Supporting Information

Felts et al. 10.1073/pnas.1003040107

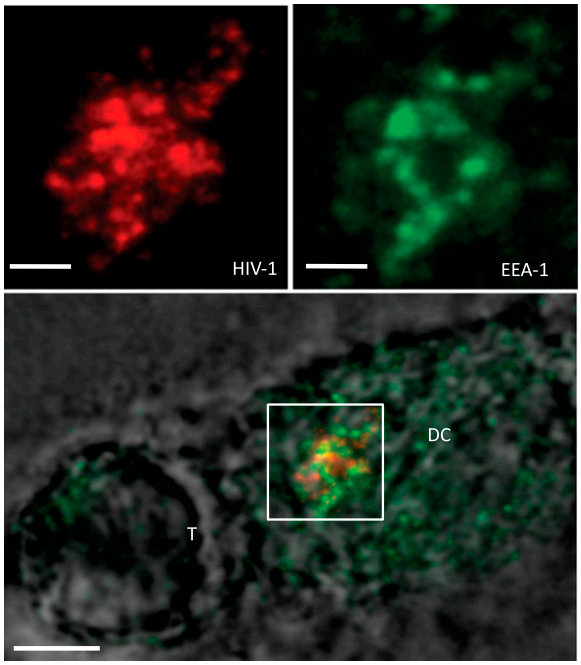


Fig. S1. Nonoverlapping localization of virus and endosomal markers in virus-pulsed dendritic cells (DC). ATTO-647N–labeled (red) HIV-1–pulsed dendritic cells were allowed to form synapses with autologous $CD4^+$ T cells as in Fig. 1. The cells were then fixed and stained for the endosomal marker EEA-1 (green). Some polarization of EEA-1 toward the T cell (T) was observed, although this was much more diffuse than that observed for HIV-1 virions and only showed modest colocalization when viewed at high resolution. (Scale bars: *Upper*, 1 μm; *Lower*, 3 μm.)

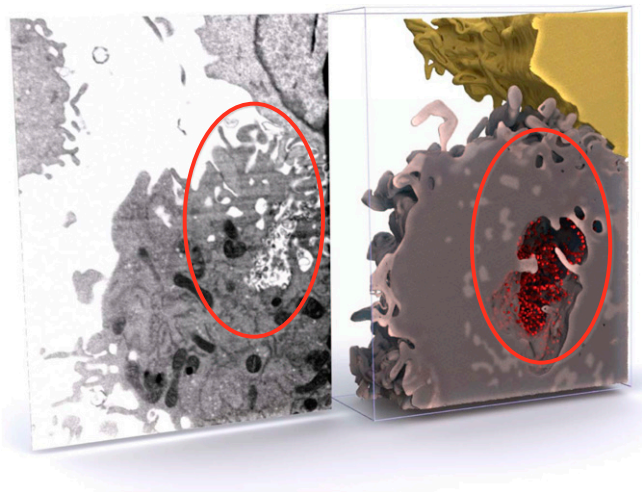


Fig. S2. 3D contacts between a dendritic cell and T cell at the synapse visualized with IA-SEM imaging. (T cell in yellow, dendritic cell in grayish pink, cell-cell contact circled in red). Single slices and 3D-volume slabs are shown side by side to highlight an example in which virion-rich compartments deep in the dendritic cell are connected to the synapse via conduits lined with HIV.

