

Figure S1 (related to Figures 3A-C): Flow cytometry plots of MT4 target cells 30h after co-culture with Jurkat donor cells. MT4 target cells infected by Jurkat donorRG cells (left) or Jurkat donorR + donorG cells (right), at different donor to target ratios. A, Diagram of MT4 target cells (blue) mixed with Jurkat donorRG (left) or Jurkat donorR + Jurkat donorG (right). **B**, Flow cytometry plots of transfected donor cells before mixing. C-F, Flow cytometry plots of target cells 30h after mixing at a donor:target cell ratio of C, 1:1, D, 1:7, **E**, 1:31, **F**, 1:127.



Figure S2 (related to Figure 3D): Schematic diagram showing the events that can occur in the computational models with their respective probabilities. **A**, An infected donor cell (grey with diamond) can form a synapse with a target cell (white) with a probability S. The target cell can be either uninfected or already infected. **B**, If cells are linked together via the synapse, the virus can be transferred from the donor cell to the target cell with a probability Q. In this picture, transfer occurs in one direction because we are concentrating on infections from the donor cell. **C**, With a probability G, a synapse can break, resulting in two uncoupled cells (see Supplementary methods for parameter details).



Figure S3 (related to Figure 3E): Flow cytometry plots of Jurkat target cells 30h after co-culture with Jurkat donor cells. Jurkat target cells infected by Jurkat donorRG cells (left) or Jurkat donorR + donorG cells (right), at different donor to target ratios. A, Diagram of Jurkat donorRG (left) or Jurkat donorR + Jurkat donorG (right) mixed with Jurkat target cells (blue). B, Flow cytometry plots of transfected donor cells before mixing. C-F, Flow cytometry plots of target cells 30h after mixing at a donor:target cell ratio of **C**, 1:1, **D**, 1:7, **E**, 1:31, **F**, 1:127.



Figure S4 (related to Figure 3F): Flow cytometry plots of Jurkat target cells 30h after co-culture with Jurkat donorRGB cells. A, Diagram of Jurkat donorRG cells mixed with Jurkat target cells (dark red). B-E, Flow cytometry plots of transfected Jurkat donorRGB cells before mixing. F-M, Flow cytometry plots of Jurkat target cells 30h after mixing with Jurkat donorRGB cells at a donor:target cell ratio of 1:7, F-I, and 1:63, J-M. Plots show target cells that are RG, B,F,J, RB, C,G,K, or BG, D,H,L. E,I,M, plots show fraction of GB cells that also express HIV(Red). Abbreviations: R = HIV(Red), G = HIV(Green), B = HIV(Blue).



Figure S5 (related to Figure 3G): Flow cytometry plots of primary CD4+ target T cells 30h after co-culture with autologous, primary CD4+ donor T cells. Primary target cells infected by primary donorRG cells (left) or primary donorR + donorG cells (right), at different donor to target ratios. **A**, Diagram of primary donorRG (left) or primary donorR + primary donorG (right) mixed with primary target cells (blue). **B**, Flow cytometry plots of transfected donor cells before mixing. **C and D**, Flow cytometry plots of target mixing at a donor:target cell ratio of **C**, 1:1 and **D**, 1:3.