

1 **Supplementary Video Legends:**

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3 **Supplementary Video 1: Morphological features of HIV-infected MDMs in 3D collagen.**

4 MDMs were either transduced with GFP-expressing lentivirus or infected with HIV-GFP or
5 HIV-GFP mutants for 2 days and embedded into 3D collagen matrices for live-cell imaging
6 studies. Two representative cellular behaviors from each condition are shown. Each individual
7 frame is a maximum intensity projection of 12 z-stacks spaced 4 μm apart (total thickness of
8 44 μm). Scale bar = 20 μm . Time is shown in minutes:seconds.

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10 **Supplementary Video 2: Morphology and behavior of HIV-infected MDMs in collagen and**
11 **infected “myeloid-like” cells in the lymph node of humanized mice.**

12 MDMs were infected with HIV-GFP for 2 days and embedded into 3D collagen matrices for
13 live-cell imaging studies (left panel). BLT humanized mice were infected by footpad injection
14 with HIV-GFP and the lymph node visualized after 6 days using MP-IVM (right panel). Each
15 individual frame is a maximum intensity projection of 12 z-stacks spaced 4 μm apart (total
16 thickness of 44 μm). Scale bar = 30 μm . Time is shown in minutes:seconds.

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18 **Supplementary Video 3: Visual characterization of MDM:T cell contact dynamics in 3D**
19 **collagen.** MDMs were either transduced with GFP-expressing lentivirus or with HIV-GFP and

20 co-cultured with Celltracker blue-labeled autologous CD4⁺ T cells in 3D collagen matrices for
21 live-cell imaging studies. In some studies, SEB was added to the co-culture to visualize stable
22 MDM:T cell contacts. Each individual frame is a maximum intensity projection of 12 z-stacks

23 spaced 4 μm apart (total thickness of 44 μm). Scale bar = 30 μm . Time is shown in
24 minutes:seconds.

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26 **Supplementary Video 4: Three distinct MDM:T cell contact behaviors following HIV**

27 **infection.** HIV-infected MDMs were co-cultured with Celltracker red-labeled autologous CD4+
28 T cells in 3D collagen. Live-cell imaging studies of three distinct MDM:T cell contact behaviors
29 (brief, scanning and stable) are shown. Yellow arrowheads indicate contacts between T cells and
30 MDMs. Each individual frame is a maximum intensity projection of 12 z-stacks spaced 4 μm
31 apart (total thickness of 44 μm). Scale bar = 20 μm . Time is shown in minutes:seconds.

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33 **Supplementary Video 5: Membranous tethering events following MDM:T cell**

34 **disengagement after LFA-1:ICAM-1 blockade.** HIV-infected MDMs were co-culture with
35 Celltracker blue-labeled autologous CD4+ T cells in 3D collagen. Live-cell imaging studies of
36 three separate tethering events are shown (yellow circles). Each individual frame is a maximum
37 intensity projection of 12 z-stacks spaced 4 μm apart (total thickness of 44 μm). Scale bar = 20
38 μm . Time is shown in minutes:seconds.

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