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Supplementary Information for

Constitutive Siglec-1 expression confers susceptibility to HIV-1 infection of human dendritic cell precursors

Nicolas Ruffin[a], Ester Gea-Mallorquí[a], Flavien Brouiller[a], Mabel Jouve[b], Aymeric Silvin[a,c], Peter See[c], Charles-Antoine Dutertre[c,d], Florent Ginhoux[c,1], and Philippe Benaroch[a,1]

Correspondence to Florent Ginhoux, and Philippe Benaroch

email: Florent_Ginhoux@immunol.a-star.edu.sg, philippe.benaroch@curie.fr

This PDF file includes:

Figures S1 to S5

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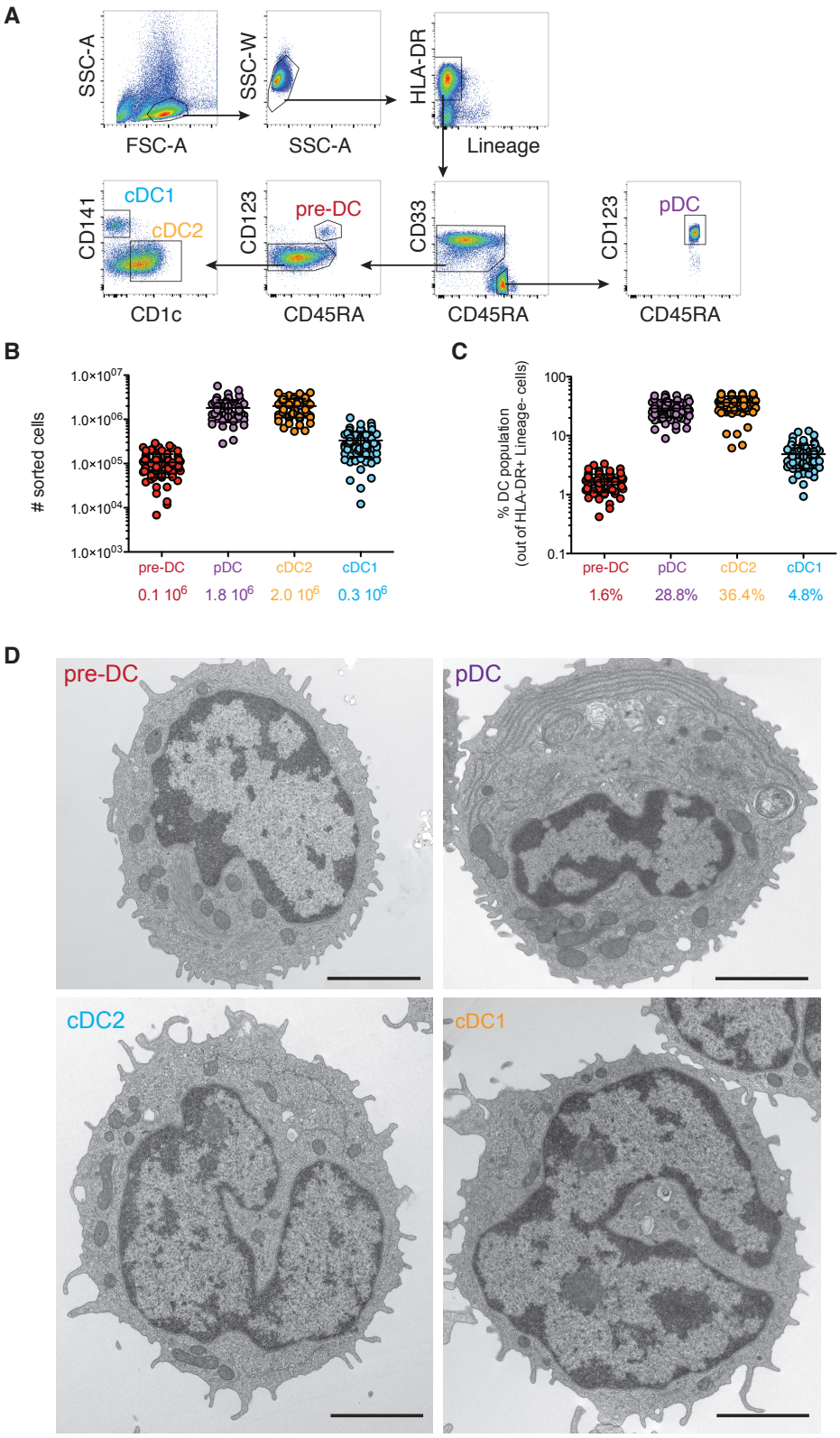

Fig. S1. Related to Figure 1. **Characterization of the sorted blood DC populations.**

(A) Gating strategy used to purify the four populations of DCs from the blood after magnetic bead-enrichment of pan-DC from PBMCs.

