Expanded View Figures

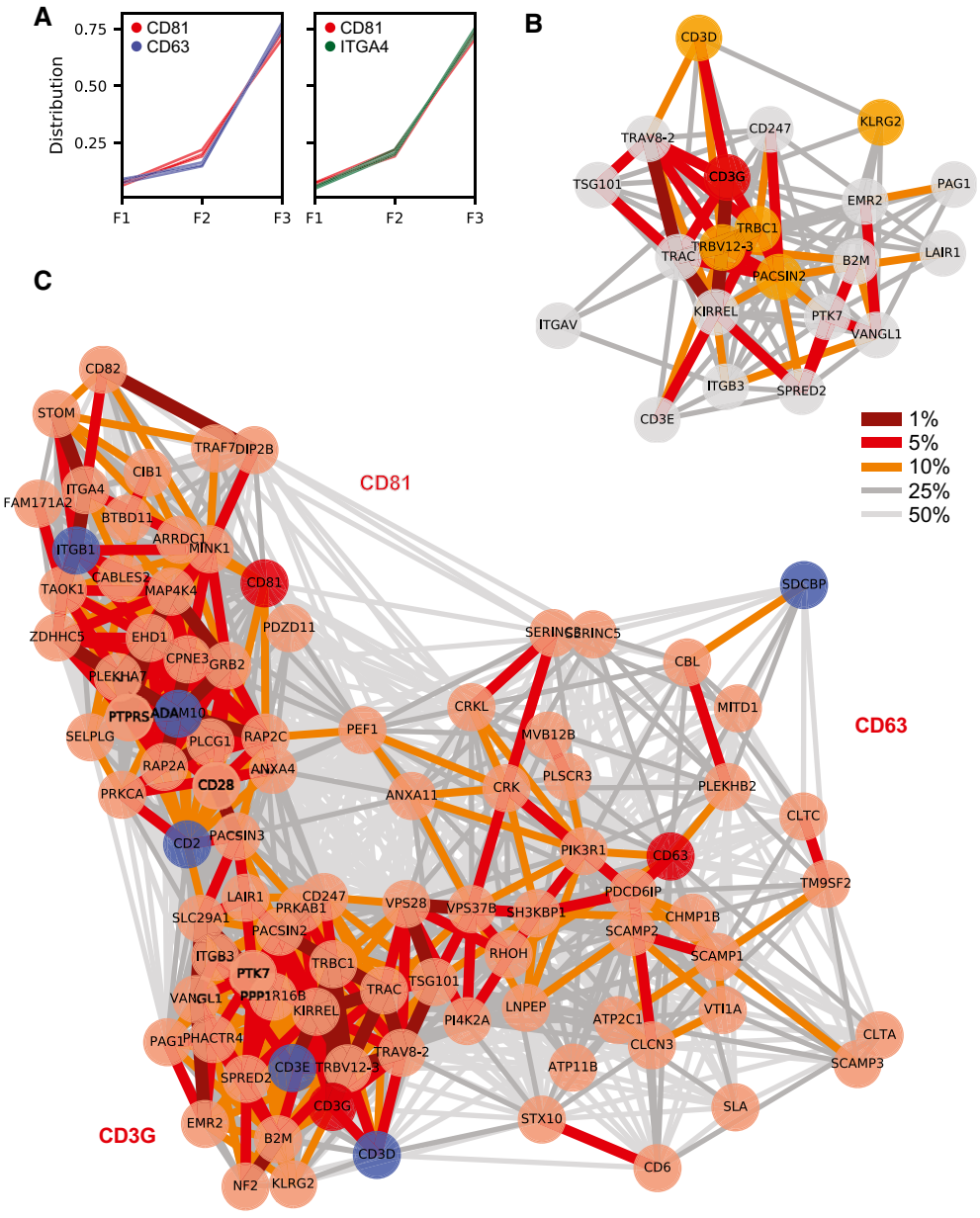

Figure EV1. Identification by unbiased proteomic analysis of groups of proteins likely released in the same EV subtypes by Jurkat cells.

A Overlay of proteomic profiles of CD63 and CD81 (left) versus CD81 and ITGB4 (right) showing the relative abundance distribution across the 3 × 3 subfractions obtained from untreated Jurkat cells. Although all three proteins are strongly enriched in F3 fractions, the profiles of CD63 and CD81 are reproducibly different, whereas profiles of CD81 and ITGB4 are extremely similar.