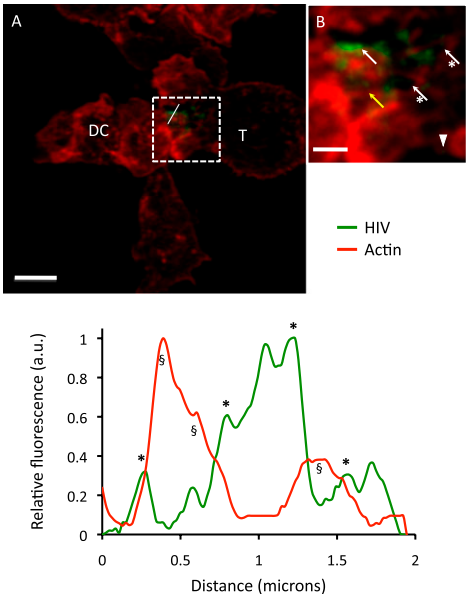


Fig. S3. HIV virions are associated with actin-rich filopodia present between pulsed dendritic cells (DC) and CD4$^+$ T cells (T). ATTO-647N–labeled HIV-1 (green)–pulsed dendritic cells were coincubated with CD4$^+$ T cells and costained for actin using fluorescent phalloidin (red). HIV virions imaged using STED were found within the dendritic cell (yellow arrow) polarized toward the T cell and at the cell membrane (white arrow). Virions were also found to associate closely with and run parallel to the filopodial extensions connecting the two cells (asterisk) and sometimes colocalized as an intact virion with the cytoskeleton of a CD4$^+$ T cell (white arrowhead). The line plot of relative fluorescence vs. distance reveals a lack of perfect correlation between the peaks corresponding to HIV virions (green) and filopodia (red). Peaks corresponding to virions (*) were ~130 nm away from the closest neighbor peaks corresponding to filopodia (§). a.u., arbitrary units. (Scale bars: *A*, 1 μm; *B*, 0.5 μm.)

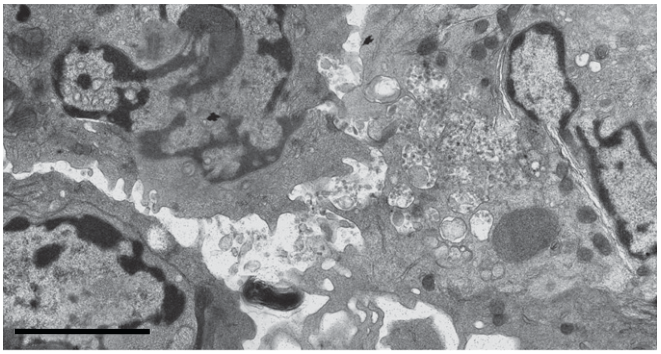


Fig. S4. TEM image from a ~200-nm thick section illustrating close interdigitating contact between CD4$^+$ T-cell and dendritic cell membranes at the synapse. (Scale bar: 2 μm.)

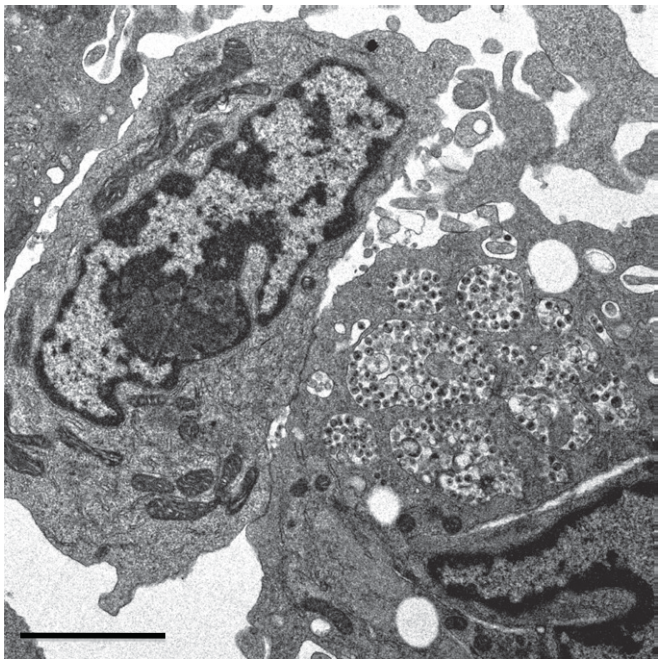


Fig. S5. Projection TEM image of synapses formed between HIV-pulsed dendritic cells and T cells preincubated with anti-gp120 antibody 2G12. Virions are polarized toward the contact region with the T cell but are not found on the T cell. (Scale bar: 2 μm.)

