

## APPENDIX

### Table of Content

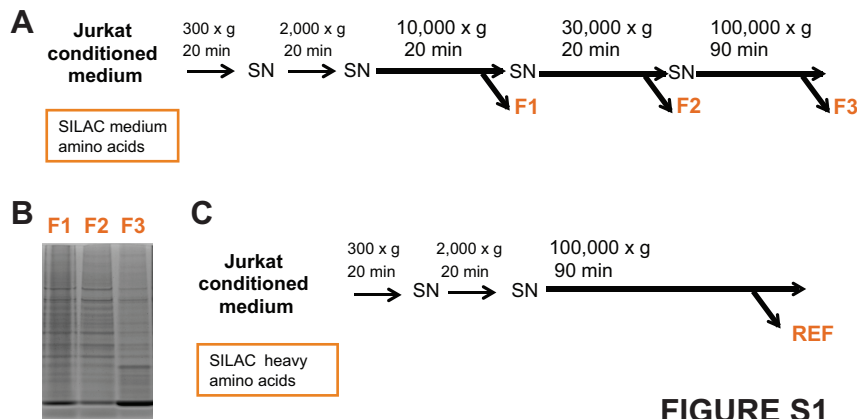
Appendix Figure S1: p1

Appendix Figure S2: p2

Appendix Table S1: p3

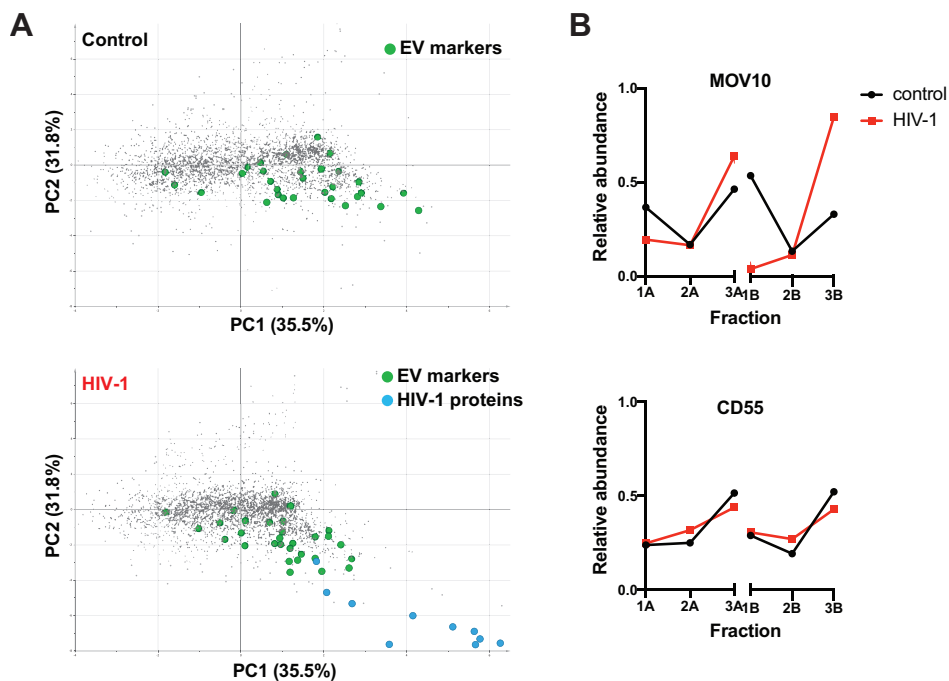
### Appendix Figure S1. Scheme of the experimental workflow.

48h CCM was obtained from Jurkat cells metabolically labeled with SILAC medium (Lys 4, Arg 6) (A) or SILAC heavy (Lys 8, Arg 10) (B) aminoacid-isotypes. CCM was centrifuged to remove cells (300g 20 min) and large vesicles + nanoparticles (2000g 20 min). (A) The supernatant was subjected to serial ultracentrifugation with increasing speeds to obtain fractions F1, F2 and F3. (B) The three fractions contain similar amount of protein as shown by gel electrophoresis and Coomassie stain. (C) For SILAC heavy-labeled cells the supernatant was subjected directly to the high-speed centrifugation to obtain the reference pellet (Ref). The three fractions in (A) were pooled with Ref fraction in three (Ctrl Jurkat analysis), or two (comparison of Ctrl and HIV-1 infected Jurkat) biological replicates. The same protocol was used for HIV-1 NL4-3 EGFP-Nef+ infected cells.