B Neighbourhood Network plot of CD3G as a single query (replicate tolerance = 50, **network members, cut-off for replicates = 2, 25% distance percentile for edges). Nodes: red = query, orange = close neighbour in all three replicates, grey = close neighbour in two out of three replicates. Edges: percentile within the local distance distribution (thicker edge and darker shade = smaller distance, i.e. closer neighbour); see Materials and Methods for details.

C Multiple query Neighbourhood Network plot for CD63, CD81 and CD3G. The top 30 close neighbours of each query were jointly used for the network layout (B ranking network members, 50% distance percentile of edges). Nodes: red = query, light red = close neighbours in 2 or 3 replicates, blue = neighbours validated by Immunoisolation, Immuno-EM or MacsPlex Exo in figure 3. Edges: percentile within the local distance distribution (thicker edge and darker shade = smaller distance, i.e. closer neighbour); see Materials and Methods for details. The three networks are remotely connected, but clearly separate.

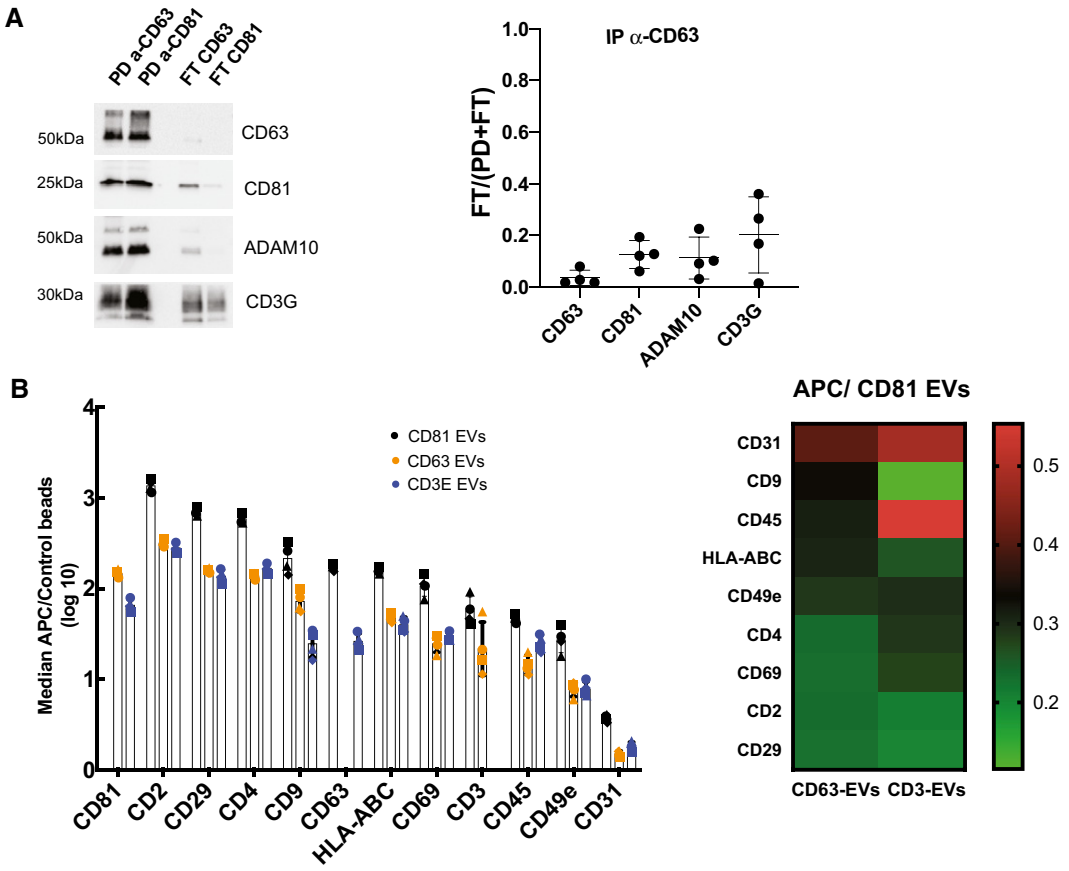

Figure EV2. Biochemical analysis of the composition of EVs released by primary CD4$^+$ T cells.

