Supporting Information

Perdomo et al. 10.1073/pnas.1206893109

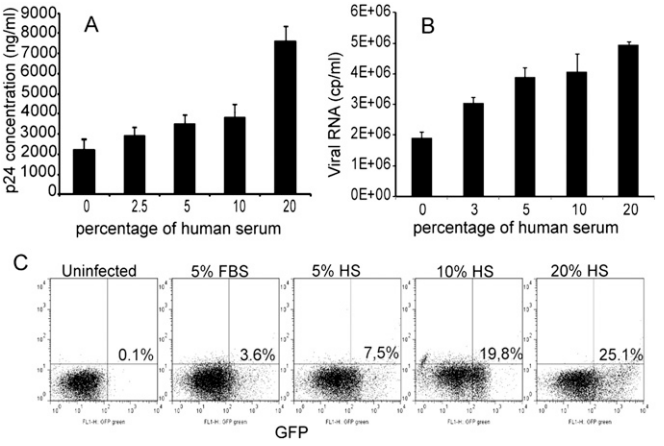

Fig. S1. Effect of human serum (HS) on HIV-1 infectivity. The HIV_{IIIB} viral output was studied in different cell lines after supplementation with heat-inactivated human serum (HIHS) and read as (*A*) p24 concentration in culture supernatant of H9 cells at day 7 postinfection. (*B*) Copies of viral RNA in culture supernatant of U87 cells at day 5 postinfection. (*C*) green fluorescent protein (GFP) expression of cell line CEM-GFP cells at day 7 postinfection. All values shown are the mean values ± the SD from four replicates. Comparisons of samples cultured at the highest serum concentration and controls were statistically significant ($P < 0.01$).

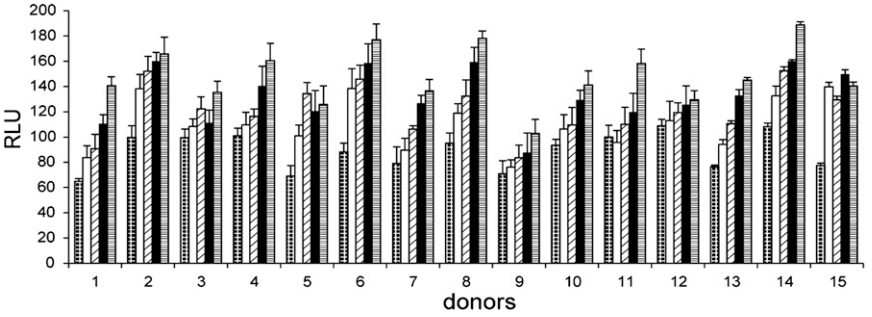

Fig. S2. Interindividual variation of HS. TZM-bl cells were infected with HIV_{IIIB} and cultured in the presence of 15 individual serum samples from uninfected individuals. Each serum sample was tested at 3% (squared bars), 5% (white bars), 10% (diagonal-line bars), 20% (black bars), or 30% (horizontal-line bars) (vol/vol). All values shown are the mean values ± the SD from four replicates.

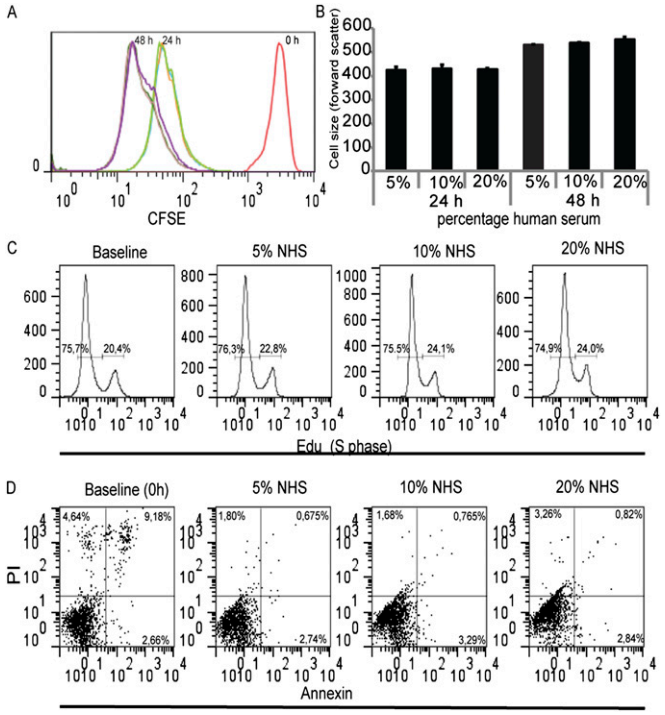

Fig. S3. Effect of human serum (HS) on cell cycle, cell proliferation, and apoptosis of uninfected TZM-bl cells. (*A*) TZM-bl cells were stained with CFSE and cultured in the presence of 5, 10, or 20% nonheat-inactivated human serum (NHS). Cell proliferation was followed after 24 and 48 h in culture by flow cytometry. Represented is the overlay of the carboxyfluorescein succinimidyl ester (CFSE) expression by progeny cells cultured at the different serum concentrations. (*B*) Cell size measured as mean of forward scatter at 24 h and 48 h in culture with NHS. (*C*) Percentage of cells on S phase of the cell cycle as determined by incorporation of the fluorescent thymidine analog 5-ethynyl-2′-deoxyuridine (Edu) after 48 h in culture with NHS. (*D*) Percentage of cells expressing annexinV (as apoptosis marker) and/or propidium iodide (PI) (as necrosis marker) after 48 h in culture with NHS.