(B) Absolute numbers of cells obtained after sorting for each of the four DC subsets (n= 84 donors). Data represent individual donors with mean ± S.D.

(C) Percentages of the different subsets among total HLA-DR^{+}Lin^{-} DCs (n=84 donors). Data represent individual donors with mean ± S.D.

(D) Electron microscopy analysis of the four DC subsets freshly sorted from blood. Representative epon sections are presented. Bar scale: 2 μM.

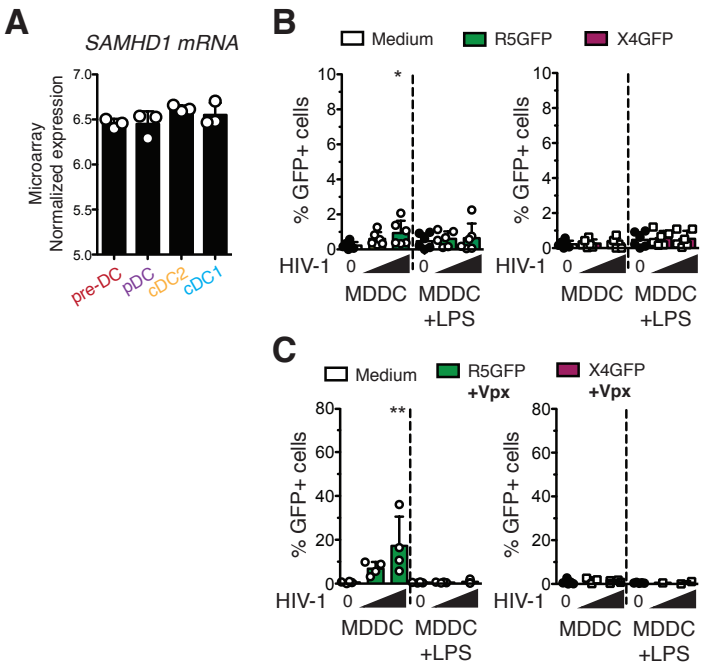

Fig. S2. Related to Figure 1. **Susceptibility of MDDCs to HIV-1 infection.**

(A) mRNA levels of SAMHD1 in the freshly sorted DC subsets, measured by RNA seq, (n=3 independent donors combined in 1 experiment). Individual donors are displayed with bars representing mean ± S.D.

(B) Quantification of GFP expression in MDDCs and LPS-activated MDDCs infected for 48 h with HIV-1 R5GFP or X4GFP alone or (C) supplemented with Vpx, n=4 from 4 independent experiments.