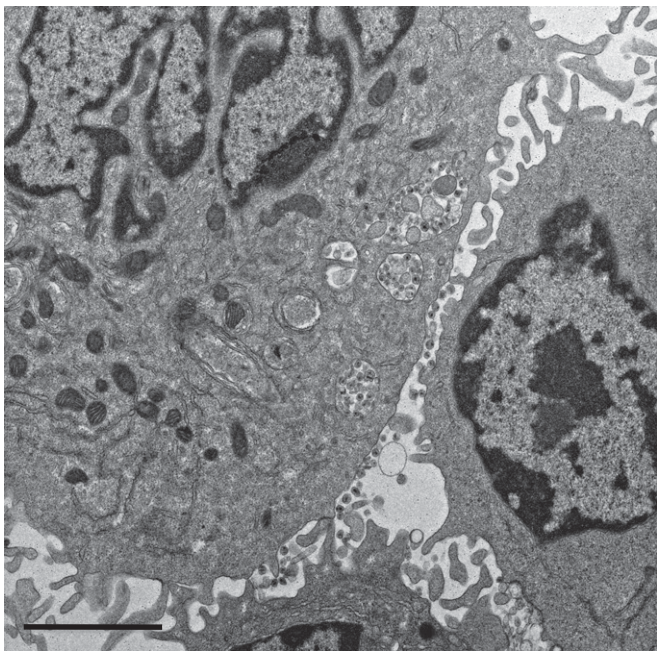


Fig. S6. Projection TEM image of synapses formed between HIV-pulsed dendritic cells and T cells preincubated with anti-gp120 CD4-induced antibody 17b. The virus distribution is similar to that seen in untreated cells (Fig. S4), with viruses present on both dendritic and T-cell surfaces at the synapse. (Scale bar: 2 μm.)

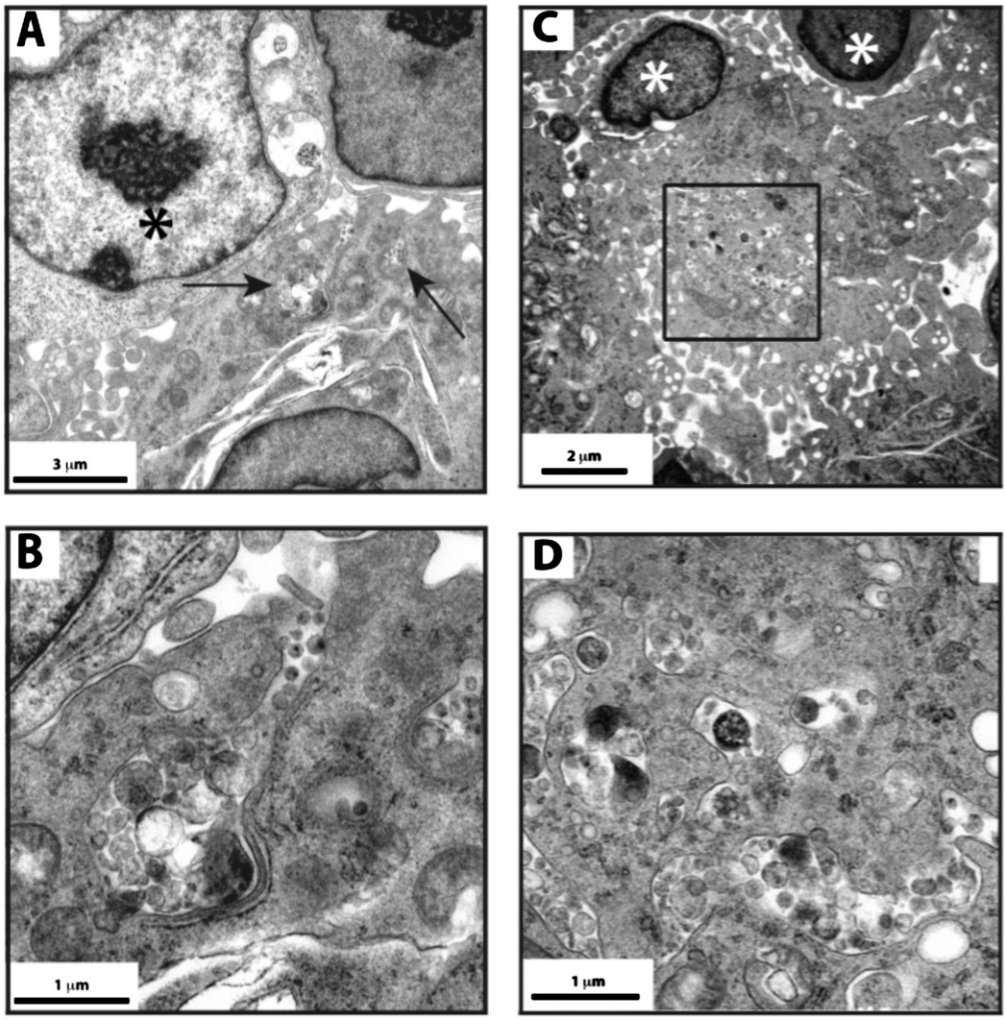
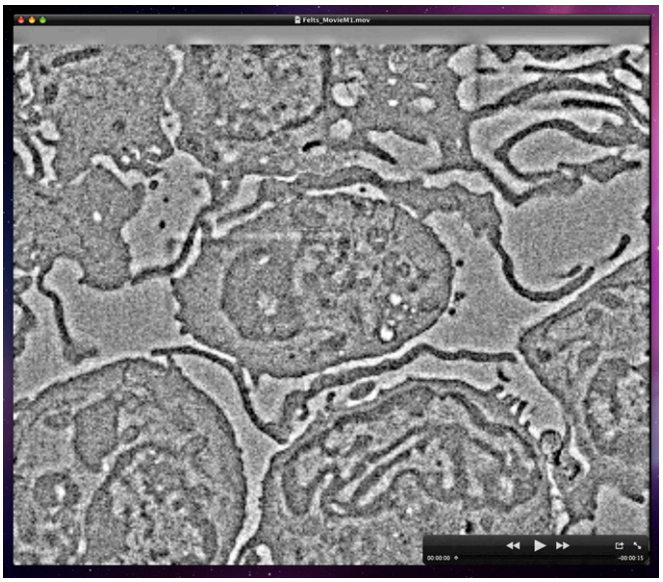
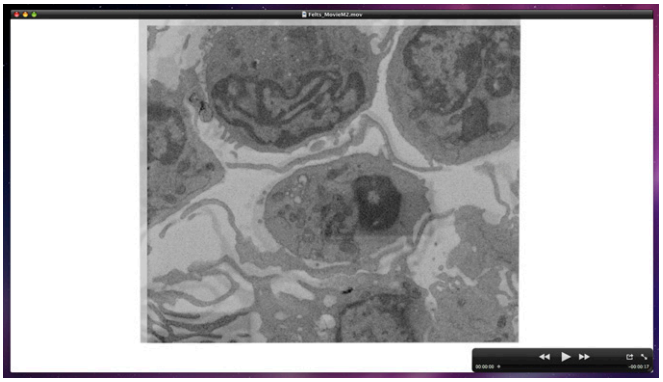