**Appendix Figure S2. Identification by unbiased proteomic analysis of candidate EV proteins changing location upon HIV infection.**

(A) Abundance profiles of over 3000 proteins in EVs recovered after 10K (F1), 30K (F2) and 100K (F3) centrifugations (see Fig S1), from non-infected and HIV-1-infected Jurkat cells were subjected to PCA. Proximity of proteins indicates similar profiles. HIV-1 proteins were identified in the same quadrant as the majority of sEVs protein markers (highlighted in green). Proteins used here as markers for sEVs were identified previously (Kowal *et al*, 2016) as specific of CD9- or CD63- or CD81-bearing sEVs in human Dendritic Cells (Table S1) (B) Proteomic abundance profiles of MOV10 and CD55 proteins across the six subfractions, showing modified distribution of MOV10 upon HIV-1 infection, whereas distribution of CD55 does not change significantly. Each profile is shown in two independent data triplets.



**Appendix Table S1. Proteins used as markers of small EVs in Fig 2.**

This table lists some proteins specifically secreted in small EVs (recovered in 100K pellets) expressing at least CD63 or CD81 or CD9 (i.e. present in the pull-down after immuno-isolation using antibodies against one of these tetraspanins), but not in small EVs devoid of all these tetraspanins (i.e. absent or very low in the flow-throughs after immuno-isolation using anti-CD9 antibodies), according to our previous proteomic analysis of Dendritic Cell-derived EVs (Kowal *et al*, 2016). A protein was considered present if identified by at least 1 peptide in at least two of the three biological replicates.

Uniprot _ gene name	major subcellular localisation (according to Uniprot)	present (Yes/No) in EVs (pull- down) CD63 <sup>+</sup> CD9 <sup>+</sup> CD81 <sup>+</sup>	Present (Yes/No) in EVs negative for (Flow- through) CD63 <sup>-</sup> CD9 <sup>-</sup> CD81 <sup>-</sup>
ABI1_ ABI1	cytosol, nucleus, plasma membrane	Y Y Y	Y Y(±) Y
ANXA7_ ANXA7	nucleus, ER, extracellular	Y Y Y	Y Y(±) Y
ARRD1_ ARRDC1	cytosol, plasma membrane	Y Y Y	N N N
CD166_ ALCAM	Single-pass transmembrane, plasma membrane	N Y Y	Y N N
CD53_ CD53	Multi-pass transmembrane, plasma membrane	Y Y Y	Y N Y
CD63_ CD63	Multi-pass transmembrane, endosomes, lysosomes, plasma membrane	Y Y Y	N N N
CD9_ CD9	Multi-pass transmembrane, plasma membrane	Y Y Y	Y N Y
CD81_ CD81	Multi-pass transmembrane, plasma membrane	Y Y Y	Y N N
CD82_ CD82	Multi-pass transmembrane, plasma membrane	Y Y Y	Y N N
CHM1A_ CHMP1A	cytosol, nucleus, endosomes, peripheral membrane	Y Y Y	N N Y
CHM2A_ CHMP2A	cytosol, endosomes, peripheral membrane	Y Y Y	N N N
CHM2B_ CHMP2B	cytosol, endosomes, peripheral membrane	Y N Y	N N N

