

1 **Supplementary Movie 1.** Volume rendered three-dimensional reconstruction of an
2 HIV-1 infected CD4 T cell engaged with an uninfected target CD4 T cell.
3 Mitochondria are red, HIV-1 Gag is green and the uninfected target CD4 T cell is
4 labeled with a cytoplasmic dye (blue). Scale bar is 5 microns. Rotation is around the
5 x axis.

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7 **Supplementary Movie 2.** Live cell imaging of mitochondria recruitment to the
8 contact zone formed between an HIV-1 infected Jurkat CD4 T cell and an uninfected
9 target T cell. Mitochondria are labeled with mitotracker red, HIV-1 infected cells
10 express Gag-GFP. Uninfected target CD4 primary T cells are unlabeled. Images
11 were recorded every 2 minutes using a DeltaVision ELITE Image Restoration
12 Microscope in Real-Time-Z-Sweep mode, in which the Z dimension is acquired
13 continuously and automatically converted to maximum intensity projection. Duration
14 of movie is 48 minutes (played at 1.5 fps) and is representative of n = 20 individual
15 contacts showing recruitment of Gag-GFP and mitochondria to the contact zone.

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17 **Supplementary Movie 3.** As described for Supplementary Movie 2. Duration of
18 movie is 34 minutes (played at 1.5 fps).

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20 **Supplementary Movie 4.** As described for Supplementary Movie 2 except primary
21 CD4 T cells were infected with HIV-1 Gag-GFP reporter virus and mixed with
22 autologous CD4 T cells. Duration of movie is 38 minutes (played at 1.5 fps) and is
23 representative of n = 25 individual contacts showing recruitment of Gag-GFP and
24 mitochondria to the contact zone.

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26 **Supplementary Movie 5.** As described for Supplementary Movie 4. Duration of
27 movie is 28 minutes (played at 1.5 fps).

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29 **Supplementary Movie 6.** Live cell imaging showing that transient contacts between
30 HIV-1 infected T cells and target cells fail to induce polarization. Duration of movie is
31 62 minutes (played at 1.5 fps).

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33 **Supplementary Movie 7.** Volume rendered three-dimensional reconstruction of a
34 polarized HIV-1 infected CD4 T cell (bottom left) treated with DMSO (solvent control)
35 that is engaged with an uninfected target CD4 T cell (top). Mitochondria are red, HIV-
36 1 Gag is green and the cytoplasmic dye labeled target CD4 cell is blue. Rotation is
37 around the x axis.

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39 **Supplementary Movie 8.** Volume rendered three-dimensional reconstruction of a
40 non- polarized HIV-1 infected CD4 T cell (bottom right) pretreated with 50 μ M Mdivi
41 that is engaged with an uninfected target CD4 T cell (top). Mitochondria are red, HIV-
42 1 Gag is green and the cytoplasmic dye labeled target CD4 cell is blue. Note the
43 elongated mitochondria tubules resulting from inhibition of Drp1 with Mdivi. Rotation
44 is around the x axis.

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