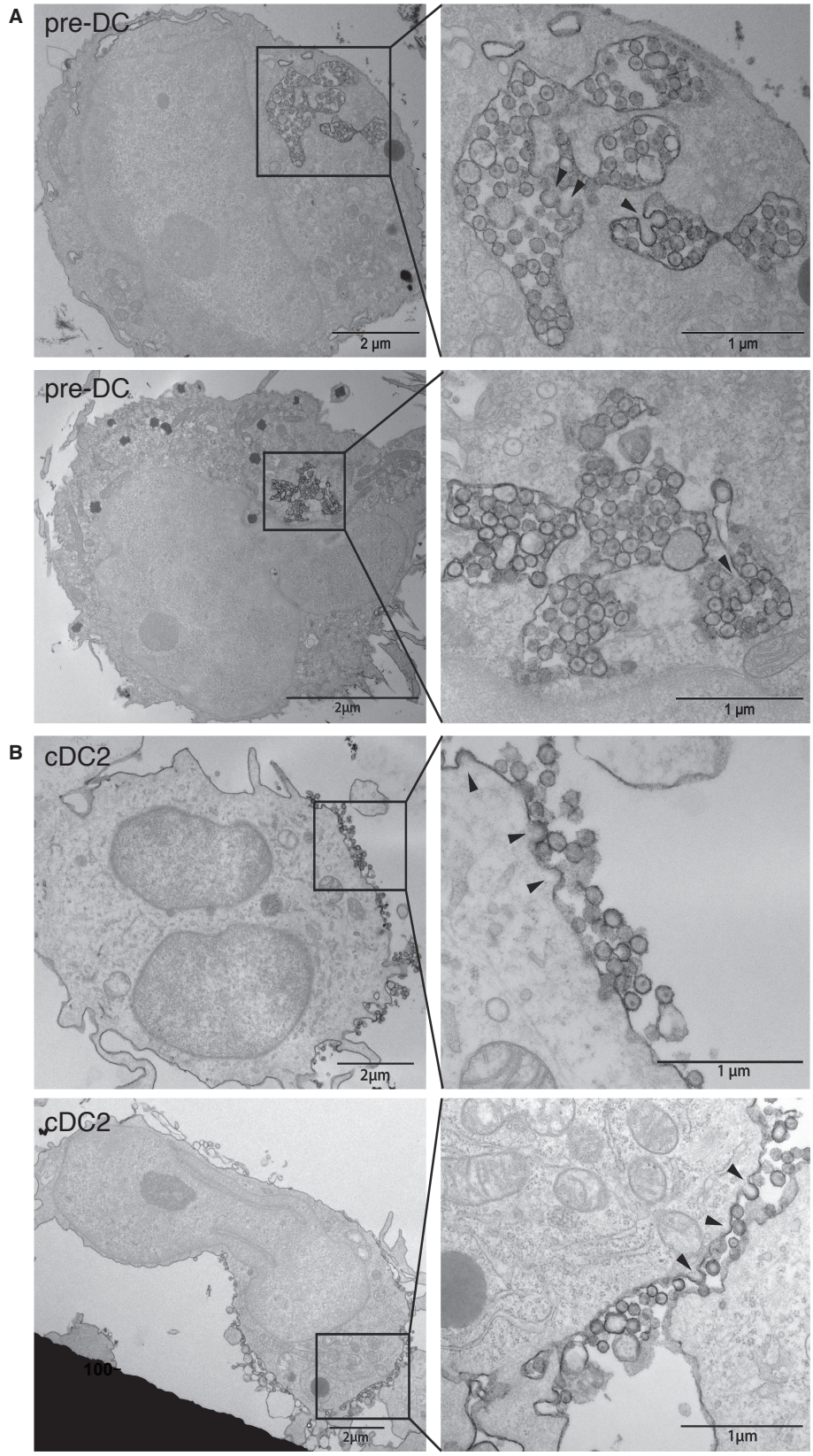

Fig. S3. Related to Figure 4. **Ultrastructural analysis of HIV-1-infected pre-DCs and cDC2s.**

(*A*) EM profiles of sorted pre-DCs or (*B*) cDC2s that were infected for 48 h with HIV-1 R5 + Vpx. Samples were prepared and processed for EM analysis as in Fig. 4*A* and *B*. Immature virions possess a darker periphery and an electron lucent centre while mature virions contain an electron dense zone in their centre (30, 31) revealing the presence of the capsid shell with sometimes its typical conical shape depending on the angle of cut in pre-DC VCC.

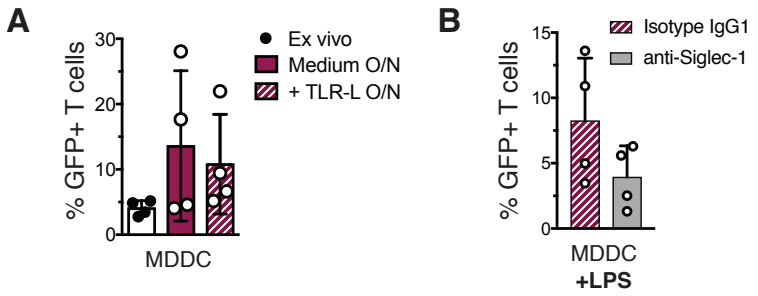

Fig. S4. Related to Figure 5. **Activated MDDCs transmit HIV-1 in *trans* to activated CD4⁺T cells through Siglec-1.**

(A) MDDCs were assayed for *trans*-infection as in Fig. 5A. GFP expression in activated T cells co-cultured directly with MDDCs or following overnight culture in medium or in the presence of LPS. Prior to CD4⁺ T cell co-culture, DC populations were exposed to HIV-1 X4GFP (without Vpx) for 2 h and washed.

