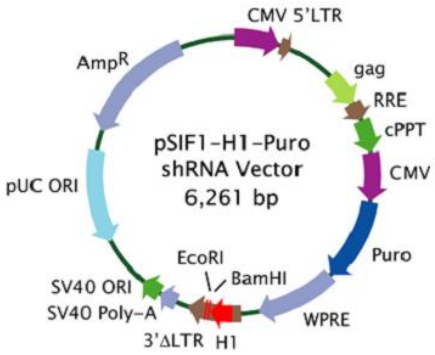
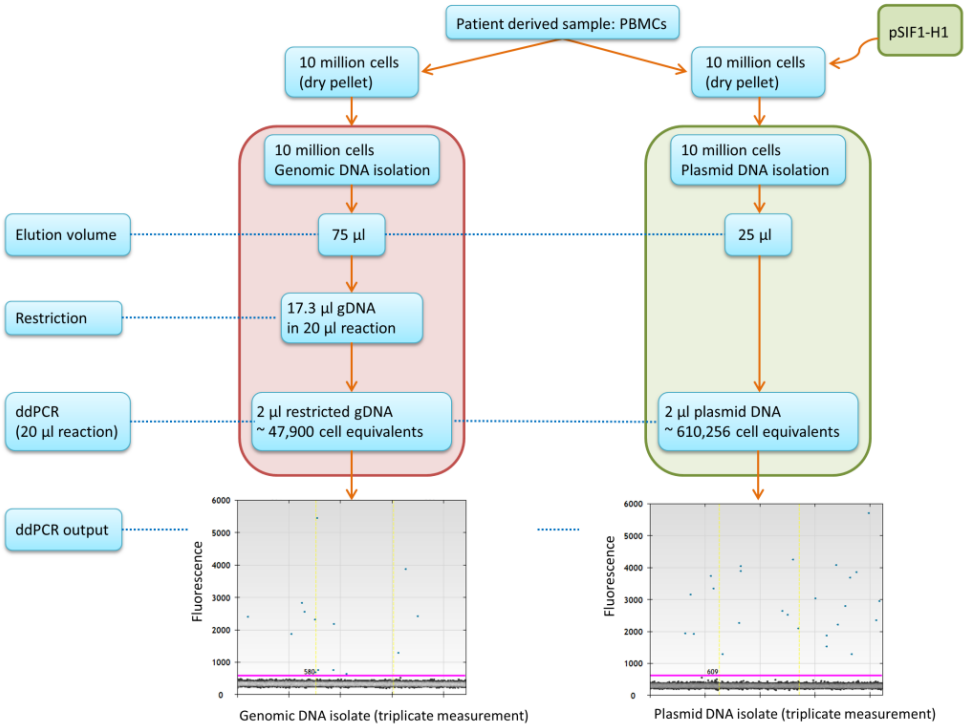
SUPPLEMENTAL FIGURES

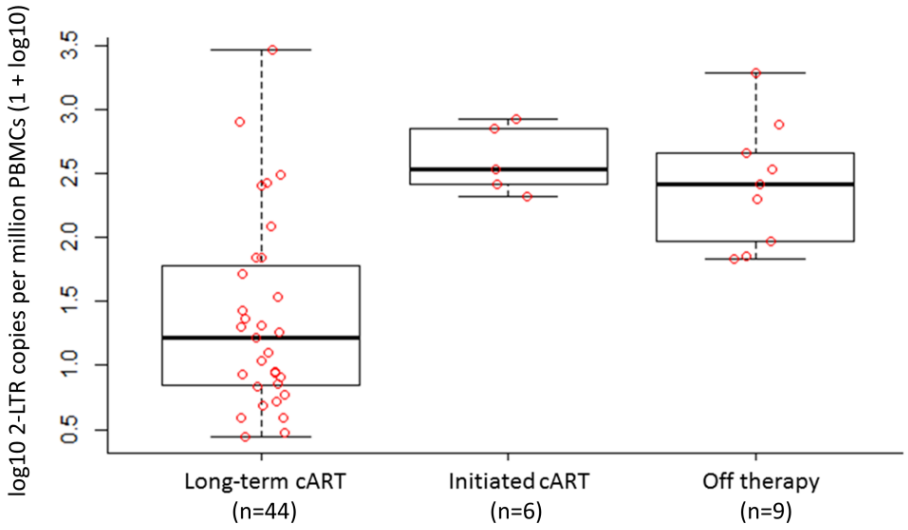


Supplemental Figure 1: Plasmid map of pSIF1-H1-Puro shRNA vector (1). The plasmid used for internal normalization to quantify 2-LTR circles per million cells in the plasmid DNA isolates.