EVs were purified by SEC from supernatant of activated CD4$^+$T cells.

A EVs were subjected to immunoisolation with beads coupled to antibodies against CD81 or CD63. Bead-associated (Pull-down: PD) vesicles and those left behind (Flow-Through: FT) were loaded on a gel for Western blot analysis with antibodies specific for CD63, CD81, ADAM10 and CD3G. A representative image and quantification (mean ± SD) of the proportion of signal in FT as compared with total (PD + FT) in samples obtained from four independent donors are shown.

B Multiplex bead-based flow cytometry assay for detection of EV surface markers. Antibody-coated capture beads were incubated with 2×10^9 particles. Captured EVs were detected with either APC-labelled anti-CD81, anti-CD63 or anti-CD3E. Left: Median APC fluorescence values for the different bead populations are shown as a ratio to the median APC fluorescence of control beads (log10 scale). Mean ± SD for four independent experiments is shown. Right: Heat-map representation of the median APC fluorescence values for the different bead populations detected with anti-CD63 or anti-CD3E antibodies relative to the values detected with anti-CD81 (mean value of 4 independent donors).

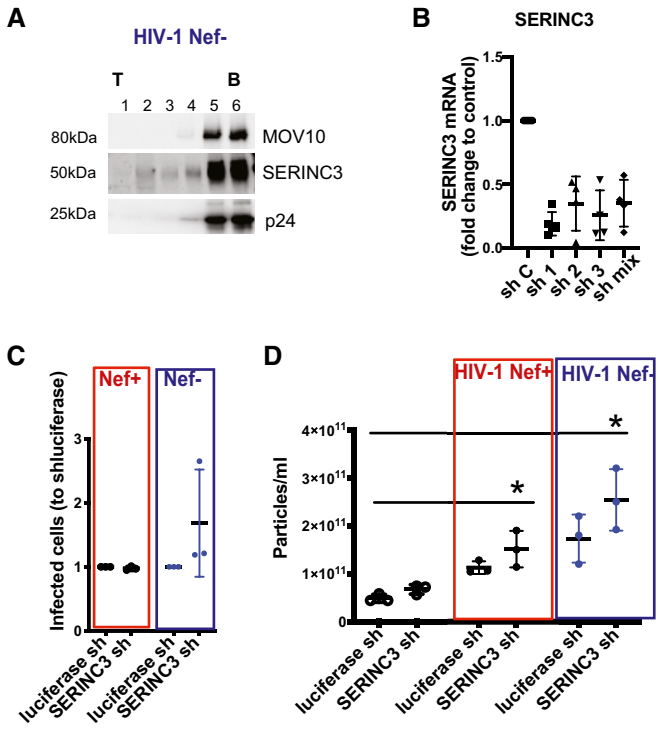

Figure EV3. Lentivirus-mediated silencing of SERINC3.

A 100K pellets from Jurkat cells infected with Nef-defective HIV-1 were subjected to iodixanol velocity gradient separation. Six fractions were recovered and analysed by Western blot for the presence of SERINC3 and p24. (T = top; B = bottom).

B Jurkat cells were transduced with lentiviruses encoding different shRNA sequences specific for SERINC3, or a control shRNA (Sh C). SERINC3 mRNA levels were determined by qPCR. Mean ± SD of three-four different experiments is shown.

C, D Control and SERINC3 KD Jurkat cells were left uninfected or infected with NL4-3 EGFP HIV-1 containing or not containing Nef. (C) Numbers of GFP^+ Ghost X4R5 cells obtained after treatment with supernatant from infected SERINC3 KD cells. Results are expressed as a ratio to the number of GFP^+ cells after treatment with supernatant from infected control cells. Mean ± SD for three independent experiments is shown This experiment confirms that SERINC3 restricts HIV-1 infectivity only for Nef-defective virus. (D) SERINC3 KD cells were infected or not infected, and numbers of secreted particles were quantified by NTA. Results are expressed as the number of secreted particles/ml of conditioned medium. Mean ± SD for three independent experiments is shown. *$P < 0.05$ (Friedman test followed by Dunn's post-test).