

HIV-1 Tat and Heparan Sulfate Proteoglycans Orchestrate the Setup of *in Cis* and *in Trans* Cell-Surface Interactions Functional to Lymphocyte Trans-Endothelial Migration

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Table S1. Quantification of the Tat-dependent activation of the various receptors and second messengers in Table 1. c and 2d) or Tat versus naïve (Figure 4a). p-value: * <0.05, ** < 0.01. n.d., not determined.

Receptors/Second Messengers	Figure 1c	Figure 2d	Figure 4a
P-ERK _{1/2}	174 ± 14 **	108 ± 8	347 ± 36 **
P-src	150 ± 28	109 ± 9	132 ± 10 *
P-FAK	192 ± 32 *	87 ± 10	165 ± 36
P-VEGFR2	n.d.	n.d.	240 ± 43 *

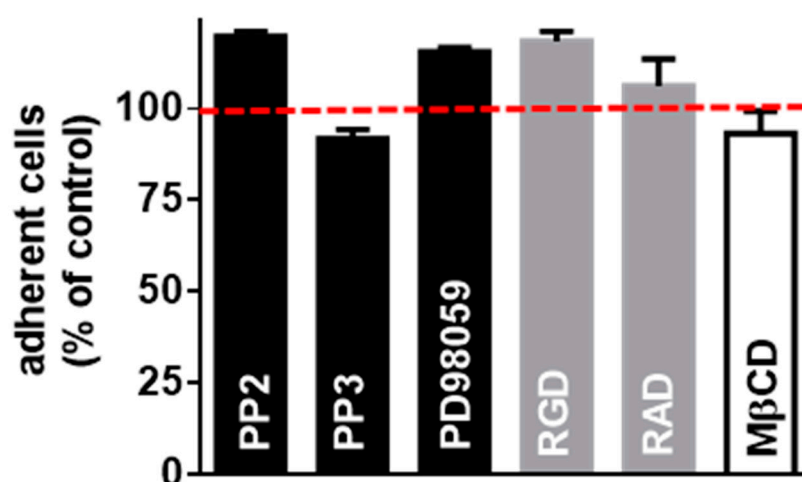


Figure S1. Inhibitors of integrin, src, ERK_{1/2} and lipid raft formation do not affect SYN-NCs adhesion to Tat. SYN-NCs were seeded onto plastic-immobilized Tat in the absence or in presence of the indicated compounds (same doses used for cell spreading assays). After 2 hours, adherent cells were counted and expressed as mean percentage ± S.E.M. in respect to the number of cells that adhered to Tat in the absence of any inhibitor (red dotted line).

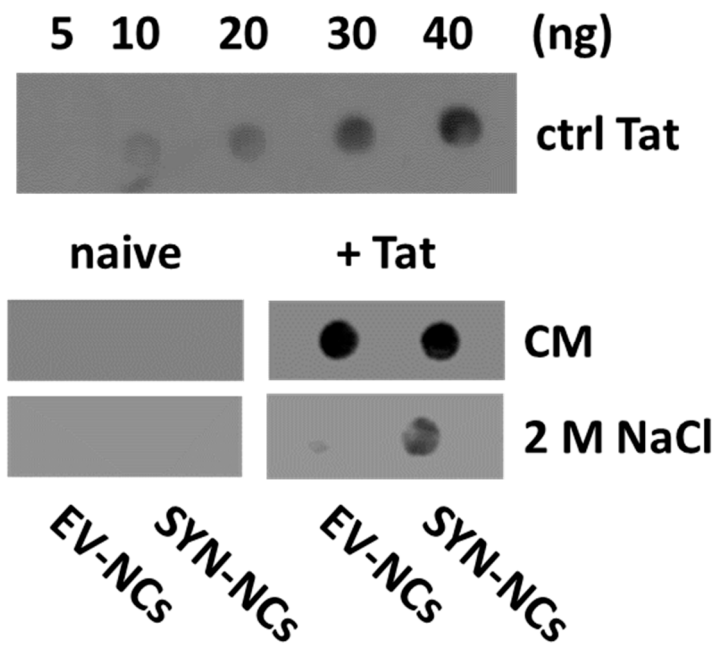


Figure S2. Coating of the lymphocyte surface with Tat. Samples (1×10^6 cells) of EV-NCs or SYN-NCs were incubated for 30 min at 37° C in the absence or in the presence of Tat (550 nM). At the end of incubation, conditioned medium was removed and cells extensively with PBS to remove un-bound Tat. Then, cells were washed twice with 1.5 M NaCl., a treatment that is known to detach Tat from HSPGs binding [1]. The entire volume of the PBS washing, the 1,5 M NaCl washing and conditioned media (CM) from the different experimental conditions were subjected to dot blot analysis with anti-Tat antibodies.

References

1. Urbinati, C.; Bugatti, A.; Oreste, P.; Zoppetti, G.; Waltenberger, J.; Mitola, S.; Ribatti, D.; Presta, M.; Rusnati, M. Chemically sulfated Escherichia coli K5 polysaccharide derivatives as extracellular HIV-1 Tat protein antagonists. FEBS letters 2004, 568, 171-177, doi:10.1016/j.febslet.2004.05.033.