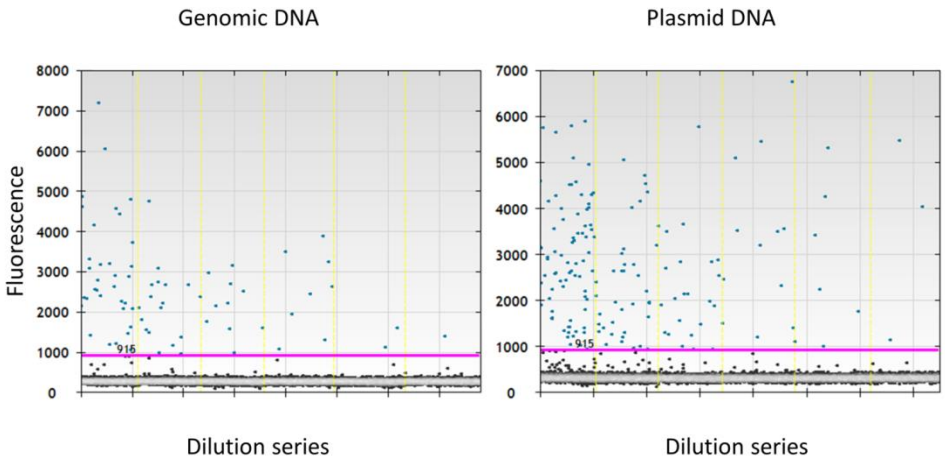


Supplemental Figure 2: Workflow representation of both DNA isolation methods in patient derived samples with ddPCR outcome. Genomic DNA isolation is shown with subsequent

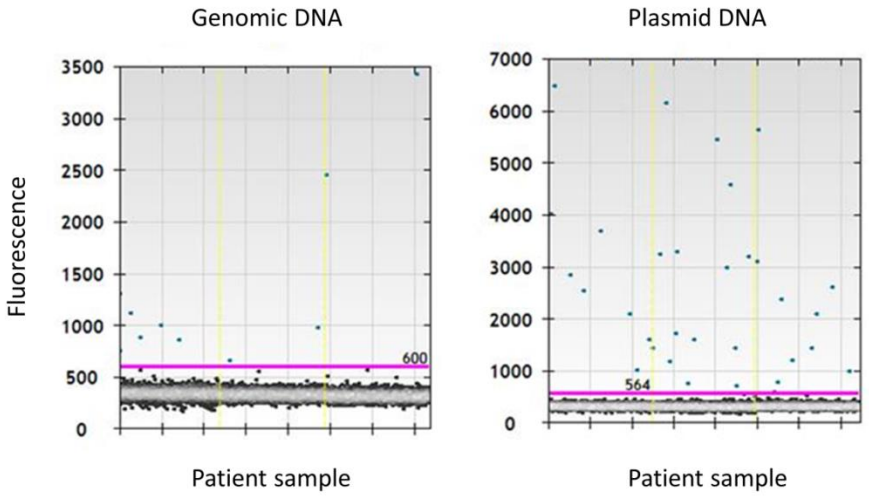
restriction and an average number of cell equivalents loaded in ddPCR well. Plasmid DNA isolation method is illustrated with spiking of a known number of pSIF1-H1 plasmid copies for data normalization in 10 million cells and an average number of cell equivalents loaded in ddPCR well. The ddPCR fluorescence intensity plots of a triplicate measurement show more positive droplets for the plasmid DNA isolate compared to the genomic DNA isolate of the same patient derived sample.



Supplemental Figure 3: Comparison of 2-LTR copies per million PBMCs between the three patient groups in plasmid DNA isolates. The highest number of 2-LTR copies per million PBMCs was measured in the group of patients with recently initiated cART and detectable VL.