Fig. S7. (*A* and *B*) TEM images of dendritic cells incubated with the actin polymerization inhibitor cytochalasin D before addition of HIV-1 BaL and then incubated with autologous $CD4^+$ T cells. The cell ultrastructure is compromised. Occasionally, viral compartments present within the dendritic cells are detected (black arrows) without polarization toward the T-cell contact zone. (*C* and *D*) TEM images of dendritic cells incubated with the actin polymerization inhibitor cytochalasin D after addition of HIV-1 BaL but before incubation with autologous $CD4^+$ T cells. The cell ultrastructure is compromised as in *A* and *B*. Occasionally, viral compartments present within the dendritic cells are detected (black square). Viruses were not detected on the T-cell membrane in either scenario when cytochalasin D was present. The T cells in both panels are indicated with a white asterisk.



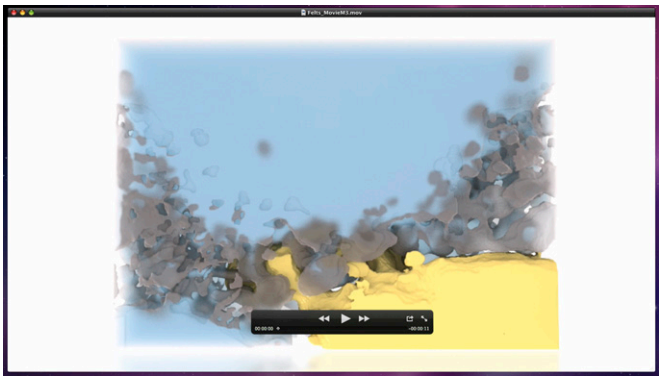
Movie S1. 3D image stack from regions of contact between T cells and dendritic cells pulsed with HIV-1 BaL, obtained using IA-SEM. The in-plane pixel size is 3.1 nm, the horizontal field of view is 6 μm wide, and the section spacing of the z-stack is 20 nm. The data in this movie correspond to the data in Fig. 2*A*.

Movie S1



Movie S2. 3D rendering of cell-cell contacts shown in Movie S1.

Movie S2



Movie S3. Segmented rendering of a 3D image stack at a dendritic cell–T-cell virological synapse. The data in this movie correspond to the data in Fig. 3*A*.

Movie S3