CHM4A_ CHMP4A	cytosol, endosomes, peripheral membrane	Y Y	Y	N	N	Y
CHM4B_ CHMP4B	cytosol, nucleus, endosomes, peripheral membrane	Y Y	Y	N	N	Y
CTL1_ SLC44A1	Multi-pass transmembrane, plasma membrane, mitochondrion	Y Y	Y	Y	Y(±)	Y
FBLN1_ FBLN1	secreted, binds to ECM	N Y	N	N	N	N
FPRP_ PTGFRN	Single-pass transmembrane, ER, Golgi	Y Y	Y	Y	Y(±)	Y
GDIB_ GDI2	cytosol, peripheral membrane	Y Y	Y	N	N	N
GRAN_ GCA	cytosol, focal adhesions	Y Y	N	N	N	N
IGSF8_ IGSF8	Single-pass transmembrane, plasma membrane	Y Y	Y	Y	N	N
IST1_ IST1	cytosol, centrosome, midbody	Y Y	Y	Y	Y(±)	Y(±)
LDHA_ LDHA	cytosol	Y Y	Y	N	N	N
M4K4_ MAP4K4	cytosol	Y Y	Y	N	N	N
MINK1_ MINK1	cytosol, plasma membrane, Golgi	Y Y	Y	N	N	N
PACN2_ PACSN2	cytosol, plasma membrane, endosomes	Y Y	Y	Y	N	Y
PDC6I_ PDCD6IP	cytosol, extracellular	Y Y	Y	Y	Y(±)	Y
PDCD6_ PDCD6	nucleus, endosome, ER	Y Y	Y	Y	Y(±)	Y
PI42A_ PIP4K2A	cytosol, plasma membrane, nucleus	Y Y	Y	Y	N	Y
PLS3_ PLSCR3	cytosol	Y Y	Y	N	N	N
PPIB_ PPIB	ER lumen	Y Y	Y	Y	Y(±)	Y(±)
PPR18_ PPP1R18	cytoskeleton	N Y	Y	N	N	N
RHG18_ ARHGAP 18	cytosol	Y Y	Y	Y	N	N
SDCB1_ SDCBP	cytosol, plasma membrane, extracellular	Y Y	Y	Y	Y(±)	Y
SELPL_ ELPLG	Single-pass transmembrane	N Y	Y	Y	N	N
SH3G1_ H3GL1	cytosol, early endosome, plasma membrane	Y Y	Y	N	N	N

<b>SRGP2_ SRGAP2</b>	<b>nucleus, plasma membrane</b>	<b>Y</b>	<b>Y</b>	<b>Y</b>	<b>Y</b>	<b>N</b>	<b>Y</b>
<b>SYK_KA RS</b>	<b>cytosol, extracellular</b>	<b>Y</b>	<b>Y</b>	<b>Y</b>	<b>Y</b>	<b>N</b>	<b>Y</b>
<b>TAOK1_T AOK1</b>	<b>cytosol</b>	<b>Y</b>	<b>Y</b>	<b>Y</b>	<b>N</b>	<b>N</b>	<b>N</b>
<b>TNAP3_T NFAIP3</b>	<b>nucleus, cytosol, lysosome</b>	<b>Y</b>	<b>Y</b>	<b>N</b>	<b>N</b>	<b>N</b>	<b>N</b>
<b>TRI25_ TRIM25</b>	<b>cytosol</b>	<b>Y</b>	<b>Y</b>	<b>Y</b>	<b>N</b>	<b>N</b>	<b>N</b>
<b>TS101_ TSG101</b>	<b>cytosol, endosomes, nucleus, centrosome</b>	<b>Y</b>	<b>Y</b>	<b>Y</b>	<b>N</b>	<b>N</b>	<b>N</b>
<b>TTYH3_ TTYH3</b>	<b>Multi-pass transmembrane, plasma membrane</b>	<b>Y</b>	<b>Y</b>	<b>Y</b>	<b>N</b>	<b>N</b>	<b>N</b>
<b>VPS28_ VPS28</b>	<b>cytosol, plasma membrane, late endosomes</b>	<b>Y</b>	<b>Y</b>	<b>N</b>	<b>N</b>	<b>N</b>	<b>N</b>
<b>VP37C_V PS37C</b>	<b>cytosol, late endosome, peripheral membrane</b>	<b>N</b>	<b>Y</b>	<b>N</b>	<b>N</b>	<b>N</b>	<b>N</b>
<b>VPS4A_ VPS4A</b>	<b>cytosol, late endosome, plasma membrane, midbody</b>	<b>Y</b>	<b>Y</b>	<b>Y</b>	<b>N</b>	<b>N</b>	<b>N</b>
<b>VPS4B_ VPS4B</b>	<b>cytosol, late endosome, plasma membrane, midbody</b>	<b>Y</b>	<b>Y</b>	<b>Y</b>	<b>N</b>	<b>N</b>	<b>N</b>