(B) GFP expression in activated T cells co-cultured with LPS-activated MDDCs. Prior to CD4⁺T cell co-culture, MDDC populations were exposed to an anti-Siglec-1 mAb or its isotype control for 30 min and then exposed to HIV-1 X4GFP (without Vpx) for 2 h and washed. For A and B, quantification of GFP⁺ T cells (CD3⁺) was performed by flow cytometry after a 48h co-culture, n=4 independent donors. Individual donors are displayed with bars representing mean ± S.D.

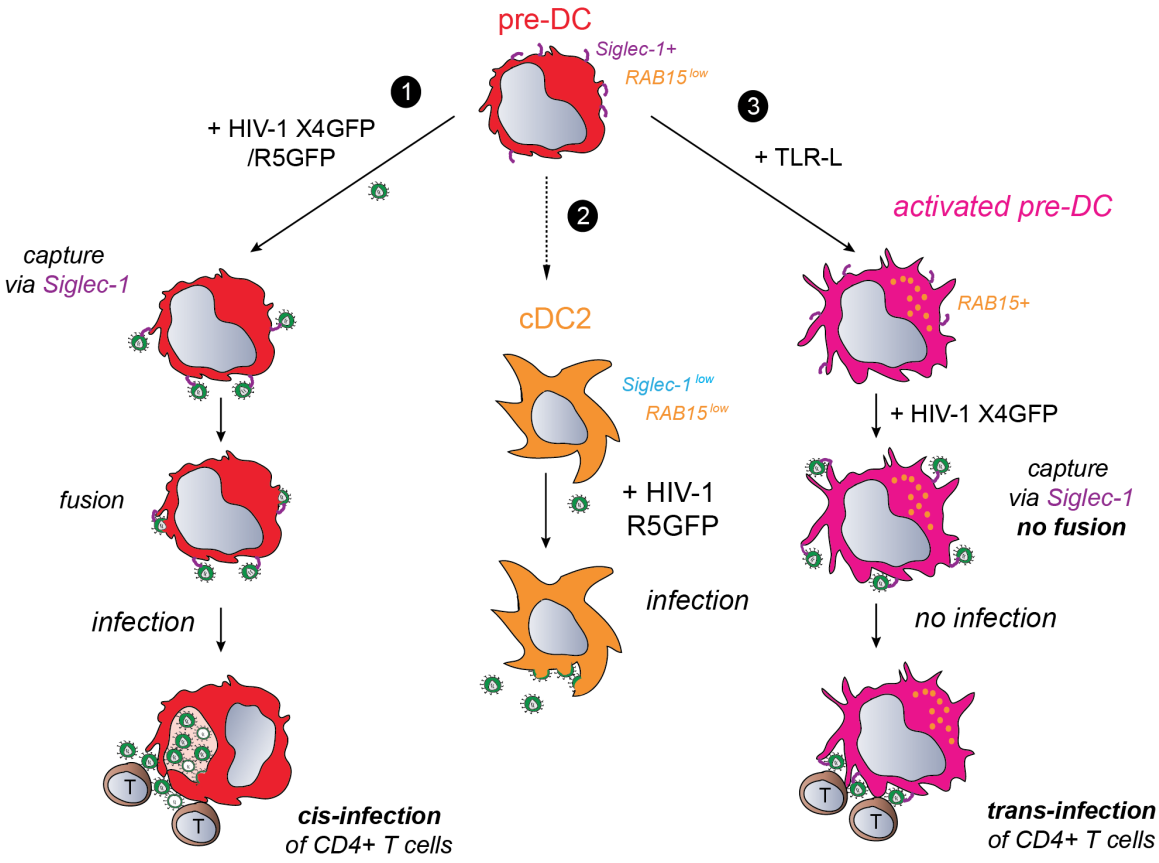

Fig. S5. Graphical Abstract. pre-DCs are endowed with unique properties with regards to HIV-1

(1) pre-DC can capture HIV-1 via their surface Siglec-1 leading to fusion and infection. They produce new viral particles in apparently intracellular virus-containing compartment and can transmit the infection in *cis* to activated T cells. (2) pre-DC can differentiate into cDC2 that are susceptible to HIV-1 R5-tropic viral infection. HIV-1-infected cDC2 produce viral particles at their plasma membrane. (3) Activated pre-DCs can capture HIV-1 via Siglec-1 and efficiently transfer in trans viral particles to activated T cells, which become infected while activated pre-DCs are resistant to viral fusion and infection, in association with their increased RAB15 expression.