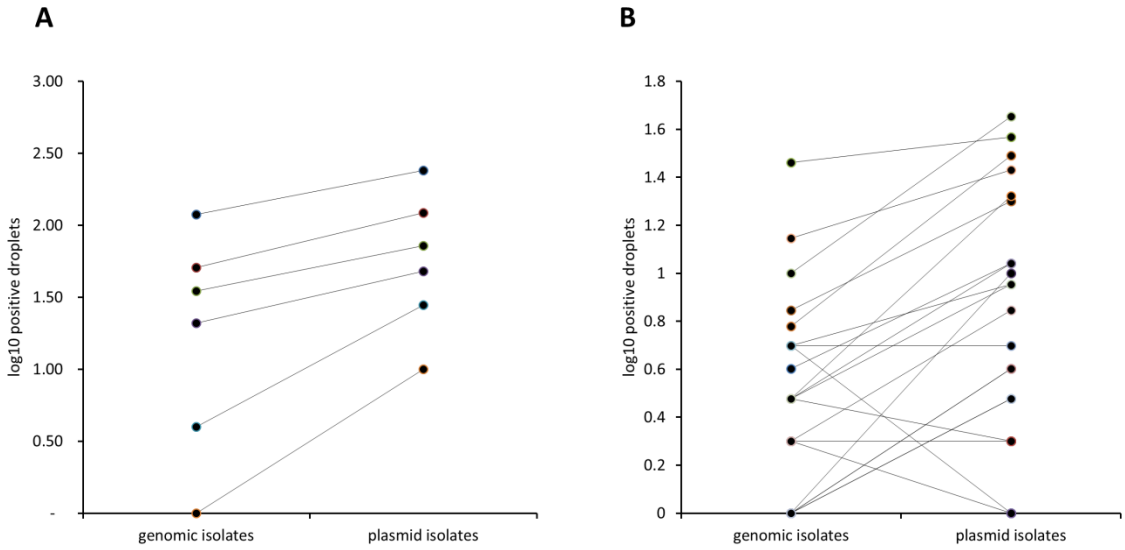


Supplemental Figure 4: Fluorescence intensity plots of ddPCRs for 2-LTR quantification in the dilution series in both DNA isolation methods. DdPCR droplets that either have no template (grey-black dots under the pink threshold line) or contain template (2-LTR) that resulted in a PCR amplification (blue dots above the pink threshold line) are shown. More positive droplets were detected in plasmid DNA isolates and a higher fluorescence amplitude of positive droplets observed compared to the genomic DNA isolates.

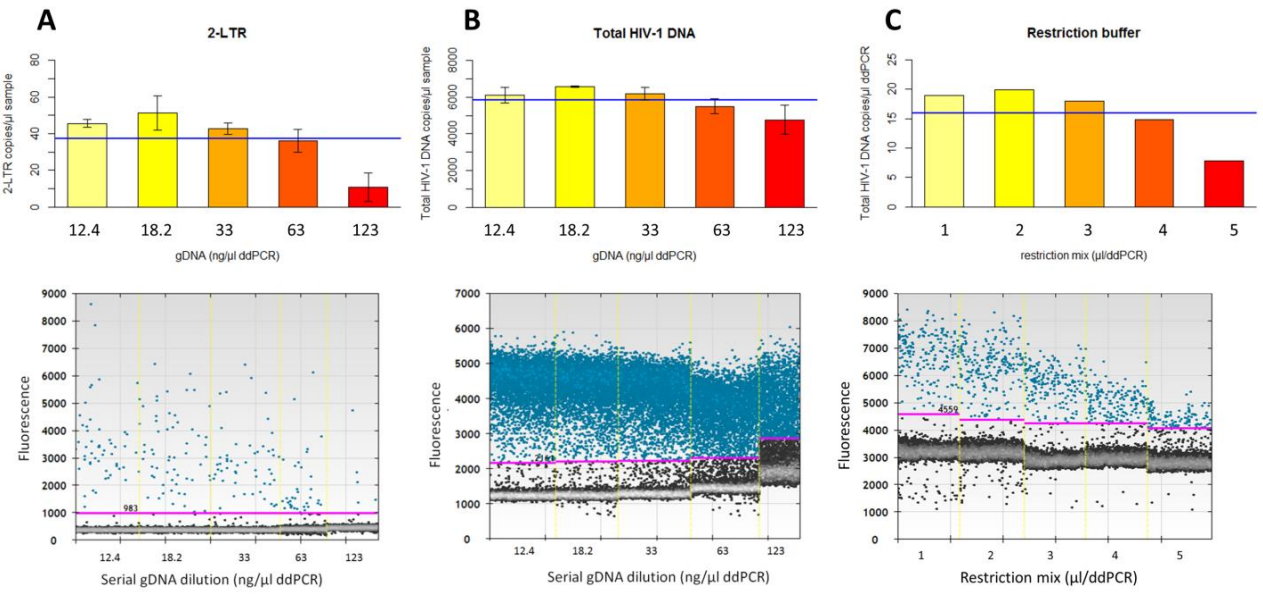


Supplemental Figure 5: Fluorescence intensity plots of ddPCRs for 2-LTR quantification in a patient derived sample in both DNA isolation methods. DdPCR droplets that either have no

template (grey-black dots under the pink line) or contain template that resulted in a PCR amplification (blue dots above the pink threshold line) are shown. The comparison of genomic versus plasmid DNA isolation on the triplicate measurement of a patient derived sample shows that the amount of positive droplets in the plasmid DNA isolates is substantially higher compared to the genomic DNA isolates. A significantly higher mean fluorescence intensity of the positive droplets was observed in plasmid DNA isolates.



Supplemental Figure 6: Comparison of the number of positive droplets in plasmid and genomic DNA isolates. More positive droplets were detected in the plasmid DNA isolates compared to the genomic DNA isolates in the dilution series (A) as well as in the patient derived samples (B).



Supplemental Figure 7: DdPCR inhibition. A & B: Quantitative output of triplicate dilution series of gDNA spiked with an equal amount of HIV-1 infected DNA assessed by the 2-LTR assay (A) and total HIV-1 DNA assay (B) with the fluorescence intensity plots. C: Quantitative output and fluorescence intensity plot of the total HIV-1 DNA assay in reaction mixtures with variable restriction digestion buffer mix (1-5 µl) showing ddPCR inhibition evident at concentrations above 2 µl. The blue horizontal lines show the mean of all measures. Error bars indicate the standard deviation within the three measurements.

References

1. **System Biosciences (SBI).** 2008. pSIF-H1 shRNA Cloning and Expression Lentivectors (Cat. #s SI100C-1, SI101B-1). User Manual SBI (ver. 4-080514).