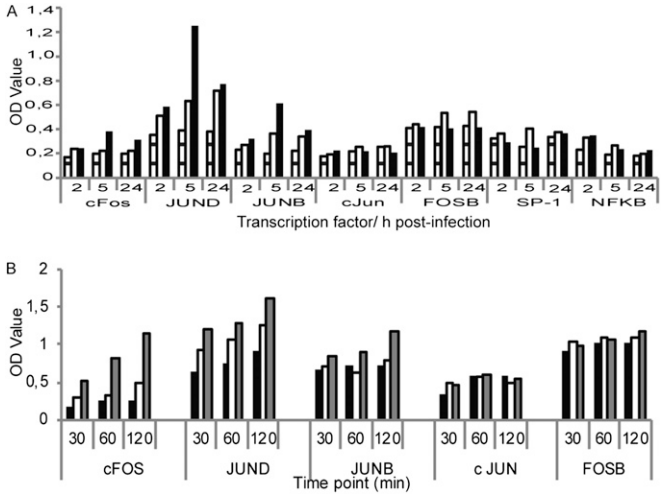

Fig. S4. Effect of HS on transcription. (*A*) TZM-bl cells were infected with HIV_{IIIB} and cultured in the presence of 2% FBS (horizontal-line bars), 5% NHS (white bars), or 20% (vol/vol) NHS (black bars). Nuclear fractions were extracted at the specified time points and tested for expression of activator protein-1 (AP-1) family, specificity protein-1 (SP-1), and nuclear factor kappa beta (NFκB) transcription factors. (*B*) TZM-bl cells were pretreated for 30 min with 20 μM of U0126 before the addition of NHS. Represented are U0126-treated cells cultured in 2% FBS (black bars), U0126-treated cells cultured with 20% NHS (white bars), and, as controls, untreated cells cultured with 20% NHS (gray bars). The nuclear extracts were collected at the specified time points and tested for AP-1 activation.

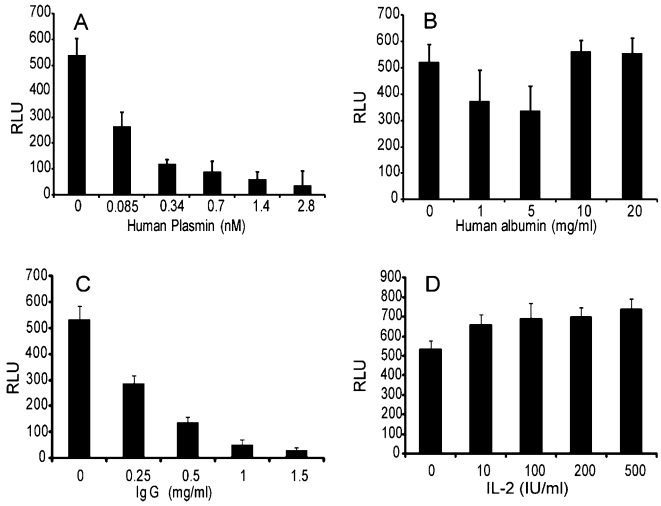

Fig. S5. Effect of individual serum components on HIV_{IIIB} infectivity. (*A*) Purified human plasmin, (*B*) purified albumin, (*C*) total purified human IgG, and (*D*) IL-2 were tested in the TZM-bl assay for their potential role in enhancing HIV infectivity. All values shown are the mean values ± the SD from four replicates.

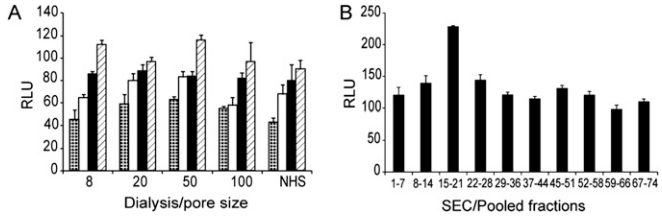

Fig. S6. Fractionation of human serum. (*A*) NHS was dialyzed with membranes of different pore size followed by test of the fractions at 5% (white bars), 10% (black bars), or 20% (diagonal-line bars) (vol/vol) on the TZM-bl cells. As a control dialyzed FBS (squared bars) was tested at 5% concentration. (*B*) Fractionation by size exclusion chromatography and test of pooled fractions in the TZM-bl assay. All values shown are the mean values ± the SD from four replicates.