

Figure S1. p24 expression in HEK-293T cells. (A) Flow cytometry analysis of p24 expression in HEK-293T cells cotransfected with the pSG3 plasmid and the different isolated or reference Env clones. The pSG3 bar corresponds to cells that do not express viral Env. Values indicate Mean fluorescence Intensity (MFI) of HEK-293 cells (2,000 total cells analyzed per point) stained with Kc57 antibody. Envs isolated from the same patient are associated with brackets. Values are mean ± S.E.M. of at least four independent experiments (n=5). (B) Flow cytometry analysis of p24 protein expression from data in panel (A) grouped by predefined patient categories. Mean values between each group are shown with no significant (ns) differences identified after comparison analysis (Kruskal-Wallis, Dunn's Multiple Comparisons Test). Values are mean ± S.E.M. of at least four independent experiments (n=5). These p24/Pr55Gag expressing cells were used to assay Env-mediated viral transfer (see Figure 5) and infection (see Figure 6).

Pérez-Yanes et al.

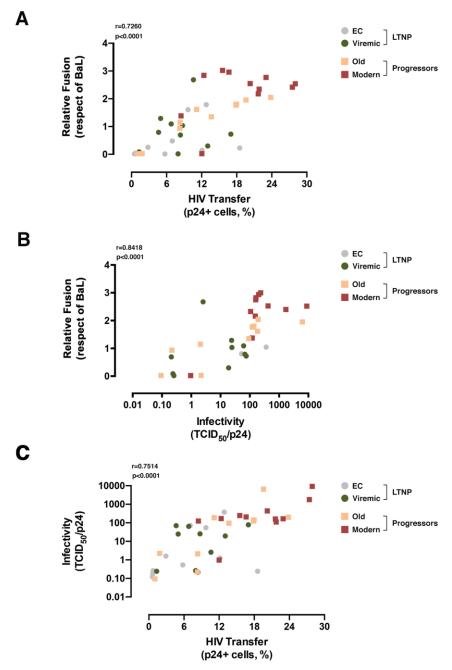


Figure S2. Analysis of the correlation of the fusion, transfer and viral infectivity Env characteristics between groups. (A) Correlation between Relative fusion and HIV Transfer of all Envs of the different groups LTNP-EC (gray circle), vLTNP (green circle), Old patients (orange square) and Modern patients (red square). The correlation was calculated with a nonparametric Spearman test. (B) Correlation between Relative fusion and Infectivity (TCID50 value normalized by viral p24 input) of all Envs of the different groups LTNP-EC (gray circle), vLTNP (green circle), Old patients (orange square) and Modern patients (red square). The correlation was calculated with a nonparametric Spearman test. (C) Correlation between Infectivty and HIV Transfer of all Envs of the differents groups LTNP-EC (gray circle), vLTNP (green circle), Old patients (orange square) and recent patients Moderns) (red square) is shown. The correlation was calculated with a nonparametric Spearman test. Values are mean ± S.E.M. of three independent experiments, as indicated in the respective experiments; p value for comparison between all groups is shown, top left.

Pérez-Yanes et al.

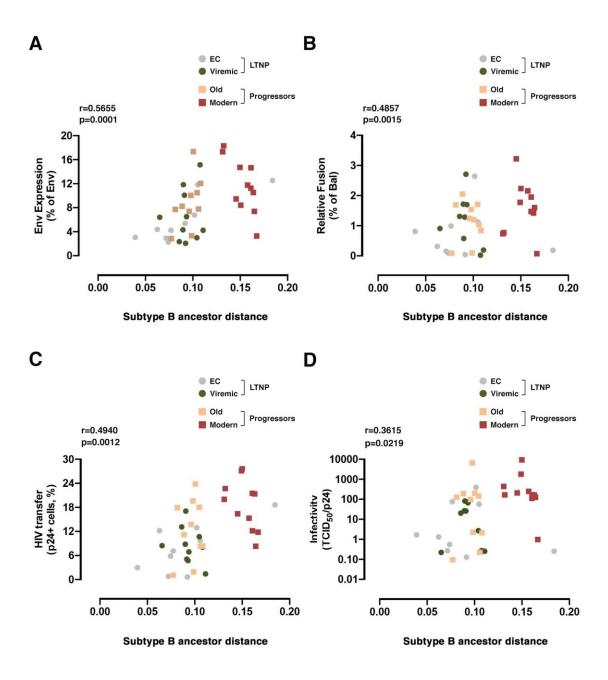


Figure S3. Correlation of the expression, fusion, transfer and viral infectivity Env characteristics with the nucleotide genetic distance to subtype B ancestor. Correlation between genetic distance to subtype B ancestor of all Envs of the different groups and Env expression (A), Relative fusion (B), HIV Transfer (C) and Infectivity (D). LTNP-ECs (gray circle), vLTNPs (green circle), Old patients (orange square) and Modern patients (red square). The correlations were calculated with a nonparametric Spearman test (p and r values are shown, top left). Values of Env expression, Relative fusion, HIV transfer and Infectivity are mean \pm S.E.M. of three independent experiments, as indicated in the respective experiments.

Pérez-Yanes et al.